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Brain edema: a valid endpoint for measuring hepatic encephalopathy?

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Abstract:	Hepatic encephalopathy (HE) is a major complication of liver failure/disease which frequently develops during the progression of end-stage liver disease. This metabolic neuropsychiatric syndrome involves a spectrum of symptoms, including cognition impairment, attention deficits and motor dysfunction which eventually can progress to coma and death. Pathologically, HE is characterized by swelling of the astrocytes which consequently leads to brain edema, a common feature found in patients with acute liver failure (ALF) as well as in cirrhotic patients suffering from HE. The pathogenic factors involved in the onset of astrocyte swelling and brain edema in HE are unresolved. However, the role of astrocyte swelling/brain edema in the development of HE remains ambiguous and therefore measuring brain edema as an endpoint to evaluate HE is questioned. The following review will determine the effect of astrocyte swelling and brain edema on neurological function, discuss the various possible techniques to measure brain edema and lastly to propose a number of neurobehavioral tests to evaluate HE.

Brain edema: a valid endpoint for measuring hepatic encephalopathy?

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List of abbreviations:

HE: hepatic encephalopathy; ALF : Acute Liver Failure; GFAP: glial fibrillary acid protein;

CSF: cerebrospinal fluid; RVD: regulatory volume decrease; MRI: magnetic resonance imaging;

DWI: diffusion weighted imaging; FLAIR: fast fluid-attenuated inversion recovery

Keywords:

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Abstract

Hepatic encephalopathy (HE) is a major complication of liver failure/disease which frequently develops during the progression of end-stage liver disease. This metabolic neuropsychiatric syndrome involves a spectrum of symptoms, including cognition impairment, attention deficits and motor dysfunction which eventually can progress to coma and death. Pathologically, HE is characterized by swelling of the astrocytes which consequently leads to brain edema, a common feature found in patients with acute liver failure (ALF) as well as in cirrhotic patients suffering from HE. The pathogenic factors involved in the onset of astrocyte swelling and brain edema in HE are unresolved. However, the role of astrocyte swelling/brain edema in the development of HE remains ambiguous and therefore measuring brain edema as an endpoint to evaluate HE is questioned. The following review will determine the effect of astrocyte swelling and brain edema on neurological function, discuss the various possible techniques to measure brain edema and lastly to propose a number of neurobehavioral tests to evaluate HE.

Astrocyte swelling and brain dysfunction

Astrocytes and disease

Astrocyte swelling has been implicated in a range of neurological disorders, involving stroke, migraine, epilepsy, and metabolic encephalopathies (including HE) (Felipo, 2013; Papadopoulos and Verkman, 2013; Thrane et al., 2014). Selective genetic (e.g. a mutation in glial fibrillary acid protein (GFAP) resulting in Alexander disease) or chemical impairment (fluorocitrate or fluoroacetate) of astrocyte function can also cause severe neurological impairment ranging from lethargy, stupor, ataxia, seizures to coma and death (Swanson and Graham, 1994; Messing et al., 2012). The cellular mechanisms causing these phenotypic manifestations can best be understood by first examining the physiological roles of astroglia.

Fundamental role of astrocytes in the central nervous system

Astrocytes are multifunctional glial cells responsible for key brain homeostatic functions such as regulating ion gradients, cerebral blood flow, blood-brain barrier (BBB) integrity, scar formation (reactive gliosis) and cellular metabolism (Ransom et al., 2003). They are electrically inactive, but signal with intracellular calcium and other secondary messengers (including adenosine triphosphate (ATP) and cyclic guanosine monophosphate (cGMP)) (Cotrina et al., 2000; Sun et al., 2013). The importance of astrocytes for more complex nervous systems is highlighted by the fact that evolution has selectively increased the size, relative abundance and complexity of these cells, whilst leaving neurons relatively unchanged (Oberheim et al., 2006). Collectively, neuroglia make up approximately 41% of the volume fraction of human cortex, compared to 27% by neurons, and 12% by interstitial fluid, with the remainder comprising blood (10%) and cerebrospinal fluid (CSF); 10%)) (Syková and Nicholson, 2008). Although astrocytes were initially seen as simple star shaped structural cells on silver-chromate and cytoskeletal (GFAP) staining, cytoplasmic labeling studies have revealed a more complex ‘bush-like’ morphology (Oberheim et al., 2008). Individual astrocytes are linked together into a syncytium by gap junctions, which have been suggested to facilitate faster ion movement, metabolic trafficking and neurovascular coupling (Rose and Ransom, 1997;

