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Review

Neuronal vs glial glutamate uptake: Resolving the conundrum

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ABSTRACT

Neither normal brain function nor the pathological processes involved in neurological diseases can be adequately understood without knowledge of the release, uptake and metabolism of glutamate. The reason for this is that glutamate (a) is the most abundant amino acid in the brain, (b) is at the cross-roads between several metabolic pathways, and (c) serves as the major excitatory neurotransmitter. In fact most brain cells express glutamate receptors and are thereby influenced by extracellular glutamate. In agreement, brain cells have powerful uptake systems that constantly remove glutamate from the extracellular fluid and thereby limit receptor activation. It has been clear since the 1970s that both astrocytes and neurons express glutamate transporters. However the relative contribution of neuronal and glial transporters to the total glutamate uptake activity, however, as well as their functional importance, has been hotly debated ever since. The present short review provides (a) an overview of what we know about neuronal glutamate uptake as well as an historical description of how we got there, and (b) a hypothesis reconciling apparently contradicting observations thereby possibly resolving the paradox.

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Abbreviations: Cre, Cyclization recombinase (Le and Sauer, 2000); EAAC1, glutamate transporter (EAAT3, slc1a1, Kanai and Hediger, 1992, Bjørås et al., 1996); EAAT, excitatory amino acid transporter (synonym to glutamate transporter); GABA, γ-aminobutyric acid; GLAST, rat glutamate transporter (EAAT1, slc1a3, Storck et al., 1992, Tanaka, 1993a); GLT-1, rat glutamate transporter (EAAT2, slc1a2, Pines et al., 1992); GLUL, glutamine synthetase; TBOA, DL-*threo*-β-benzyloxyaspartate.

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1. Introduction

It was noted early on that glutamate is a key player in brain metabolism (Krebs, 1935) and that brain cells have a remarkable ability to take up glutamate from the extracellular fluid (Stern et al., 1949). Unexpectedly, glutamate was found to have an excitatory effect on neurons (Hayashi, 1954; Lucas and Newhouse, 1957; Curtis et al., 1959, 1960) and investigators started to speculate whether glutamate might be a neurotransmitter (for review see: Roberts et al., 1981; Schousboe, 1981; Danbolt, 2001; Watkins and Evans, 1981; Fonnum, 1984; Erecinska and Silver, 1990; Broman et al., 2000; McKenna, 2007; Hertz, 2013). As glutamate uptake turned out to be the only mechanism able to remove extracellular glutamate, it followed that the brain had to be dependent on glutamate uptake in order to control the excitatory action of glutamate (Logan and Snyder, 1971, 1972; Wofsey et al., 1971; Balcar and Johnston, 1972; Johnston, 1981). In agreement, glutamate uptake was shown to protect neurons in culture against glutamate (Frandsen and Schousboe, 1990; Rosenberg et al., 1992). This realization lead to a strong interest in glutamate uptake as perturbations in glutamate uptake might cause or aggravate neurological disease. For instance, because the uptake process is driven by the transmembrane ion gradients of K^+ and Na^+ (Kanner and Schuldiner, 1987; Nicholls and Attwell, 1990; Barbour et al., 1991; Danbolt, 2001), it follows that a compromised brain energy supply (e.g. ischemia) will impair the transport process (for review see: Szatkowski and Attwell, 1994; Rossi et al., 2000; Danbolt, 2001; Larsson et al., 2004; Grewer and Rauen, 2005; Tzingounis and Wadiche, 2007). A full discussion about molecular properties and roles of glutamate transporters in disease, however, is beyond the scope of this review as that topic is covered elsewhere (for review see for example: Lipton and Rosenberg, 1994; Danbolt, 2001; Sattler and Rothstein, 2006; Beart and O'Shea, 2007; Sheldon and Robinson, 2007; Bröer and Palacin, 2011; Vandenberg and Ryan, 2013; Robert and Sontheimer, 2014; Robert et al., 2014; Karki et al., 2015; Soni et al., 2014; Takahashi et al., 2015).

