J. exp. Biol. (1981), **95**, 181–193
With 4 figures
Printed in Great Britain

GLIAL VERSUS NEURONAL UPTAKE OF GLUTAMATE

BY D. NEIL CURRIE AND JOHN S. KELLY

Department of Pharmacology, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, U.K.

SUMMARY

Inactivation of amino acid neurotransmitters is generally held to be via high-affinity uptake into pre-synaptic neurones and glia, a model well established for monoaminergic systems. GABA, for instance, is taken up at high affinity by glia and GABAergic neurones, and the two uptake systems have been distinguished by the use of competitive analogues *in vivo* and *in vitro*. Consequently, autoradiographic location of neuronal GABA uptake has become a useful method for recognition of GABAergic neurones. However, in the case of glutamate, the site(s) of high-affinity uptake are not well established and there are contradictions in the literature between results obtained by different methods. Biochemical studies on compartmentation in the metabolism of glutamate and the related amino acids GABA, aspartate and glutamine indicate strongly that glutamate uptake is exclusively located in glia. This is in accordance with the hypothesis of a neuronal-glial cycle in which glutamate passes from neurones to glia, and is returned to the neurones as glutamine. *In vitro* studies of uptake into isolated cell types show a high glial capacity for glutamate uptake. Studies of neuronal glutamate uptake *in vitro* are few and the results, although equivocal, indicate a lack of high-affinity uptake. Autoradiography of uptake of glutamate, or the non-metabolisable analogue, D-aspartate, has been performed *in vivo* by several groups of workers at the light and EM level. Some have reported only glial uptake, while others have reported a predominantly neuronal uptake of glutamate and have used this as a criterion for the presence of glutamatergic neurones. Considerable support for this interpretation comes from the finding that transection of the relevant pathways leads to a substantial reduction in the target tissue capacity for high-affinity glutamate uptake. Selective killing of supposed glutamatergic populations of neurones such as cerebellar granule neurones also leads to loss of glutamate uptake.

Results are reported from a preliminary autoradiographic study of uptake of glutamate, glutamine and taurine into well-defined cerebellar cell cultures. The uptake of glutamate into granule neurones was minimal, although there was a substantial uptake into glia. The uptake of glutamine and taurine showed no specific distribution. It is possible that granule neurones *in vitro* are too immature to express sites for glutamate uptake, however sites for neuronal uptake of GABA are present from the first day of culture. Therefore these observations add some further weight to the view that high affinity glutamate uptake is exclusively located in glia. If glial uptake also predominates *in vivo* then the reductions in glutamate uptake which follow the loss of certain neuronal populations, thought to use glutamate as their transmitter, may be explained in terms of a glial–neuronal interaction. Thus the capacity of glial glutamate uptake would be dependent either on the level of release of glutamate from adjacent nerve terminals or on specific diffusible or contact-dependent factors.

Several amino acids have been implicated as neurotransmitters in the central nervous system on the basis of electrophysiological and neurochemical data (Curtis & Johnston, 1974). Termination of their synaptic activity is generally proposed to be via high-affinity uptake into surrounding glia and the presynaptic neurones, as has been found for monoamine transmitters (Iversen, 1974). Thus, gamma-aminobutyric acid (GABA) is well established as an inhibitory transmitter in spinal cord and brain and a specific high-affinity GABA uptake into glia and GABAergic neurones has been demonstrated (e.g. Kelly & Dick, 1976). Glycine has been found to act as an inhibitory transmitter in the spinal cord (Curtis *et al.* 1968) but not in cortical areas (Kelly & Krnjevic, 1968) and high-affinity glycine uptake is similarly restricted in distribution (Johnston & Iversen, 1971). Glutamate and aspartate have been proposed as neurotransmitters, due primarily to their excitatory action when iontophoresed onto many types of CNS neurones (Curtis & Johnston, 1974). Support for a transmitter role for these acidic amino acids came initially from the demonstration of a specific sodium-dependent high-affinity uptake system in brain slices and synaptosomes (Balcar & Johnston, 1972; Logan & Snyder, 1972).

In the last decade, a great deal of information on the uptake and metabolism of transmitter amino acids has emerged from several lines of investigation. These include biochemical studies of metabolic compartmentation in brain tissue, autoradiographic studies of uptake localization, and studies of single cell types maintained in culture. However, some areas of uncertainty remain, in particular with respect to the role of glutamate. Several studies have now shown that lesions of excitatory pathways in the brain lead to decreases in glutamate content, high-affinity glutamate uptake and stimulus-induced glutamate release. In particular, substantial decreases in glutamate content and high-affinity glutamate uptake have been correlated with the loss of cerebellar granule cells, whether caused by virus infection (Young *et al.* 1974), X-irradiation (Valcana, Hudson & Timiras, 1972), or neurological mutation (McBride, Aprison & Kusano, 1976). Transection of the lateral olfactory tract caused a reduction in glutamate and aspartate content in guinea-pig and rat pre-pyriform olfactory cortex (Harvey *et al.* 1975; Collins, 1979). Lesions of the entorhinal input to the dentate gyrus result in a decrease of glutamate uptake and of Ca^{2+}-dependent, K^{+}-induced release (Nadler *et al.* 1976). Glutamate uptake, and its loss after lesions of excitatory inputs to the hippocampus were shown in autoradiographic studies to be localized in the known target areas of these projections (Storm-Mathisen, 1977).

Aspartate is taken up by a high-affinity transport system which appears to be the same as that for glutamate and therefore cannot be distinguished in uptake studies. In addition, aspartate release is often found to be associated with glutamate release (e.g. Baughman & Gilbert, 1980). However, differential neural sensitivity to the excitatory actions of aspartate or glutamate has been found in some regions of the central nervous system (Watkins, 1978) and at least three different types of receptor are now postulated (Watkins, 1980).

Metabolic compartmentation of brain amino acids

The three putative transmitter amino acids – glutamate, aspartate and GABA – comprise, with the physiologically inactive glutamine, 70% of the free amino acid in the adult brain. Although there may be considerable variation in the levels of each

component, the total concentration of this amino acid system remains constant under a variety of conditions (including neuronal division and degeneration) which suggests the existence of a homeostatic mechanism (Margolis, Heller & Moore, 1968). Glutamate is formed by reductive amination of α-ketoglutarate (via glutamate dehydrogenase) and glutamate and aspartate can interchange with TCA cycle intermediates via a transaminase. Glutamine is produced by further amination of glutamate (glutamine synthetase) and GABA is formed by decarboxylation of glutamate (glutamic acid decarboxylase, GAD).

A compartmentation of glutamate metabolism in the brain was suggested some time ago, in an attempt to explain anomalies in precursor/product relations (Berl & Clarke, 1969; Balázs & Cremer, 1973; Berl, Clarke & Schnieider, 1975). For instance, radioactive glutamate administered intracranially labels brain glutamine to a greater extent than it mixes with the main pool of glutamate resulting in a far higher specific activity in the product than in its immediate precursor. Similarly, several TCA cycle inhibitors, such as fluoroacetate, were found to inhibit the labelling of glutamine from a variety of precursors far more effectively than that of glutamate, an intermediate in glutamine synthesis (Clarke, Nicklas & Berl, 1970). However, the labelling of GABA, which also derives from glutamate, was hardly affected by fluoroacetate. These results have been explained in terms of a separate TCA cycle, with its own (small) pool of glutamate, which is particularly active in glutamine synthesis and makes little contribution to brain glutamate or GABA pools. This 'small' compartment of glutamate metabolism is preferentially labelled by acetate and glutamate, whereas glucose labels both compartments equally well.

Several lines of evidence have pointed to a neuronal location for the large compartment which synthesizes GABA and glutamate and to a glial site for the small compartment. For instance, a tetrodotoxin-sensitive increase in neuronal respiration can be caused by veratrine alkaloids, which act on neuronal ion channels. Selective inhibitors of glutamate metabolism in the small compartment, such as fluoroacetate, do not prevent the veratrine-stimulated increase in neuronal respiration (Benjamin & Quastel, 1972). Malonate, on the other hand, prevents the veratrine stimulated increase in respiration and the stimulated release of glutamate, aspartate and GABA, but not the release of glutamine.