Rouach et al., 2008). Astrocytes have an intricate subcellular anatomy, with thousands of specialized processes that express a different subset of transporters depending on whether they abut blood vessels (peri-vascular end-feet), CSF (sub-ependymal end-feet) or synapses (peri-synaptic processes). Peri-vascular processes, for instance, are selectively endowed with ion and water transport mechanisms (including Na^+-K^+-$2Cl^-$ cotransporter isoform 1(NKCC1), inwardly rectifying potassium channel Kir4.1, glutamate transporter-1 (GLT1), glucose transporter (GLUT), Na^+-K^+-ATPase (NKA), system N transporter (SN1)), including the main brain water channel, aquaporin 4 (AQP4) (Figure 1). Metabolically, astrocytes are estimated to be responsible for about 30% of cerebral metabolism, and are amongst the most glycolytically active cells in the brain (Kasischke et al., 2004; Pellerin et al., 2007). Finally, a large body of evidence indicates that astrocytes play an active role in synaptic transmission (tripartite synapse), for instance by regulating neurotransmitter turnover and perhaps gliotransmission, along with fine-tuning the structural and electrochemical synaptic environment (Araque et al., 1999; Nedergaard and Verkhratsky, 2012). Therefore, astrocytes are critical cells which play a pivotal role in health and disease (Rose et al., 2013).

Astrocytes: susceptible to swelling

Astrocyte responses to osmotic stress have been extensively studied *in vitro* and *in situ*. Astrocytes are thought to be able to cope with brief exposures (30-90 min) to mild or moderate amounts of swelling by offloading osmolytes, including potentially excitotoxic neurotransmitters, a mechanism known as regulatory volume decrease (RVD) (Kimelberg, 1987; Ordaz et al., 2004; Thrane et al., 2011; Anderova et al., 2014). Conversely, hyper-osmotic stress may be able to induce a regulatory volume increase (RVI) by uptake of osmolytes and water (Evanko et al., 2004; Risher et al., 2009). The molecular mechanisms underlying this response are incompletely understood, and thought to involve osmo- or stretch-sensitive intracellular signaling cascades involving $[Ca^{2+}]_I$ transients, AQP4 and volume-regulated anion channels (VRACs) (Mulligan and MacVicar, 2006; Thrane et al., 2011; Qiu et al., 2014; Voss et al., 2014). The high expression of water transporting membrane proteins has also led many authors to suggest that astrocytes are more susceptible to swelling than neurons when the capacity for RVD is exhausted (Häussinger, 2000; Bosoi and Rose, 2013; Papadopoulos and Verkman, 2013; Thrane et al., 2015).

Astrocyte swelling and brain edema

On a larger scale, brain edema is believed to preferentially enter via astrocyte membranes by virtue of their strategic perivascular location and high water permeability (Papadopoulos and Verkman, 2013). A range of studies have shown that deleting or inhibiting AQP4 reduces the amount of brain edema following many types of insults, including hypoosmotic stress, stroke, traumatic brain injury, hepatic (but not hyperammonemic) encephalopathy and meningitis (Manley et al., 2000; Amiry-Moghaddam et al., 2003; Papadopoulos and Verkman, 2005; Fukuda et al., 2013; Rangroo Thrane et al., 2013; Rao et al., 2014). Conversely, AQP4 deletion can also slow edema resorption, and this mechanism likely explains why $AQP4^{-/-}$ animals display worse vasogenic edema compared to $AQP4^{+/+}$ animals in the context of tumors, abscesses and following subarachnoid hemorrhage (Papadopoulos et al., 2004; Bloch et al., 2005; Tait

