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Letter to the Editor

Autoradiography of [^{3}H]Aspartate and Glutamate Transport in Schizophrenia

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Sir

Quantitative autoradiography is uniquely useful in being able to show the distribution of important binding sites *in situ*, at the same time providing information on their affinities and pharmacological profiles. The results are, however, strongly influenced by the choice of radioligands. This is particularly relevant for the studies of glutamate transport (Scarr *et al*, 2005).

GLAST (EAAT1) and GLT (EAAT2) are by far the most abundant excitatory amino-acid transporters (EAAT's) in the CNS (see for reviews, Danbolt, 2001; Shigeri *et al*, 2004). The principal EAAT in forebrain regions is GLT while GLAST predominates in cerebellum (see for review, Danbolt, 2001). The regional distribution of [^{3}H]aspartate-marked sites as studied by autoradiography therefore differs from that of GLT but is remarkably similar to that of GLAST (cerebellar cortex $\gg$ forebrain structures: Killinger *et al*, 1996; Balcar *et al*, 2001; Takamoto *et al*, 2002; Balcar, 2002).

D-Aspartate (Davies and Johnston, 1976) has long been used as a radioligand in autoradiographic studies (Parsons and Rainbow, 1983; see for reviews, Balcar *et al*, 2001; Balcar, 2002) mainly because Na^+-dependent glutamate transport was thought to have about equal affinity for L- (not D-) glutamate, L-aspartate or D-aspartate ('stereoselective anomaly'; Cooper *et al*, 1998; Balcar *et al*, 2001; Balcar, 2002). However, assumption that [^{3}H]aspartate, in the presence of Na^+, would always label equally well all EAAT's may not be correct. Affinities of L- and D-aspartate for the [^{3}H]aspartate-labelled binding sites are about 50 times greater ($IC_{50} < 1\,\mu M$) than the corresponding affinity of L-glutamate (Balcar *et al*, 2001; Takamoto *et al*, 2002) and greater than the affinities of glutamate and aspartate in uptake/transport studies (see for reviews, Bridges *et al*, 1999; Danbolt, 2001; Balcar *et al*, 2001). The high affinity makes [^{3}H]aspartate a convenient radioligand producing adequate labelling at low concentrations. However, neither the regional distribution of [^{3}H]-aspartate binding nor, indeed, its substrate specificity, suggest that it labels preferentially GLT (Bridges *et al*, 1999; Balcar *et al*, 2001). If [^{3}H]D-aspartate labels mostly a variant of GLAST (Takamoto *et al*, 2002), the autoradiography could severely underestimate the most abundant EAAT (GLT), particularly at the glutamatergic synapses in the cerebral cortex (Minelli *et al*, 2001, Sullivan *et al*, 2004).

Glutamate transport may be altered in schizophrenia: chronic neuroleptics reduce glutamate transport (Schneider *et al*, 1998; De Souza *et al*, 1999, Schmitt *et al*, 2003; see for review, Balcar and Nanitsos, 2005) while increased levels of EAAT's have been reported in post mortem schizophrenic brains from nonmedicated patients (Matute *et al*, 2005). Most of the changes, however, affect GLT—not GLAST—particularly in the cerebral cortex (rat: 70% reduction by chronic clozapine, Melone *et al*, 2001, 2003; humans: GLT in tissue from patients with schizophrenia 2–4 times greater, compared to controls, Matute *et al*, 2005). Given that binding experiments using 40 nM [^{3}H]D-aspartate (Scarr *et al*, 2005) may not adequately label the most important glutamate transporter in the cerebral cortex (GLT), suggesting that glutamate transport in cortical areas affected by schizophrenia is not changed (Scarr *et al*, 2005) seems premature.

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