The high-affinity uptake of extracellular glutamate seems from biochemical studies to be linked with the small (glial) compartment of glutamate metabolism. Thus, when glutamate was applied to brain slices or intracranially, it labelled mainly glutamine. Exogenous glutamate has been found to exchange poorly with endogenous glutamate, i.e. with the large (neuronal) compartment of glutamate metabolism, and to be a poor precursor for GABA (Benjamin & Quastel, 1972). In contrast, glutamine is generally a better precursor for neuronal glutamate metabolism and GABA synthesis than glutamate itself (e.g. Berl, Lajtha & Waelsch, 1961). Indeed, glutamine may be superior to glucose as a precursor of neuronal stimulus-evoked glutamate release (Hamberger *et al.* 1979).

These biochemical observations were drawn together in a proposal linking neurones and glia which would account not only for the regulation of the levels of the physiologically active amino acids, but also for the removal of tissue ammonia (Benjamin & Quastel, 1975). This hypothesis stemmed from the observation under a variety of

experimental conditions of a striking constancy in total nitrogen content of the sum of tissue ammonia, glutamate, glutamine, GABA and aspartate. Inactivation of synaptically-released glutamate, and maintenance of the low ($< 10\ \mu M$) extracellular glutamate concentration is proposed to be via uptake into surrounding glial cells. Addition of ammonia to glutamate by the glial enzyme glutamine synthetase would yield glutamine which can be released to the extracellular space. The high extracellular level of glutamine ($\sim 500\ \mu M$) is available for neuronal re-uptake and metabolism to glutamate, GABA and the other components of the large compartment. Similarly, GABA released by neurones and taken up by glia could be returned to the large glutamate pool via glutamine (Van den Berg & Garfinkel, 1971). This simple arrangement is consistent with the biochemical data on compartmentation and accounts for most of the apparently anomalous aspects of glutamate metabolism. The cellular locations of the biochemical compartments have been inferred, but cannot be established directly by precursor/product experiments on brain slices. For confirmation of the glutamate-glutamine cycle hypothesis, it is essential to examine the uptake kinetics of glutamate, GABA and glutamine into neurones and glial cells and the activities of the relevant enzymes in each cell type.

Quantitative cellular studies

Several *in vitro* preparations enriched in glial or neuronal cells or cell fragments have been used to investigate and quantify the cellular locations of aspects of glutamate metabolism.

Studies on glial material began with the demonstration of high-affinity uptake of glutamate and GABA into a bulk preparation of glial cells from adult rat brain (Henn & Hamberger, 1971). Human gliomas show high-affinity uptake of glutamate and GABA in slices (Snodgrass & Iversen, 1974) and when cultured (Stewart *et al.* 1976). Several studies have demonstrated sodium-dependent transport of glutamate and GABA at high-affinity into tumour cell lines of glial origin (Hutchison *et al.* 1974; Schrier & Thompson, 1974; Balcar, Borg & Mandel, 1977). Primary cultures of normal astrocytes also exhibit high-affinity uptake of GABA (Schousboe, Hertz & Svenneby, 1977) and glutamate (Hertz *et al.* 1978). This preparation has virtually the same homogeneity as clonal cell lines and in addition seems more faithfully to reflect *in vivo* kinetic properties, particularly when quantifying the capacity (V_{max}) of the uptake system (Schousboe, 1977). From these uptake data and the astrocytic activities of glutamine synthetase, glutamate dehydrogenase, glutamate oxaloacetate transferase and other enzymes involved in metabolism of glutamate, Hertz (1977) calculated that the glial capacity for uptake and metabolism of glutamate was sufficient to account for the known flux between neuronal and glial metabolism. In contrast, glial uptake of GABA and its metabolism via GABA trans-aminase, although significant, could only account for a small part of the total GABA degradation *in vivo*, implying a requirement for neuronal GABA uptake and breakdown. The neuronal-glial cycle of glutamate and glutamine proposed by Benjamin & Quastel (1975) is supported by these results. However, the fact that significant levels of low-affinity glutamine uptake and of glutaminase have been found in cultured astrocytes (Schousboe *et al.* 1979*a*) may indicate that some portion of glutamine breakdown can occur in glia.

Most quantitative studies of glutamate and GABA uptake into neuronal elements have involved the use of synaptosomal preparations. High-affinity uptake of GABA, glutamate and glycine has been found in synaptosomes from spinal cord and brain (Iversen & Johnston, 1971; Logan & Snyder, 1972; Bennett, Logan & Snyder, 1973). Separation by density-gradient centrifugation of synaptosome populations accumulating different amino acids has been claimed (Arregui *et al.* 1972), although there is a contrary report (Balcar & Johnston, 1973). Contamination of synaptosomal preparations by a proportion of glial particles must be borne in mind when assessing these results (Cotman, Herschman & Taylor, 1971; Schon & Kelly, 1975; Henn, Anderson & Rustad, 1976). However, localization of glutamate uptake in a small proportion of nerve terminals in a synaptosome preparation has been observed (Beart, 1976). A five times greater activity for glutamate uptake in synaptosomes compared with bulk-isolated glia has been reported (Weiler, Nystrom & Hamberger, 1979) although, as the authors point out, membrane damage in the glial fraction may cause losses in apparent uptake capacity.

In order to obtain comparable data for neurones to those found for glia grown in primary culture, it is necessary to study a preparation of neurones grown in the absence of glia. Neuronal primary culture systems have been reported by several groups (e.g. Dichter, 1978; Pettman, Louis & Sensenbrenner, 1979; Currie & Dutton, 1980) which contain a small and quantifiable proportion of glia. However, none have been used for such a study, although the work of Lasher (1975) on GABA uptake demonstrated the usefulness of this approach.

Two groups have followed up the possible glutamatergic nature of granule cells by investigating the uptake of labelled amino acid into separated cerebellar perikarya. Campbell & Shank (1978) found that a population separated from 10-day-old mice containing predominantly granule cells showed high-affinity uptake for [^{14}C]glutamate with a V_{max} of about one-third that of a glial-enriched (although heterogeneous) population. The other kinetic parameters were identical and included an unusual two-component high-affinity uptake system. A parallel tissue culture study showed the granule fraction, although remarkably homogeneous, to include 5% astrocytes and other cells, while the glial-enriched fraction was much more heterogeneous and contained an unstated proportion of astrocytes (Campbell & Williams, 1978). It is difficult, on the basis of these workers' results, to exclude the possibility that the glutamate uptake shown by the granule cell population may be a high level of uptake into a small minority of glial cells, or subcellular glial fragments. East, Dutton & Currie (1980) showed the distribution of glutamate uptake into separated fractions of cerebellar perikarya isolated from 10- to 11-day-old rats to coincide with that of β-alanine (see below), the highest levels occurring in the glial-enriched fractions. There was no uptake of glutamate or β-alanine by fractions of granule neurones. Although it is possible that the mildly trypsinized perikarya used by Campbell & Shank (1978) and East *et al.* (1980) had lost the sites responsible for neuronal glutamate uptake, the latter found GABA uptake to be retained by fractions enriched in Purkinje cells. Granule cells isolated from animals of this age could also be too immature to express the ability to take up glutamate.