et al., 2010). Astrocyte swelling is accompanied by a shift of fluid from either interstitial or intravascular to the intracellular (astrocytic) compartment (Thrane et al., 2014). This fluid shift can have several detrimental effects. Net fluid entry to the brain from the vascular compartment (*vasogenic or osmotic edema*) increases the brain volume, raising intracranial pressure, and causing potentially fatal brainstem compression; complications which develop in 25% of patients with ALF (Lee, 2012). However, astrocyte swelling can hypothetically also occur if there is an isolated fluid shift from the interstitial to the intracellular (cytosol) compartment, with no net fluid entry into the brain. This is termed *cytotoxic edema*, and when seen in isolation it does by definition not lead to raised intracranial pressure (Simard et al., 2007). However, this definition of cytotoxic edema is perhaps controversial, as cytotoxic swelling would arguably always be accompanied by some degree of net brain edema through other mechanisms (e.g. osmotic gradient across the BBB). Moreover, cytotoxic edema has several direct detrimental effects, such as increasing the concentration of ions and neurotransmitters in the now shrunken interstitial space. This can potentially lower the seizure threshold (increased $[K^+]_o$) and cause excitotoxicity (increased $[Glutamate]_o$ and consequent NMDA receptor activation). Additionally, cytotoxic edema can dilute intracellular ion and metabolite concentrations and thereby impair cellular metabolism. Generally, any form of tissue edema will also increase the distance for oxygen and metabolite diffusion, exposing micro-watershed areas to hypoxia (Takano et al., 2007; Thrane et al., 2013).

Brain edema and the 'glymphatic' system

Brain edema can also develop by net salt and water entry into the parenchyma in the presence of an intact BBB, termed *ionic edema* by some authors, to distinguish it from isolated (cytotoxic) extra-to-intra-cellular redistribution (Simard et al., 2007; Iliff et al., 2012; Thrane et al., 2014). One recent hypothesis that might explain this apparent paradoxical observation, proposes that physiological interstitial fluid turnover in the brain parenchyma is facilitated by continuous influx of peri-arterial CSF and efflux via the perivascular space of a subset of large veins (Iliff et al., 2012) or the recently discovered brain glymphatic vessels (Louveau et al., 2015). This pathway, termed the glymphatic system, might also explain the preponderance of astrocytes for swelling (Manley et al., 2000; Amiry-Moghaddam et al., 2003) and the extensive molecular machinery astrocytes express for volume regulation (Iliff et al., 2012; Nedergaard, 2013). This hypothesis is also particularly interesting in the context of astrocyte swelling, because the main gateway for net water entry into the brain parenchyma is thought to be via the AQP4-enriched perivascular membranes of astrocytes (Nielsen et al., 1997; Nagelhus and Ottersen, 2013; Papadopoulos and Verkman, 2013). Moreover, a derangement of the polarized perivascular expression of salt and water transporters along with decreased glymphatic interstitial fluid turnover appears to be a consistent feature of traumatized, infarcted, aged and even sleep-deprived brain tissue, which might make it more prone to astrocyte swelling and edema formation (Iliff et al., 2012; Wang et al., 2012; Ren et al., 2013; Xie et al., 2013). Taken together, recent studies therefore highlight how astrocyte water transport, and consequently volume change, is likely to be linked to vascular perfusion and highly compartmentalized, with most of it happening in the small perivascular and perisynaptic processes, rather than astrocyte cell bodies. Future studies should therefore aim to use experimental models that best recapitulate the intimate neuro-

glio-vascular interplay seen in living brain tissue extrapolating findings from cell culture or even brain slice studies may sometimes lead to false conclusions.

