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Astrocyte membrane properties are altered in a rat model of developmental cortical malformation but single-cell astrocytic glutamate uptake is robust

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ABSTRACT

Developmental cortical malformations (DCMs) are linked with severe epilepsy and are caused by both genetic and environmental insults. DCMs include several neurological diseases, such as focal cortical dysplasia, polymicrogyria, schizencephaly, and others. Human studies have implicated astrocyte reactivity and dysfunction in the pathophysiology of DCMs, but their specific role is unknown. As astrocytes powerfully regulate glutamate neurotransmission, and glutamate levels are known to be increased in human epileptic foci, understanding the role of astrocytes in the pathological sequelae of DCMs is extremely important. Additionally, recent studies examining astrocyte glutamate uptake in DCMs have reported conflicting results, adding confusion to the field. In this study we utilized the freeze lesion (FL) model of DCM, which is known to induce reactive astrocytosis and cause significant changes in astrocyte morphology, proliferation, and distribution. Using whole-cell patch clamp recording from astrocytes, we recorded both UV-uncaging and synaptically evoked glutamate transporter currents (TCs), widely accepted assays of functional glutamate transport by astrocytes. With this approach, we set out to test the hypothesis that astrocyte membrane properties and glutamate transport were disrupted in this model of DCM. Though we found that the developmental maturation of astrocyte membrane resistance was disrupted by FL, glutamate uptake by individual astrocytes was robust throughout FL development. Interestingly, using an immunolabeling approach, we observed spatial and developmental differences in excitatory amino acid transporter (EAAT) expression in FL cortex. Spatially specific differences in EAAT2 (GLT-1) and EAAT1 (GLAST) expression suggest that the relative contribution of each EAAT to astrocytic glutamate uptake may be altered in FL cortex. Lastly, we carefully analyzed the amplitudes and onset times of both synaptically- and UV uncaging-evoked TCs. We found that in the FL cortex, synaptically-evoked, but not UV uncaging-evoked TCs, were larger in amplitude. Additionally, we found that the amount of electrical stimulation required to evoke a synaptic TC was significantly reduced in the FL cortex. Both of these findings are consistent with increased excitatory input to the FL cortex, but not with changes in how individual astrocytes remove glutamate. Taken together, our results demonstrate that the maturation of astrocyte membrane resistance, local distribution of glutamate transporters, and glutamatergic input to the cortex are altered in the FL model, but that single-cell astrocytic glutamate uptake is robust.

1. Introduction

Developmental cortical malformations (DCMs) are a common cause of intractable epilepsy and are linked to environmental and genetic insults occurring in utero or during early post-natal development (Guerrini and Dobyns, 2014; Jacobs et al., 1999b; Palmini et al., 1995). DCMs include focal cortical dysplasia, polymicrogyria, and schizencephaly, all of which are characterized by disorganized cortical architecture and severe, often intractable, epilepsy (Barkovich et al., 2005; Raymond et al., 1995; Sisodiya, 2000; Tassi et al., 2002). The molecular, cellular, and network level changes that make the malformed brain prone to ictogenesis are largely unknown. Changes in neuronal morphology and location, synapse number and function, and neurotransmitter receptor expression and localization have all been reported in different DCMs and may be mechanistically linked to cortical hyperexcitability (reviewed by Marin-Valencia et al. (2014) and Aronica et al. (2012)). Changes in astrocyte properties are also of great interest in understanding the pathogenesis of DCMs.

Astrocytes remove the bulk of extracellular glutamate (Holmseth et al., 2012a; Petr et al., 2015) and buffer extracellular potassium in

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tiled, non-overlapping domains (Halassa et al., 2007). Glutamate uptake by astrocytes is mediated by the excitatory amino acid transporters (EAATs), GLT-1 and GLAST. EAATs are sodium-dependent and can rapidly bind and transport glutamate into astrocytes (Danbolt, 2001). EAATs are developmentally regulated, greatly increasing their expression during development (Ullensvang et al., 1997). The proper function of EAATs ensures rapid clearing of extracellular glutamate, limits post-synaptic glutamate receptor activation, and enables recycling of glutamate (Fonnum, 1984; Oliet et al., 2001; Petroff et al., 2002). In the injured or epileptic brain, astrocytes become reactive and many of their properties are disrupted. Reactive astrocytes express high levels of glial fibrillary acidic protein (GFAP), have hypertrophied morphology, and exhibit an altered protein expression profile (reviewed by Sofroniew (2009)). Functional glutamate transport may also be disrupted, though changes in glutamate transporter expression in reactive astrocytes remain debatable. In human DCMs, astrocytes are often reactive (Kakita et al., 2004) and GLT-1 expression has been reported to be decreased (Ulu et al., 2010). Importantly, elevated extracellular glutamate levels have been reported in human epileptic foci (Cavus et al., 2005), suggesting that glutamate is dysregulated and that lowering glutamate levels may be a therapeutic target for DCM-associated hyperexcitability.