Autoradiographic localisation of GABA uptake in vivo *and* in vitro

Information on the cellular location of amino acid uptake has also been provided by autoradiographic studies. Uptake of labelled GABA in brain tissue has been investigated by autoradiography after incubation of slices (Iversen & Bloom, 1972), ventricular injection (Hökfelt & Ljungdahl, 1970, 1972; Schon & Iversen, 1972) and direct microinjection (Kelly & Dick, 1976). The contribution of neuronal and glial components of GABA uptake were clarified by the finding that their different substrate specificities could be distinguished by the use of GABA analogues (Kelly & Dick, 1976). Thus, glial uptake of [^{3}H]GABA was inhibited by β-alanine, while labelled β-alanine was selectively accumulated by glia (Schon & Kelly, 1975; Kelly & Dick, 1976; Weitsch-Dick, Jessell & Kelly, 1978). Similarly, diaminobutyric acid (DABA) showed specificity for the high-affinity GABA transport sites of known and putative GABAergic neurones. Aminocyclohexanecarboxylic acid (ACHC) has also been found to be an effective competitive inhibitor of neuronal GABA uptake (Bowery, Jones & Neal, 1976) and a wide range of further analogues of varying selectivity have been reported (e.g. Schousboe *et al.* 1979*b*).

In cell culture, GABA is taken up by astrocytes and some types of neurone which can plausibly be identified as cells known to be GABAergic *in vivo*. Thus, uptake into cerebellar interneurones (Lasher, 1974; Messer, 1977), cortical inhibitory neurones (White, Snodgrass & Dichter, 1980) and olfactory bulb granule neurones (Currie & Dutton, 1980) has been shown in dissociated cell cultures. Neuronal and glial uptake have been distinguished using competitive analogues (ACHC and β-alanine) in cultures of cerebellum and olfactory bulb (Currie & Dutton, 1980) (see Fig. 2).

The presence of neuronal GABA uptake in GABAergic cells accords well with the calculation of Hertz (1977) that glial GABA uptake, although substantial, is not sufficient to account for more than a fraction of the neuronal release. There is no evidence that non-GABAergic cells which receive GABA innervation possess GABA uptake sites; the granule cells of the cerebellum, for instance, receive GABAergic input from Golgi cells, but show no GABA uptake (Kelly & Dick, 1976; Currie & Dutton, 1980). The evidence on GABA uptake from studies *in vivo* and *in vitro* using kinetic and autoradiographic approaches is thus broadly in agreement. In the case of the major putative excitatory amino acid transmitter, glutamate, the situation is more problematic.

Autoradiographic localization of glutamate transport in situ

It might be thought that the most direct way to determine whether high-affinity glutamate uptake is located in neurones or glia would be via autoradiography of uptake into intact tissue at the light, and particularly the electron, microscopic level. However, results have not been unequivocal.

Exclusively glial uptake has been found in several studies, including uptake into Müller glial cells in retina (Ehinger & Falck, 1971), Bergmann glial cells in cerebellum (Hökfelt & Ljungdahl, 1972) and satellite glial cells in sensory ganglia (Schon & Kelly, 1974). An EM autoradiographic study of glutamate uptake into brain after microinjection or topical application found silver grains to be located over glial cells

McLennan, 1976). Unfortunately, there have been no glutamate analogues described which distinguish between neuronal and glial uptake, a facility which clarified the distribution of GABA uptake.

The most extensive autoradiographic investigation of glutamate uptake is that of Storm-Mathisen and his co-workers (Storm-Mathisen, 1977; Storm-Mathisen & Iversen, 1979) who have adopted a 10 min incubation of thin (200 μm) slices of rat hippocampus with labelled glutamate. Under these conditions, the majority of label in some regions was associated with axons and boutons in a 'hypothetical grain analysis' of EM autoradiographs. There was minimal labelling of cell bodies or dendrites. The label was concentrated over areas in which glutamatergic projections terminate, e.g. the inner zone of the area dentata molecular layer which is a target of pyramidal axons from zones CA3 and CA4 (Storm-Mathisen & Iversen, 1979). Particularly important was the large decrease in labelling found some days after transection of the contralateral and ipsilateral projections of these pyramidal cells (Storm-Mathisen, 1977). Similar observations of loss of glutamate uptake on denervation have been made in several systems, including lesions of corticostriatal paths (McGeer *et al.* 1977; Divac, Fonnum & Storm-Mathisen, 1977), corticofugal fibres (Lund, Karlsen & Fonnum, 1978) and hippocampal afferents to septum and hypothalamus (Storm-Mathisen & Woxen Opsahl, 1978). This association of substantial loss of glutamate uptake with removal of excitatory synaptic input represents the most compelling body of evidence in favour of a predominantly neuronal location of glutamate uptake.

Similar evidence for the cerebellum has been provided by the observation of a large decrease in glutamate uptake and glutamate content associated with viral-induced loss of granule neurones (Young *et al.* 1974). However, interpretation of these cerebellar results is complicated by the finding that loss of glutamate content in agranular mutant mice extended to the deep cerebellar nuclei which contain no granule neurones (Roffler-Tarlov & Sidman, 1978). It has recently been reported that cerebellar uptake of labelled D-aspartate, a non-metabolisable substrate for the glutamate uptake system, was confined to glia in a light and EM autoradiographic study (Gordon *et al.* 1980).

Autoradiography of glutamate uptake in vitro

The pattern of GABA uptake *in vitro* was found to reflect faithfully *in vivo* behaviour. It may be possible, therefore, to resolve the site of high-affinity glutamate uptake in dissociated cell cultures where cells can be readily distinguished, and where the cellular composition of the culture can be manipulated. Accordingly, a study has been undertaken of glutamate uptake into cerebellar cell cultures which are enriched in granule neurones. These small excitatory interneurones are generally accepted as glutamatergic on the basis of the considerable (circumstantial) evidence reviewed earlier.

The system of defined cell cultures of cerebellum used here has been described previously (Currie, 1980; Currie & Dutton, 1980). Taking advantage of the limited and well-established range of cell populations in the cerebellum, it has been possible to define the composition of such cultures in terms of known cell types (Currie, 1980) using reliable criteria such as GABA uptake autoradiography (Currie & Dutton, 1980) and immunological markers (Raff *et al.* 1979). Cells are prepared from one-week old

rat cerebellum by a controlled trypsinisation procedure and seeded at $2 \times 10^5/cm^2$ on 13 mm glass cover slips coated with poly-L-lysine. Addition of fluorodeoxyuridine after, in this case, 48 h allowed a substantial population of astrocytes to grow up, but prevented overgrowth which adversely affects neuronal survival. Cultures such as those shown here contain 75–80% granule neurones with 15–20% astrocytes and 5–8% GABAergic neurones, probably stellate and basket cells (Currie & Dutton, 1980); fibroblasts, oligodendrocytes and macrophages are present at less than 1%.

Granule neurones in cell culture are small, round or ovoid, phase-bright, bipolar cells which have a tendency to aggregate after some days in culture. As shown in Fig. 1, they bind tetanus toxin, a neuronal marker (Dimpfel, Huang & Habermann, 1977; Mirsky *et al.* 1978), but do not show neurone-specific GABA uptake (Fig. 2). The minor population(s) of tetanus-positive neurones which show GABA uptake, inhibitable by ACHC but not by β-alanine (Fig. 2), are probably stellate and basket neurones (Currie & Dutton, 1980). These neurones are usually larger than granule cells, and show no tendency to aggregate with each other or with granule neurones. Specific GABA uptake sites are present in these cells from the earliest times in culture: Fig. 2(*c*) shows that GABA uptake can be detected after only 3 h in culture.

When a similar procedure was used to study [^{3}H]glutamate uptake (Figs. 3*a*, *b*) high levels of labelling were found over the underlying background cells, but not over the granule neurones. In a parallel culture (Figs. 3*c*, *d*) the flattened cells could be labelled by an antibody to glial fibrillary acidic protein (anti-GFAP) and therefore may be identified as astrocytes (Antanitus, Choi & Lapham, 1975). They also show glial-specific GABA uptake (Fig. 2*b*) (Currie & Dutton, 1980). The absence of [^{3}H]glutamate uptake into granule neurones is shown again in Figs. 4(*a*), (*b*), where unlabelled neuronal processes can clearly be seen running over labelled astrocytes. This pattern of uptake is similar to that shown by β-alanine and GABA inhibited by ACHC (Currie & Dutton, 1980); both are taken up at high-affinity by the glial GABA transport system, but not by the neurones. Neuronal uptake of glutamine is predicted by the glutamate/glutamine cycle hypothesis; when cultures were incubated with [^{3}H]glutamine and processed for autoradiography in parallel (Fig. 4*c*), grains were found over both granule neurones and glia. Studies of labelled glutamate uptake by autoradiography must be tempered by the knowledge that glutamate is rapidly metabolized into other components, particularly glutamine, which may pass between cells during the incubation period. Such an effect might account for misleading distributions of label after incubations of intact tissue. However cells in monolayer culture are accessible to a large volume of medium which will by dilution tend to prevent significant intercellular transfer of label.