Astrocyte swelling results in brain dysfunction

Astrocyte swelling can lead to neurological dysfunction in several ways. Prolonged osmotic and/or metabolic stress has also been shown to cause the generation of reactive oxygen species (ROS), apoptotic pathways (such as mitochondrial permeability transition pore (MPTP)) and/or inflammatory signals (such as tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), transforming growth factor-β (TGF-β), matrix metallopeptidase-9 (MMP-9) and interleukin-6 (IL-6)) (Schliess et al., 2004; Simard et al., 2007; Thrane et al., 2014). These mechanisms likely have physiological, as well as pathophysiological consequences. For example, perisynaptic astrocyte processes swell briefly and reversibly during normal synaptic transmission and this is thought to represent buffering of extracellular potassium and sodium released by active neurons (Binder et al., 2006; Haj-Yasein et al., 2012; Karus et al., 2015). However, prolonged osmotic stress and astrocyte swelling near the synapse could set up a vicious cycle where shrinkage of the interstitial space, volume-regulated excitotoxic neurotransmitter offloading, extracellular K^+ accumulation, lactic acidosis, neuronal Na^+ and Cl^- accumulation impairing inhibitory neurotransmission, ROS, and inflammatory signals further compound brain edema by promoting astrocytes to swell more readily (Mulligan and MacVicar, 2006; Cauli et al., 2007; Rodrigo et al., 2010) (Figure 1).

Techniques to measure brain edema

It is evident that the development of intracranial hypertension is associated with brain edema in ALF, however in chronic liver disease, where intracranial hypertension is rarely observed, brain edema is also present (O'Grady, 2008; Rovira et al., 2008; Shah et al., 2008; Sugimoto et al., 2008; Bosoi et al., 2012; Bosoi and Rose, 2013; Butterworth, 2015; Dam et al., 2015). The pressure-volume relationship between intracranial pressure and brain volume indicates that either low-grade edema or age-induced brain atrophy could explain the difference between ALF and chronic liver disease (Bosoi and Rose, 2013). To date several methods have been used to measure brain edema (i.e. brain water content) either directly or indirectly: 1) Direct/absolute value of the amount of water in the brain can be measured through; i) dry/wet weight technique (Marmarou et al., 1978), ii) specific gravity method (Marmarou et al., 1978; Hayazaki et al., 1995) and iii) brain water mapping using magnetic resonance imaging (MRI) (Shah et al., 2003, 2008; Neeb et al., 2006; Dam et al., 2015); 2) Indirect/relative information regarding the content of water in the brain can be calculated using several advanced MRI techniques; i) magnetization transfer (MT), ii) diffusion weighted or tensor imaging (DWI or DTI) and iii) fast fluid-attenuated inversion recovery (FLAIR) Table 1) (Häussinger et al., 1994; Córdoba et al., 2001; Spahr et al., 2002; McPhail et al., 2012; Braissant et al., 2013; Cudalbu, 2013).

The dry/wet weight and specific gravity methods are performed *ex-vivo*, using dissected tissue from sacrificed animals. The dry/wet weight method is the easiest way to measure the amount of water in extracted brain tissue. In this technique, brain samples are weighed before and after drying for 24 hours at 100°C (Marmarou et al., 1978) and the difference in weight reflects the amount of water (evaporated) in the tissue. Aside

from its simplicity, this technique is not sensitive enough for precisely measuring water evaporation in small regions and therefore is limited to whole or half brains (at least for rodents).

The specific gravity method involves placing small pieces of brain tissue into a graduated cylinder containing a layered mixture (gradient) of organic solvents of known density (kerosene and bromobenzene). The equilibration depth of the inserted tissue is recorded after 2 minutes (representing the specific gravity). This technique is excellent for measuring small changes in water of many different (including small) brain regions. The initial set-up for making the kerosene/bromobenzene gradient columns is cumbersome, but worth the investment. Following the complex mixing process, each column must be carefully calibrated but can be used to measure the specific gravity of about 20-25 samples. The percentage of water is calculated taking into consideration the specific gravity of the solid dry tissue (Marmarou et al., 1978). This method provides absolute water content in the brain and has been shown to detect changes in water content of 1-2% in a rat model of chronic HE (Bosoi et al., 2012).