To better understand the role of glutamate uptake in the pathogenesis of DCMs, we have utilized the freeze lesion (FL) model of DCM. In the FL model, a freezing probe is placed on the skull of a rat pup on the day of birth (P0) resulting in the formation of a cortical microgyrus (Dvorák and Feit, 1977). The microgyrus is bordered both medially and laterally by normally-laminated cortex. This normally-laminated cortex surrounding the microgyrus forms the paramicrogyral zone (PMZ). The PMZ is known to be hyperexcitable beginning approximately two weeks following FL (Jacobs et al., 1996). Electrical stimulation of ascending cortical axons generates epileptiform, polyphasic cortical field potentials in the PMZ (Hablitz and DeFazio, 1998). Interestingly, cortical field potentials recorded in the microgyrus are not epileptiform, indicating heterogeneity in the malformed and surrounding tissue (Dulla et al., 2013; Jacobs et al., 1999a). In vivo, FL animals have an enhanced response to kainic acid treatment (Andresen et al., 2014) and, when given multiple FL, animals have spontaneous seizures (Kamada et al., 2013). Although the FL does not etiologically replicate human DCMs, it does provide an excellent opportunity to probe the molecular and cellular consequences of neonatal injury prior to the onset of network dysfunction in a model of DCM.

Because DCMs arise in the developing brain (both in humans and in animal models), astrocytes are exposed to injury before they are fully matured (Morel et al., 2014). Thus, astrocyte development is disrupted by FL, likely resulting in altered functional maturation of glutamate uptake. However, characterizing the function of individual astrocytes in DCM is complicated by astrocyte reactivity, proliferation, and heterogeneity following injury (Bordey et al., 2001; Shimizu-Okabe et al., 2007). Perhaps because of this, studies of astrocytic glutamate uptake in FL cortex have reached conflicting conclusions. Specifically, studies have shown that the effects of the EAAT inhibitor DL-*threo*-β-benzyloxyaspartate (TBOA) on network activity are potentiated following FL. Additionally, blockade of EAATs evokes spontaneous network discharges in FL but not in sham-injured cortex (S. L. Campbell and Hablitz, 2008). Furthermore, a recent study has shown that the amplitude of glutamate transporter currents is decreased in individual PMZ astrocytes (Campbell et al., 2014). These studies concluded that glutamate uptake is decreased, thus increasing glutamate accumulation in the extracellular space and contributing acutely to hyperexcitability. However, recent work from our group has demonstrated intact astrocytic glutamate uptake in the PMZ and suggested that FL specifically affects the developmental switch from GLAST-mediated to GLT-1-mediated uptake (Armbruster et al., 2014). We also showed that the local density of astrocytes is decreased in the PMZ and that the functional domain of astrocytes is increased. This suggests that there may be disruptions in astrocyte territories which may lead to subsequent disruptions in their homeostatic functions (Armbruster et al., 2014; Dulla et al., 2013). Finally, it remains unclear how alterations in astrocyte coverage or function during the FL latent period influence network development. Thus, questions remain about whether an alteration in glutamate uptake contributes to the development of hyperexcitable neuronal networks in a model of developmental cortical malformation, and whether a reduction in glutamate uptake contributes acutely to hyperexcitability.

In this study, we seek to determine whether the maturation and function of individual astrocytes is disrupted by FL, potentially contributing to pathological network development and hyperexcitability in the mature malformed cortex. We use whole-cell astrocyte electrophysiology to measure astrocytic membrane resistance in lesioned cortex before and after the onset of hyperexcitability, simultaneously measure uncaging-evoked glutamate transporter currents in the same astrocytes, compare synaptically-released to uncaging-evoked glutamate transients in the mature FL cortex, and examine glutamate transporter immunoreactivity throughout lesion development. We demonstrate that the rate of glutamate clearance is unchanged by FL both before and after the onset of hyperexcitability, though astrocyte membrane resistance is altered during the latent period, at postnatal day (P) 7. Interestingly, we find that synaptically-activated transporter current (STC) are larger in amplitude and more easily evoked in the FL cortex. Our findings suggest that decreased astrocytic uptake may not contribute significantly to acute PMZ hyperexcitability and they support the idea that hyperexcitability in the mature PMZ is caused by increases in excitatory input.

2. Materials and methods

2.1. Animals and freeze lesion surgery

Experimental microgyri in primary somatosensory cortex (left hemisphere) were induced in P0 Sprague–Dawley rat pups (Jackson Laboratories) by freeze lesioning as described previously (Jacobs and Prince, 2005). All protocols were approved by the Tufts Institutional Animal Care and Use Committee. Briefly, animals were anesthetized by hypothermia, an incision into the scalp was made, and a 5×2 mm copper probe cooled to -50 to -60 °C was placed onto the exposed skull for 5 s (approx. 1 mm right of midline, between bregma and lambda). Sham operated littermates were generated in a similar manner with a room temperature probe. After freeze-lesioning, the incision was closed using surgical glue, and pups were warmed and returned to the dam. At approximately two weeks of age, a fully formed microgyrus was present.

2.2. Slice preparation

Cortical brain slices, 400 μm thickness, containing the sensorimotor cortex were prepared from P6–8 and P27–33 Sprague–Dawley rats of either sex. Rats were anesthetized with isoflurane, decapitated, and the brains were rapidly removed and placed in cold slicing solution (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 0.5 $CaCl_2$, 11 glucose, 234 sucrose, and 26 $NaHCO_3$, equilibrated with 95% O_2:5% CO_2. The brain was glued to a Vibratome VT1200S (Leica) and slices were cut in a coronal orientation. Slices were then placed into a recovery chamber containing artificial cerebrospinal fluid (aCSF, in mM: 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 10 glucose, and 26 $NaHCO_3$ equilibrated with 95% O_2:5% CO_2) and 0.5 mM sulforhodamine 101 (SR-101) for 5 min at 32 °C. Following SR-101 incubation, slices were equilibrated in ACSF at 32 °C for 1 h. Slices were allowed to return to room temperature and used for electrophysiology.