From these results, it must be concluded that the granule cell does not show high-affinity glutamate uptake as it develops *in vitro*. It is possible that such uptake sites develop at a later stage of maturation, and that these studies must be extended to longer times *in vitro*. However, neuronal GABA uptake is present from earliest times in culture. Dissociated cerebral cortex neurones have been reported to show a similar lack of high-affinity glutamate uptake after three weeks in culture (White *et al.* 1980).

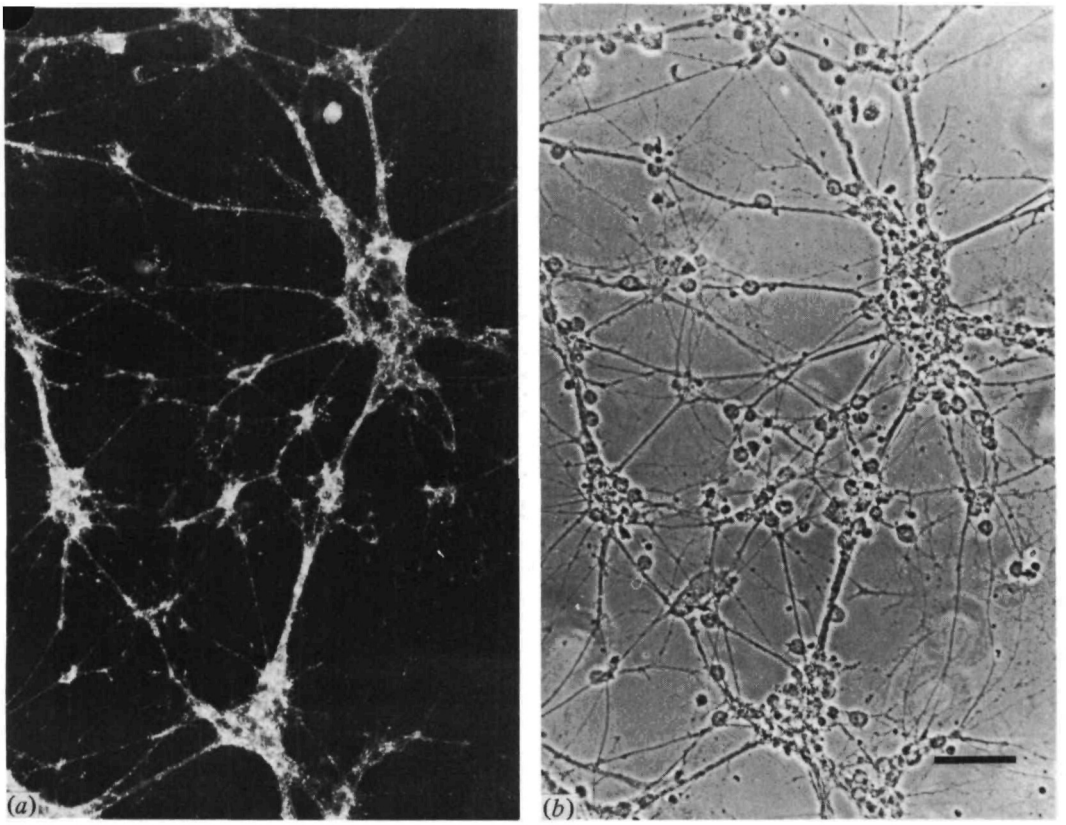

Fig. 1. Tetanus toxin labelling of 6-day rat cerebellar neurons after 8 days in culture. (*a*) Fluorescence and (*b*) phase-contrast images of the same field showing surface labelling of aggregated granule neurone cell bodies and their processes. Scale bar 50 μm. Living cultures grown on 13 mm glass cover slips were incubated with tetanus toxin followed by rabbit anti-tetanus, then goat anti-rabbit Ig rhodamine conjugate, fixed in 5 % acid ethanol and mounted in glycerol on microscope slides.

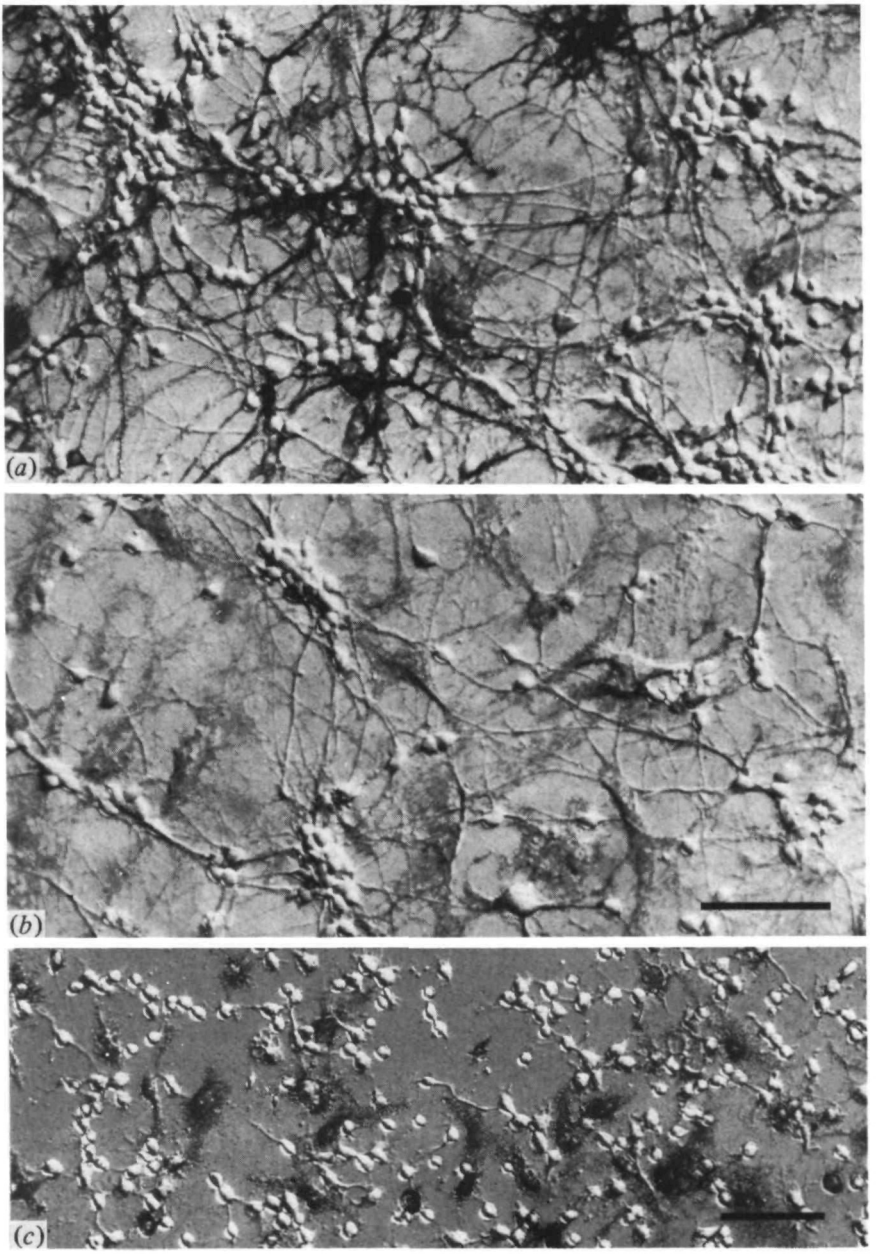

Fig. 2. (*a*) Autoradiography of [^{3}H]GABA uptake into a cerebellar culture (9-day rat, 7 days *in vitro*) in the presence of β-alanine, which competes for glial GABA uptake, showing several GABA-labelled neurones. (*b*) GABA uptake in the presence of ACHC, a neuronal uptake inhibitor, showing labelling of glia. Scale bar 50 μm. (*c*) GABA uptake into cerebellar cells (8-day rat) is detectable 3 h after plating, scale bar 50 μm. Cultures grown on 13 mm glass cover slips were incubated (30 min, 37 °C) with 0·2 μM [^{3}H]GABA (50–60 Ci/mmol), rinsed, fixed in 2·5 % glutaraldehyde, coated with Ilford L-4 emulsion, exposed for 10 days at −20 °C (5 days for [^{3}H]glutamate labelling, Figs. 3 and 4), and developed in Kodak D19.