Relevant to the present review is the fact that both glial cells (e.g. Henn et al., 1974) and neurons (their axon-terminals in particular; Wofsey et al., 1971; Beart, 1976) possess high-affinity glutamate uptake mechanisms. This was clear already in the 1970s, but the relative importance of the neuronal (axon-terminal) and the astroglial uptake systems has been debated ever since.

2. Early observations of neuronal glutamate uptake

Evidence for significant glutamate uptake in neurons came from several sources: **(a)** It was noted that neurons cultured alone are able to take up glutamate with high affinity (e.g. Drejer et al., 1982; Waniewski and Martin, 1983; Sher and Hu, 1990; Frandsen and Schousboe, 1990; Balcar, 1991; Wang et al., 1998a; Plachez et al., 2004). **(b)** Axon-terminals are believed to reseal during homogenization of brain tissue and thereby form metabolically active structures. These pinched off nerve endings are called "synaptosomes" (Gray and Whittaker, 1962; Whittaker, 1969), and they can be used in uptake assays (Gfeller et al., 1971; Logan and Snyder, 1972; Levi and Raiteri, 1973a, 1973b; Dodd et al., 1981; Erecinska et al., 1996; Robinson, 1998; Raiteri and Raiteri, 2000). Although it was understood that fragments of some of the smallest astrocyte branches can reseal like axon-terminals, and that this would contaminate the synaptosome preparations (e.g. Delaunoy et al., 1979; Henn et al., 1976; Nakamura et al., 1993), it was believed that the resealing frequency of astrocytes was lower than that of nerve terminals. If so, then nerve terminal uptake would be relatively better preserved in the homogenates than that of other cellular structures. **(c)** Radiolabeled excitatory amino acids (L-[^{3}H]glutamate and D-[^{3}H]aspartate) accumulated preferentially in nerve terminals also when presented to brain slices *in vitro* (Beart, 1976; Storm-Mathisen and Iversen, 1979; Taxt and Storm-Mathisen, 1984), or infused *in vivo* at low concentrations and for short duration (Storm-Mathisen and Wold, 1981). While a predominance of neuronal over astroglial uptake in synaptosome preparations could be disregarded as a consequence of differences in cellular geometry and thereby in resealing frequency (see section 10 below), a predominance of neuronal uptake in hippocampal slices was harder to explain without assuming that axon-terminals had a considerable uptake activity. **(d)** Interruption of putative glutamatergic fibers caused a marked decrease in glutamate uptake activity in the target regions of these fibers (Fig. 1). The uptake activities in the target areas were determined using synaptosomes or brain slices (Divac et al., 1977; McGeer et al., 1977; Storm-Mathisen, 1977; Fonnum et al., 1981; Taxt and Storm-Mathisen, 1984). Thus, destruction of

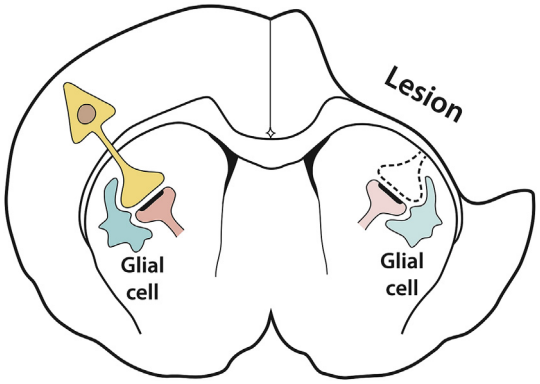

Fig. 1. Diagrammatic representation of lesions of the neocortex made to destroy the cell bodies that give rise to the glutamatergic corticostriatal fibers. When the neocortex is removed, the axon-terminals projecting to the striatum degenerate. This results in a strong reduction in synaptosomal glutamate uptake activity in striatum on the lesioned side. However, there is also a reduction in the expression of EAAT1 and EAAT2 in the striatum (Levy et al., 1995). This motivated investigation into whether neurons influence expression of EAAT2 in astrocytes (see Fig. 2). (Modified from a figure provided by Line ML Boulland. Copyright: Neurotransporter AS, Oslo, Norway; Reproduced with permission).