Non-invasive *in vivo* measurements of water content in the human brain with MRI are often applied to explore brain swelling in human HE studies (Häussinger et al., 1994; Córdoba et al., 2001; Shah et al., 2003, 2008; Rovira et al., 2008). MRI is a non-invasive technique applicable *in vivo* and therefore be implicated in longitudinally studies on the same individual. MRI is primarily focused on imaging the single proton of the hydrogen nucleus (^{1}H). Since hydrogen is by far the most common nucleus in the human body, it has become a valuable target for *in vivo* imaging. Several refined MRI techniques are presently available to detect subtle changes of approximately 1% in total brain water content. MT, FLAIR and DWI imaging are all sensitive to changes in brain tissue water of ~1 %. Although sufficiently sensitive, they all lack specificity in regards the etiology of the water accumulation. MT and FLAIR are both sensitive to exchange properties between bound and free protons, and therefore the output is determined by factors besides the total water content. The images, therefore, provide only indirect evidence of brain swelling. DWI allows the mapping of the diffusion process of primarily water molecules. However, molecular diffusion in tissues is not free, but reflects interactions with, for example, macromolecules, fibers, membranes. DWI can demonstrate changes in intra- or extracellular volume but no firm conclusions on the absolute water content can be drawn. Hence, the direct assessment of cerebral water changes in HE is tedious. A quantitative estimation of brain tissue water contents (water mapping) was validated on 1.5 and 3 Tesla platforms in Juelich in Germany from 2004-2008 (Neeb et al., 2006; Shah et al., 2008). This water mapping method enables an automated assessment of global water content changes in both grey and white matter as well as changes in the spatial distribution of water in the brain. It is sensitive and specific and can be performed within a clinically relevant measurement time (less 20 min). The water mapping method was applied to fifty-four patients with various grades of HE in 2008 (Shah et al., 2008).The average white matter water content was 2.1% higher in patients with overt HE compared to the healthy control group. There was no significant difference in grey matter water.

In conclusion, we believe that brain water mapping is the most precise and accurate method that can be applied to patients with HE for absolute quantification of their cerebral hydration status. However, increased water content remains an unspecific phenomenon that also occurs in trauma, tumors, focal inflammation and late stages of

cerebral ischemia. For measuring brain edema in animal models a multimodal approach would be the most suitable. For example, an approach combining *in vivo* and longitudinal measurements with an *ex vivo* technique measuring the absolute water value in the brain. This combination allows monitoring of the progression of the syndrome longitudinally and therefore provides additional information on the temporal resolution of the onset of brain edema. Since none of these techniques provides information on the type of the edema or which cell is involved, it would be also very useful to combine these techniques with electron microscopy (Kato et al., 1992).

Measuring hepatic encephalopathy in rodents; neurophenotyping

In humans, diagnosing HE, in particular minimal/covert HE, involves using sophisticated neuropsychometric and neurophysiological tests, such as the psychometric hepatic encephalopathy score (PHES) (Weissenborn et al., 2001; Ferenci et al., 2002; Amodio et al., 2008), the inhibitory control test (ICT) (Bajaj et al., 2007), the critical flicker frequency (CFF) test (Kircheis et al., 2002), the continuous reaction time (CRT) (Lauridsen et al., 2013) and the EncephalApp (Smart-phone based Stroop test) (Bajaj et al., 2013). Overall, these tests evaluate cognition, psychomotor processing speed, visuomotor coordination, memory, attention as well as motor function. As with HE patients, the best methods to evaluate HE in rodents are through the use of various behavioral tests (neurophenotyping). In general, behavioral measurements in rodents can be divided into three categories : 1) Cognitive function/learning and memory; 2) Motor function: and 3) Anxiety (Table 2). In the following sections, different tests used to evaluate the parameters in each category are discussed.