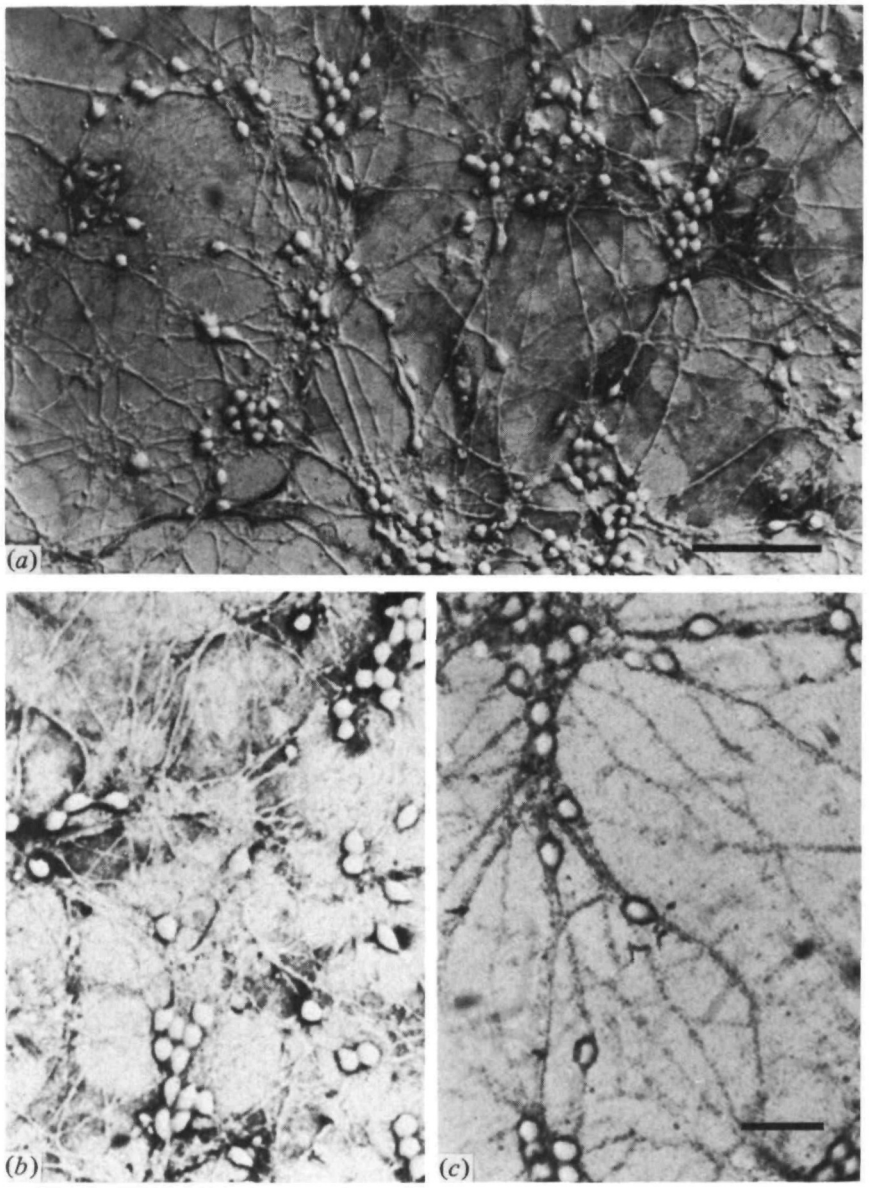

Fig. 4. Uptake of [^{3}H]glutamate and [^{3}H]glutamine. (*a*) Low power Nomarski view of cerebellar culture (8-day rat, 7 days *in vitro*) incubated with [^{3}H]glutamate, showing unlabelled neuronal processes growing over labelled glia. Method as in Fig. 3. Scale bar 50 μm. (*b*) Higher-power bright-field view of same culture incubated with [^{3}H]glutamate. (*c*) Parallel culture incubated with 0·5 μM [^{3}H]glutamine (20 Ci/mmol, 30 min, 37 °C) and processed as in Fig. 2, showing uptake into neuronal processes and cell bodies in addition to glia. The paucity of grains directly over labelled neuronal cell bodies is an artifact due to flow of the liquid emulsion before it sets. Scale bar 20 μm.

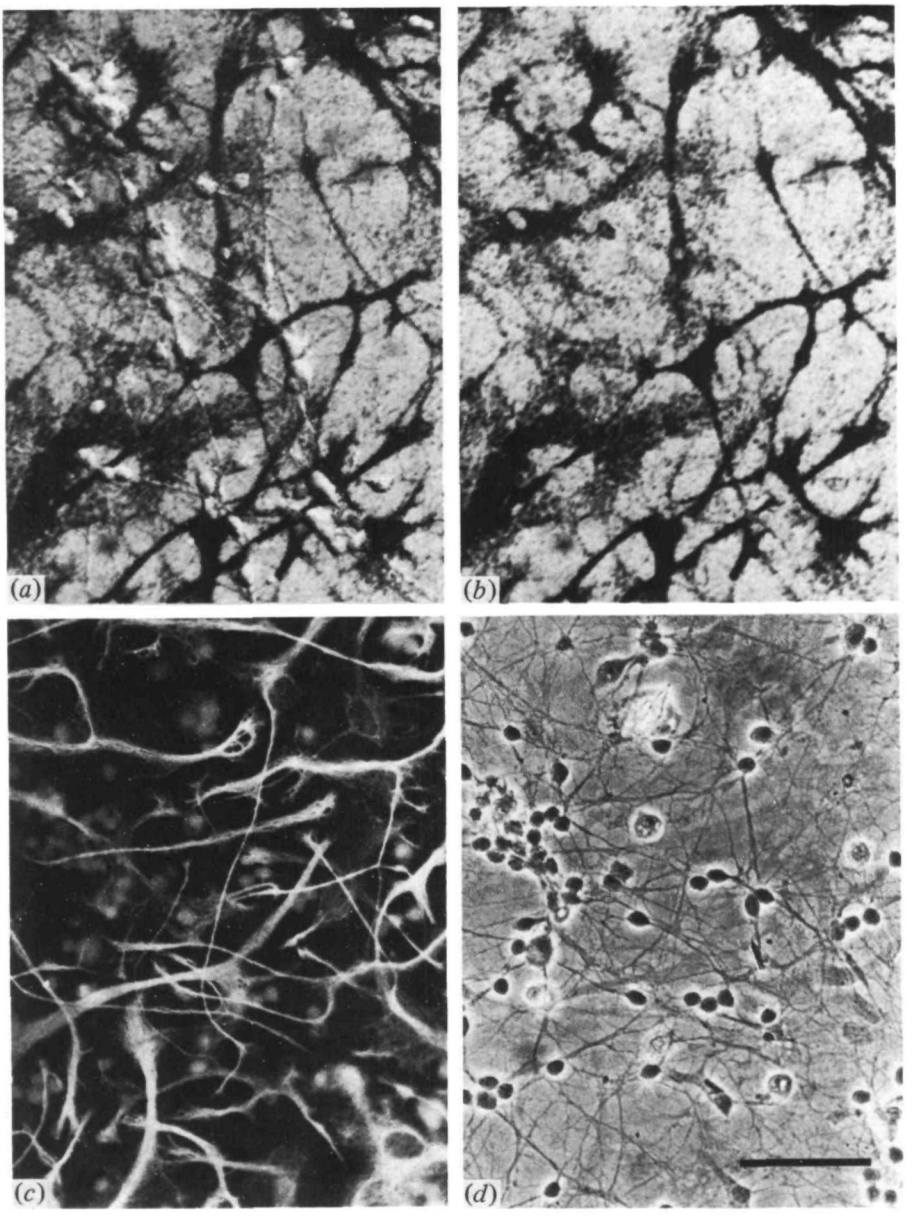

Fig. 3. Comparison of [^{3}H]glutamate uptake and GFA labelling in parallel cerebellar cultures (8-day rat, 7 days *in vitro*). (*a*) Nomarski and (*b*) bright-field views of an autoradiograph showing heavy labelling of glial cells in a culture incubated with 0·3 μM [^{3}H]glutamate (35 Ci/mmol, 30 min, 37 °C) and further processed as in Fig. 2. It can be seen that the cell bodies and processes of the granule neurons, visible in (*a*), remain unlabelled. (*c*) Rhodamine fluorescence and (*d*) phase-contrast images showing a parallel culture at the same magnification in which the background glial cells have been labelled with the astrocyte marker anti-GFAP. (Acid alcohol fixation followed by incubation with rabbit anti-GFAP, then goat anti-rabbit Ig – rhodamine-conjugate). Scale bar 50 μm.

DISCUSSION

There is persuasive, although not yet conclusive, evidence that glutamate is an excitatory transmitter in several brain pathways, and that it is inactivated by high-affinity uptake into adjoining cells. Knowledge of the cellular location of glutamate uptake sites is thus central to an understanding of a major excitatory transmitter system. The existence of neuronal uptake which is restricted to glutamatergic neurones would have the additional methodological advantage that localisation of glutamate uptake could be used to identify such neurones, as has already been possible for GABA, glycine and the monoamine systems.

However, several lines of evidence suggest that glutamate uptake is restricted to glia. Biochemical studies have established that extracellularly applied glutamate is almost exclusively taken up into the small compartment of glutamate metabolism and converted to glutamine via the enzyme glutamine synthetase. A recent histochemical study indicates that this enzyme is to be found only in glia (Norenberg & Martinez-Hernandez, 1979); it has since been used as a marker for astrocytes (White, Dutton & Norenberg, 1981). Externally applied glutamate does not mix with the large intracellularly synthesized pool of glutamate from which GABA is produced. Extracellular glutamate is a very poor precursor for glutamate released by Ca^{2+}-dependent stimulation; better precursors are glucose and, particularly, glutamine (Hamberger *et al.* 1979).

The biochemically-defined compartments of glutamate metabolism involve, of course, considerable simplification. There can, for instance, be no unitary pool: GABA synthesis is associated with the large (neuronal) pool, but only a fraction of the neurones release GABA, and are rich in the enzyme glutamate decarboxylase. However, putative glutamate-releasing neurones from a substantial proportion of the total neurones and any proposal regarding glutamate uptake into these cells must be reconcilable with the established biochemical data. There are few studies of the possible heterogeneity of glia between different brain areas.

The results shown here support the conclusion that high-affinity glutamate uptake is solely a glial property. The lack of glutamate uptake shown by the supposedly glutamatergic granule neurones is in complete contrast with the neuronal-specific uptake of GABA into GABAergic neurones from the first hours in culture. The major site of glutamate uptake in cerebellar cultures is the population of astrocytes which can be labelled with an antibody to glial fibrillary acidic protein. Glutamate uptake into freshly isolated cerebellar perikarya was also concentrated in glial fractions, and no transport into granule neurones occurred (East *et al.* 1980). Autoradiographic studies *in vivo* have shown glutamate (or D-aspartate) uptake to be located in cerebellar glia, particularly the characteristic Bergmann glial cells, but not in neurones (Hökfelt & Ljungdahl, 1972; Gordon *et al.* 1980). The restriction of glutamate uptake to glia is thus supported by several lines of evidence; however certain studies have indicated a contrary conclusion.

An autoradiographic investigation of exogenous glutamate uptake into hippocampal slices has indicated a primarily neuronal uptake site (Storm-Mathisen & Iversen, 1979), the largest proportion of labelling occurring in nerve terminals, with no

labelling of the parent cell bodies as commonly found for neuronal GABA uptake. It would be useful to extend the culture studies to hippocampus, and to longer times *in vitro*, as it is possible that one-week-old cultures of cerebellar granule neurones have not developed sufficient synapses to show detectable uptake. A nerve terminal uptake site would be in accordance with the basic compartmentation evidence and glutamate/glutamine cycle hypothesis if terminal-accumulated glutamate were exclusively re-released without mixing with the larger main neuronal glutamate pool, or entering metabolism via the transaminase. However, this scheme cannot easily be reconciled with the observation that external glutamate is a very poor precursor for glutamate released from hippocampal and other tissues by Ca^{2+}-dependent stimulation (Hamberger *et al.* 1979).

The main body of evidence against an exclusive uptake of glutamate by glia comes from denervation studies in several brain areas. The great reductions in glutamate uptake capacity found after transection of certain projections (Storm-Mathisen, 1977; McGeer *et al.* 1977; Lund Karlsen & Fonnum, 1978) or selective loss of a neuronal population (Young *et al.* 1974) are probably accompanied by a *proliferation* of glial cells.

One hypothesis which might account for these denervation effects is that the presence of glutamate-releasing nerve terminals stimulates the uptake of glutamate into glia. An uptake system of high capacity is necessary to remove all extracellular glutamate released by neurones (Hertz, 1977). It is plausible that a regulatory mechanism ensures that the uptake sites for glutamate are concentrated in the glia in close proximity to glutamatergic terminals. The presence of high-affinity glycine uptake in the spinal cord and not in the brain could be an example of such control (Johnston & Iversen, 1971). The capacity of glial glutamate uptake (i.e. the number of sites) could be controlled directly by the level of extracellular glutamate, by specific factors released from glutamatergic neurones, or by a contact-dependent interaction between nerve terminals and adjacent glia. The interesting observation that astrocytes from different brain areas differ in their glutamate uptake capacity (Schousboe & Divac, 1979) suggests that glial cells are not homogeneous in this respect. Similar interactions of neurones and glia have been proposed previously (Henn, 1976). Studies in defined cell cultures offer good possibilities for resolving such cell–cell interactions.

The initial observations on glutamate uptake in culture were made by DNC in collaboration with Dr G. R. Dutton. Gifts of materials are gratefully acknowledged from: Dr B. Anderton and Dr J. Kahn (anti-GFAP), Dr N. G. Bowery (ACHC), Dr R. Mirsky (anti-tetanus) and Dr R. O. Thompson (tetanus toxin). This work was partially supported by an MRC grant to the authors.

REFERENCES

ANTANITUS, D. S., CHOI, B. H. & LAPHAM, L. W. (1975). Immunofluorescence staining of astrocytes *in vitro* using antiserum to glial fibrillary acidic protein. *Brain Res.* **89**, 363–367.

ARREGUI, A., LOGAN, W. J., BENNETT, J. P. & SNYDER, S. H. (1972). Specific glycine-accumulating synaptosomes in the spinal cord of rats. *P.N.A.S.* **69**, 3485–3489.

BALÁZS, R. & CREMER, J. E. (1973). *Metabolic Compartmentation in the Brain*, pp. 1–383. London: MacMillan Press.