Cognitive function/learning and memory

Eight-Arm Maze

This test (Olton and Samuelson, 1976), which assesses spatial memory, consists of eight horizontal arms placed radially around a central platform above the floor. Food is placed at the end of all arms, and the animal must learn to enter each arm a single time. Errors are defined as repeat entries into already-visited arms. Although the simplest strategy to solve this task would be to enter adjacent arms, rodents do not typically adopt this tactic. As such, the analysis of arm entries can yield insight into such processes as planning and decision making and impulsivity in the rodent. Different variables are commonly used for the analysis of the performance, including: number of errors in each session (entering an arm that has been visited previously counted as an error) and the total number of errors during eight sessions; number of correct choices in the first eight arm entries; location of the first error in each session; time taken to visit each arm (total time to complete the session divided by the total number of arm entries); number of sessions to reach the criterion of one error or less, averaged over four consecutive days of training. Since the task is motivated by appetite, food restriction regimen are required, which represents a disadvantage of this behavioral test. Indeed, food restriction contrasts with cognitive tasks which are aversively motivated.

Morris Water Maze

This is a behavioral procedure widely used to study spatial learning and memory (Morris, 1984; D'Hooge and Deyn, 2001). Animals are placed into a pool of water in

which a platform is hidden beneath the surface. The animal must learn to use spatial cues located in the testing room to navigate to the platform. Longer latencies indicate poorer performance. Variations in the experimental protocol allow the experimenter to determine whether the observed impairments are the result of working (more than once a day) or reference memory (once a day) systems. Cognitive flexibility can be assessed using a water maze paradigm in which the hidden platform is continually re-located. The earliest measure of learning is escape latency, which is the time it takes to find the platform. However, this measure is confounded by swimming speed, not necessarily a cognitive factor. Path length between point of origin and platform is a parameter more closely related to spatial learning. Stress of swimming may be a disadvantage of this test.

Object Recognition test

This is a fast and efficient test to assess working memory (Ennaceur and Delacour, 1988). The animal is first placed into an arena containing two identical objects. After a predetermined period of exploration, the animal is removed from the arena, and a delay is imposed. Following the delay, the animal is placed back into the arena, where one of the objects has been replaced by a novel object. Since rodents are curious, they typically avoid familiar objects and explore novel objects. The amount of time investigating the novel object is taken as the measure of working memory. Lower exploration of the novel object is thus interpreted as poorer working memory performance (for further details: (Antunes and Biala, 2011).

Motor function

Locomotor activity

This test is commonly used in rodents to qualitatively and quantitatively measure general locomotor activity and willingness to explore (Denenberg, 1969; Stanford, 2007). This test uses an arena with walls to prevent escape. Generally, the field is marked with a grid and square crossings. This test measures exploratory behavior in a novel, enclosed environment. Rearing and time spent moving are used to assess the activity of the rodent. The apparatus is equipped with infrared beams or video cameras with associated software that can be used to automate the assessment process.

Rotarod

The rotarod test is used to assess motor coordination and balance (Jones and Roberts, 1968). The test animal (usually a rodent) is placed on a cylinder that rotates at gradually increasing speed until the animal can no longer maintain itself on the cylinder. The speed of the rotarod may either be held constant or accelerated. The length of time that a given animal stays on the rotating rod is a measure of its balance and coordination. However, the physical condition of the animal may represent a bias to the analysis.

Arm Grip test

This test measures forepaw strength (Meyer et al., 1979). Animals are allowed to grasp the grip strength meter with their forepaws. They are then gently pulled from the base of the tail until the grip is released. A grip strength meter measures the maximum force applied to the meter.

Gait
This test consists of images of the underside of the animal that are taken as the animal ambulates on a clear treadmill. Measurements of stride length, base width, and fore and hind paw overlap give an indication of gait.

Anxiety
Elevated Plus Maze
This test has become the benchmark for assessing anxiety in rodents (Pellow et al., 1985). The test creates an approach-avoidance conflict between the natural tendency of the rodent to explore and its aversion for open spaces. The elevated plus maze itself consists of two enclosed arms and two open arms (arms without walls). Anxiety is typically measured by the amount of time the animal explores the open arms. The more anxious the animal, the less it will explore the open arms.

Open Field test
This test, which is used to assess motor function (see previous section), may also be used to assess anxiety (Prut and Belzung, 2003). The latter is assessed by including additional measures of defecation, time spent in the center of the field and the first few minutes of activity. Anxiety is measured by the amount of time the animal avoids the exposed center area of the field and remains in close proximity to the walls.