BALCAR, V. J., BORG, J. & MANDEL, P. (1977). High affinity uptake of L-glutamate and L-aspartate by glial cells. *J. Neurochem.* **28**, 87–93.

BALCAR, V. J. & JOHNSTON, G. A. R. (1972). The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *J. Neurochem.* **19**, 2657–2666.

BALCAR, V. J. & JOHNSTON, G. A. R. (1973). High affinity uptake of transmitters: studies on the uptake of L-aspartate, GABA, L-glutamate and glycine in cat spinal cord. *J. Neurochem.* **20**, 529–539.

BAUGHMAN, R. W. & GILBERT, C. D. (1980). Aspartate and glutamate as possible neurotransmitters of cells in layer 6 of the visual cortex. *Nature, Lond.* **287**, 848–850.

BEART, P. M. (1976). The autoradiographic localization of L-[^{3}H]glutamate in synaptosomal preparations. *Brain Res.* **103**, 350–355.

BENJAMIN, A. M. & QUASTEL, J. H. (1972). Locations of amino acids in brain slices from the rat: tetrodotoxin-sensitive release of amino acids. *Biochem. J.* **128**, 631.

BENJAMIN, A. M. & QUASTEL, J. H. (1975). Metabolism of amino acids and ammonia in rat brain cortex slices *in vitro*: a possible role of ammonia in brain function. *J. Neurochem.* **25**, 197.

BENNETT, J. P. JR., LOGAN, W. J. & SNYDER, S. H. (1973). Amino acids as central nervous transmitters: the influence of ions, amino acid analogues, and ontogeny on transport systems for L-glutamic and L-aspartic acids and glycine into central nervous synaptosomes of the rat. *J. Neurochem.* **21**, 1533–1550.

BERL, S. & CLARKE, D. D. (1969). Compartmentation of amino acid metabolism. In *Handbook of Neurochemistry*, vol. II (ed. A. Lajtha), p. 447. New York: Plenum Press.

BERL, S., CLARKE, D. D. & SCHNEIDER, D. (1975). *Metabolic Compartmentation and Neurotransmission: Relation to Brain Structure and Function.* New York: Plenum Press.

BERL, S., LAJTHA, A. & WAELSCH, H. (1961). Amino acid and protein metabolism. Cerebral compartments of glutamic acid metabolism. *J. Neurochem.* **7**, 186.

BOWERY, N. G., JONES, G. P. & NEAL, M. J. (1976). Selective inhibition of neuronal GABA uptake by cis-1,3-aminocyclohexanecarboxylic acid. *Nature, Lond.* **264**, 281–284.

CAMPBELL, G. LE M. & SHANK, R. P. (1978). Glutamic acid uptake by cerebellar granule and glial enriched populations. *Brain Res.* **153**, 618–622.

CAMPBELL, G. LE M. & WILLIAMS, M. P. (1978). *In vitro* growth of glial cell-enriched and depleted populations from mouse cerebellum. *Brain Res.* **156**, 227–239.

CLARKE, D. C., NICKLAS, W. J. & BERL, S. (1970). Tricarboxylic acid cycle metabolism in brain. Effects of fluoroacetate and fluorocitrate. *Biochem. J.* **120**, 345.

COLLINS, G. G. S. (1979). Effect of chronic bulbectomy on the depth distribution of amino acid transmitter candidates in rat olfactory cortex. *Brain Res.* **171**, 552–555.

COTMAN, C. W., HERSCHMAN, H. & TAYLOR, D. (1971). Subcellular fractionation of cultured glial cells. *J. Neurobiol.* **2**, 169–180.

CURRIE, D. N. (1980). *Identification of Cell Type by Immunofluorescence in Defined Cell Cultures of Cerebellum: Tissue Culture in Neurobiology* (ed. E. Giacobini *et al.*). New York: Raven Press.

CURRIE, D. N. & DUTTON, G. R. (1980). [^{3}H]GABA uptake as a marker for cell type in primary cultures of cerebellum and olfactory bulb. *Brain Res.* **199**, 473–481.

CURTIS, D. R., HÖSLI, L., JOHNSTON, G. A. R. & JOHNSTON, I. H. (1968). The hyperpolarization of spinal motoneurones by glycine and related amino acids. *Expl Brain Res.* **5**, 235–258.

CURTIS, D. R. & JOHNSTON, G. A. R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.* **69**, 94–188.

DICHTER, M. A. (1978). Rat cortical neurons in cell culture: culture methods, cell morphology, electrophysiology and synapse formation. *Brain Res.* **149**, 279–294.

DIMPFEL, W., HUANG, R. T. C. & HABERMANN, E. (1977). Gangliosides in nervous tissue cultures and binding of ^{125}I-labelled tetanus toxin: a neuronal marker. *J. Neurochem.* **29**, 329–334.

DIVAC, I., FONNUM, F. & STORM-MATHISEN, J. (1977). High affinity uptake of glutamate in terminals of corticostriatal axons. *Nature, Lond.* **266**, 377–378.

EAST, J. M., DUTTON, G. R. & CURRIE, D. N. (1980). Transport of GABA, β-Alanine and Glutamate into Perikarya of Postnatal Rat Cerebellum. *J. Neurochem.* **34**, 523–530.

EHINGER, B. & FALCK, B. (1971). Autoradiography of some suspected neurotransmitter substances: GABA, glycine, glutamic acid, histamine, dopamine and L-DOPA. *Brain Res.* **33**, 157–172.

GORDON, R. D., WILKINS, G. P., GALLO, V., LEVI, G. & BALÁZS, R. (1980). High affinity transport of L-glutamate and D-aspartate into cell types of the cerebellum. *Neurosci. Letts.* (Suppl.) **5**, S78.

HAMBERGER, A. C., CHIANG, G. H., NYLEN, E. S., SCHEFF, S. W. & COTMAN, C. W. (1979). Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. *Brain Res.* **168**, 513–530.

HARVEY, J. A., SCHOLFIELD, C. N., GRAHAM, L. T. JR. & APRISON, M. H. (1975). Putative transmitters in denervated olfactory cortex. *J. Neurochem.* **24**, 445–449.

HENN, F. A. (1976). Neurotransmission and glial cells. A functional relationship? *J. Neurosci. Res.* **2**, 271–282.

HENN, F. A., ANDERSON, D. J. & RUSTAD, D. G. (1976). Glial contamination of synaptosomal fractions. *Brain Res.* **101**, 341–344.

HENN, F. A. & HAMBERGER, A. (1971). Glial cell function: Uptake of transmitter substances. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2686–2690.

HERTZ, L. (1977). Biochemistry of glial cells: In *Cell Tissue and Organ Cultures in Neurobiology* (S. Fedoroff and L. Hertz), pp. 39–71. New York: Academic Press.

HERTZ, L., SCHOUSBOE, A., BOECHLER, N., MUKERJI, S. & FEDOROFF, S. (1978). Kinetic characteristics of the glutamate uptake into normal astrocytes in cultures. *Neurochem. Res.* **3**, 1–14.

HÖKFELT, T. & LJUNGDHAL, A. (1970). Cellular localization of gamma-aminobutyric acid [^{3}H-GABA] in rat cerebellar cortex: An autoradiographic study. *Brain Res.* **22**, 391.

HÖKFELT, T. & LJUNGDHAL, A. (1972). Autoradiographic identification of cerebral and cerebellar cortical neurones accumulating labelled gamma-aminobutyric acid (^{3}H-GABA). *Expl Brain Res.* **14**, 354.

HUTCHISON, H. T., WERRBACH, K., VANCE, C. & HABER, B. (1974). Uptake of neurotransmitters by clonal lines of astrocytoma and neuroblastoma in culture. I. Transport of γ-aminobutyric acid. *Brain Res.* **66**, 265–274.

IVERSEN, L. L. (1974). Uptake mechanisms for neurotransmitter amines. *Biochem. Pharmac.* **23**, 1927–1935.