In conclusion, evaluating the behavioral phenotype in rodent models of liver disease is a valid way to define the presence of HE. However, due to costs, space and infrastructure, neurophenotyping is not routinely performed in laboratories. Furthermore, with increasing use of these protocols, standardizing these tests will be an imperative.

Hepatic encephalopathy and brain edema

Undoubtedly, in the development of intracranial hypertension in ALF, brain edema plays a "physical" contributing role to the associated encephalopathy. However the pathophysiological role of astrocyte swelling/brain edema in neurological deterioration remains elusive. Even in the absence of intracranial hypertension, it is suggested that astrocyte swelling can have important functional consequences and that HE represents a clinical manifestation of astrocyte swelling (Häussinger, 2000).
It is well documented that cirrhotic patients with brain edema present HE (Kumar et al., 2008; O'Grady, 2008; Rovira et al., 2008; Shah et al., 2008; Sugimoto et al., 2008; Bosoi and Rose, 2013; Dam et al., 2015, 2015)
. However, it is not known whether all patients with HE have an increase in brain water. In rats with bile-duct ligation (BDL)-induced CLD, a type-C model of HE, brain edema and HE are present (Bosoi et al., 2014). However, rats with portacaval anastomosis (PCA), a type-B model of HE, brain edema is not present (Bosoi et al., 2012). This raises the question regarding the role of brain edema in the neurological alterations related to HE. Other studies suggest that brain edema is not implicated in the pathogenesis of HE; in rats with ALF, it was shown that following attenuation of brain edema with the hypertonic solution mannitol, motor tract function did not improve (Oria et al., 2010). In addition, the same authors demonstrated that following an acute injection of ammonia to PCA rats, severe alterations of the motor tract function developed, without the

development of brain edema (Oria et al., 2010). It is however worth noting that in this study, attenuation in brain water was only measured in the cortex and brain stem whereas brain water content in the red nucleus, substantia nigra and basal ganglia (regions implicated in the modulation of the motor tract function) were not evaluated. In another study by Wright and colleagues, BDL and sham-operated controls were challenged with lipopolysaccharides (LPS) and both groups developed brain edema. However, only the BDL rats presented with a neurological decline (Wright et al., 2007). In addition, acute hyperammonemia induced in mice resulted in severe encephalopathy without brain edema or astrocyte swelling (Rangroo Thrane et al., 2013). Furthermore, $Aqp4^{-/-}$ mice have 2-3% increased brain water content compared to wild-type animals, but no obvious neurological phenotype (Nagelhus and Ottersen, 2013). However, there are many studies implicating brain edema in the pathogenesis of HE. Rovira et al., elegantly demonstrated a decrease in brain volume and improvement in HE following liver transplantation (Rovira et al., 2007). Furthermore, BDL rats treated with ammonia-lowering agents or antioxidants result in an attenuation in brain edema as well as an improvement in neurological status (Bosoi et al., 2011, 2012). Whether these discrepancies are model-specific (i.e. HE type A vs B vs C) remains to be determined.

Conclusion

The role of brain edema, as a neuropathological feature or a cause of HE, remains a controversial topic. The correlation between brain edema and HE is strong, with ample supporting studies demonstrating that brain edema leads to neuronal dysfunction. We suggest that different degrees of astrocyte swelling/brain edema may inflict differential effects (physical stress as well as metabolic alterations) on cerebral function. Therefore, brain edema remains a valid endpoint in the evaluation of HE. However, in the setting of liver disease/failure, other factors in addition to brain edema may play a role in the severity of HE. In effect, brain edema may play a predisposing or precipitating role in the pathogenesis of severe/overt HE.

Understanding the obvious limitations of assessing similar neuropsychological tests to rodents as in humans, the worthiest assessment of HE in rodents remains evaluating behavioural changes. Measuring changes in cognitive function, learning, memory, anxiety and motor function are valid parameters in the assessment of HE. In fact, developing and standardizing a battery of tests to assess HE in small animals is highly warranted and worth considering in the future.

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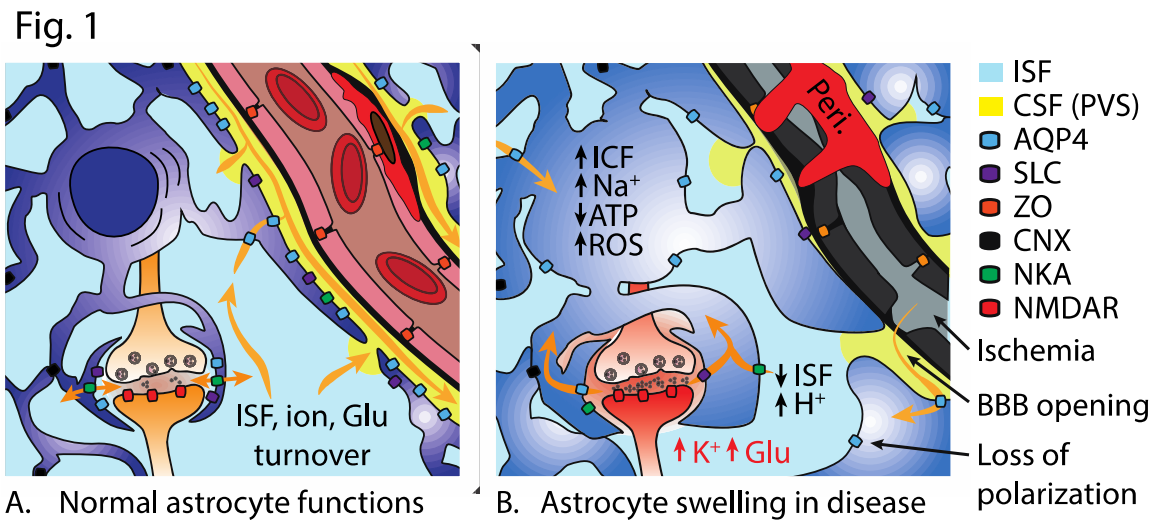

Figure 1. Effects of astrocyte swelling on brain function. *Left*, physiological salt and water turnover in the brain is functionally and anatomically compartmentalized to the peri-synaptic and peri-vascular regions. *Right*, proposed effects of astrocyte swelling on brain function include both acute alterations in ion concentrations, metabolism and blood flow, followed by more chronic or adaptive changes in transporter expression and polarization. Glutamate (Glu), interstitial fluid (ISF), intracellular fluid (ICF), adenosine triphosphate (ATP), reactive oxygen species (ROS), blood-brain barrier (BBB), cerebrospinal fluid (CSF), perivascular space (PVS), aquaporin 4 (AQP4), solute carrier family protein (SLC, e.g. Na^+-K^+-Cl^--cotransporter), zonula occludens (ZO, tight junctions), connexin (CNX, gap junctions), Na^+-K^+-ATPase, N-methyl-D-aspartate receptor (NMDAR), pericyte (Peri.)

Table 1: Short description of different techniques used to measure brain edema.

Technique	**Type of measurement**	**Edema measurement**	**Type of edema**	**Cell Specificity**
Water mapping	Direct – in vivo Longitudinal	% value	no	No
Dry/wet weight	Direct – ex vivo End point	% value	no	No
Specific gravity	Direct – ex vivo End point	% value	no	No
DTI	Indirect – in vivo Longitudinal	n/a	indication	indication
MT, FLAIR	Indirect – in vivo Longitudinal	n/a	No	No
Electron microscopy	Direct – ex vivo End point	n/a	No	Yes

DTI: diffusion tensor imaging; MT : magnetization transfer; FLAIR : fast fluid-attenuated inversion recovery

Table 2 : Behavioral measurements in rodents

Category	Test
Cognitive function/Learning and memory	Eight-Arm Maze Morris Water Maze Object recognition test
Motor function	Locomotor activity Rotarod Arm Grip Gait
Anxiety	Elevated Plus Maze Open Field