IVERSEN, L. L. & BLOOM, F. E. (1972). Studies of the uptake of ^{3}H-GABA and [^{3}H]glycine in slices and homogenates of rat brain and spinal cord by electron microscopic autoradiography. *Brain Res.* **41**, 131.

IVERSEN, L. L. & JOHNSTON, G. A. R. (1971). GABA uptake in rat central nervous system: comparison of uptake in slices and homogenates and the effect of some inhibitors. *J. Neurochem.* **18**, 1939–1950.

JOHNSTON, G. A. R. & IVERSEN, L. L. (1971). Glycine uptake in rat central nervous system slices and homogenates: evidence for different uptake systems in spinal cord and cerebral cortex. *J. Neurochem.* **18**, 1951–1961.

KELLY, J. S. & DICK, F. (1976). Differential labelling of glial cells and GABA-inhibitory interneurons and nerve terminals following the microinjection of [^{3}]β-alanine, [^{3}H]DABA and [^{3}H]GABA into single folia of the cerebellum. *Cold Spring Harb. Symp. quant. Biol.* **40**, 93–106.

KELLY, J. S. & KRNJEVIC, K. (1968). Effect of γ-aminobutyric acid and glycine on cortical neurones. *Nature, Lond.* **219**, 1380–1381.

LASHER, R. S. (1974). The uptake of [^{3}H]GABA and differentiation of stellate neurons in cultures of dissociated postnatal rat cerebellum. *Brain Res.* **69**, 235–254.

LASHER, R. S. (1975). Uptake of GABA by neuronal and non-neuronal cells in dispersed cultures of postnatal rat cerebellum. *J. Neurobiol.* **6**, 597–608.

LOGAN, W. J. & SNYDER, S. H. (1972). High affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous system. *Brain Res.* **42**, 413–431.

LUND KARLSEN, R. A. & FONNUM, F. (1978). Evidence for glutamate as a neurotransmitter in cortico-fugal fibres to the dorsal lateral geniculate body and the superior colliculus in rats. *Brain Res.* **5**, 457–467.

MCBRIDE, W. J., APRISON, M. H. & KUSANO, K. (1976). Contents of several amino acids in the cerebellum, brain stem and cerebrum of the 'staggerer', 'weaver' and 'nervous' neurologically mutant mice. *J. Neurochem.* **26**, 867–870.

MCGEER, P. L., MCGEER, E. G., SHERER, U. & SINGH, K. (1977). A glutamergic corticostriatal path? *Brain Res.* **128**, 369–373.

MCLENNAN, H. (1976). The autoradiographic localization of L[^{3}H]glutamate in rat brain tissue. *Brain Res.* **115**, 139–144.

MARGOLIS, R. K., HELLER, A. & MOORE, R. Y. (1968). Effects of changes in cellular composition following neuronal degeneration of amino acids in brain. *Brain Res.* **11**, 19.

MESSER, A. (1977). The maintenance and identification of mouse cerebellar granule cells in monolayer culture. *Brain Res.* **130**, 1–12.

MIRSKY, R., WENDON, L., BLACK, P., STOLKIN, C. & BRAY, D. (1978). Tetanus toxin: a cell surface marker for neurons in culture. *Brain Res.* **148**, 251–259.

NADLER, J. V., VACA, K. W., WHITE, W. F., LYNCH, G. S. & COTMAN, C. W. (1976). Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. *Nature, Lond.* **260**, 538–540.

NORENBERG, M. D. & MARTINEZ-HERNANDEZ, A. (1979). Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.* **161**, 303–310.

PETTMANN, B., LOUIS, J. C. & SENSENBRENNER, M. (1979). Morphological and biochemical maturation of neurones cultured in the absence of glial cells. *Nature, Lond.* **281**, 378–380.

RAFF, M. C., FIELDS, K. L., HAKOMORI, S. I., MIRSKY, R., PRUSS, R. M. & WINTER, J. (1979). Cell-type-specific markers for distinguishing and studying neurons and the major classes of glial cells in culture. *Brain Res.* **174**, 283–308.

ROFFLER-TARLOV, S. & SIDMAN, R. L. (1978). Concentrations of glutamic acid in cerebellar cortex and deep nuclei of normal mice and weaver, staggerer and nervous mutants. *Brain Res.* **142**, 269–283.

SCHON, F. & IVERSEN, L. L. (1972). Selective accumulation of [^{3}H]GABA by stellate cells in rat ecerebellar cortex *in vivo*. *Brain Res.* **42**, 503.

SCHON, F. & KELLY, J. S. (1974). Autoradiographic localization of [^{3}H]GABA and [^{3}H]glutamate o satellite glial cells. *Brain Res.* **66**, 275.

CHON, F. & KELLY, J. S. (1975). Selective uptake of [^{3}H]β-alanine by glia – Association with glial uptake system for GABA. *Brain Res.* **86**, 243–257.

SCHOUSBOE, A. (1977). Differences between astrocytes in primary cultures and glial cell lines in uptake and metabolism of putative amino acid transmitters. In *Cell Tissue and Organ Cultures in Neurobiology* (ed. S. Fedoroff and L. Hertz), pp. 441–446. New York: Academic Press.

SCHOUSBOE, A. & DIVAC, I. (1979). Differences in glutamate uptake in astrocytes cultured from different brain regions. *Brain Res.* **177**, 407–409.

SCHOUSBOE, A., HERTZ, L. & SVENNEBY, G. (1977). Uptake and metabolism of GABA in astrocytes cultured from dissociated mouse brain hemispheres. *Neurochem. Res.* **2**, 217–229.

SCHOUSBOE, A., HERTZ, L., SVENNEBY, G. & KVAMME, E. (1979*a*). Phosphate activated glutaminase activity and glutamine uptake in primary cultures of astrocytes. *J. Neurochem.* **32**, 943–950.

SCHOUSBOE, A., THORBEK, P., HERTZ, L. & KROGSGAARD-LARSEN, P. (1979*b*). Effects of GABA analogues of restricted conformation on GABA transport in astrocytes and brain cortex slices and on GABA receptor binding. *J. Neurochem.* **33**, 181–189.

SCHRIER, B. K. & THOMPSON, E. J. (1974). On the role of glial cells in the mammalian nervous system. Uptake, excretion, and metabolism of putative neurotransmitters by cultured glial tumor cells. *J. biol. Chem.* **249**, 1769–1780.

SNODGRASS, S. R. & IVERSEN, L. L. (1974). Amino acid uptake into human brain tumors. *Brain Res.* **76**, 95–107.

STEWART, R. M., MARTUZA, R. L., BALDESSARINI, R. J. & KORNBLITH, P. L. (1976). Glutamate accumulation by human gliomas and meningiomas in tissue culture. *Brain Res.* **118**, 441–452.

STORM-MATHISEN, J. (1977). Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy. *Brain Res.* **120**, 379–386.

STORM-MATHISEN, J. & IVERSEN, L. L. (1979). Uptake of [^{3}H]glutamic acid in excitatory nerve endings: light and electronmicroscopic observations in the hippocampal formation of the rat. *Neuroscience* **4**, 1237–1253.

STORM-MATHISEN, J. & WOXEN OPSAHL, M. (1978). Aspartate and/or glutamate may be transmitters in hippocampal efferents to septum and hypothalamus. *Neurosci. Letts* **9**, 65–70.

VALCANA, T., HUDSON, D. & TIMIRAS, P. S. (1972). Effects of X-irradiation on the content of amino acids in the developing rat cerebellum, *J. Neurochem.* **19**, 2229–2232.

VAN DEN BERG, C. J. & GARFINKEL, D. (1971). A simulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. *Biochem. J.* **123**, 211–218.

WATKINS, J. C. (1978). In *Kainic Acid as a Tool in Neurobiology* (ed. E. G. McGeer, J. W. Olney and P. L. McGeer), pp. 37–69. New York: Raven Press.

WATKINS, J. C. (1980). NMDA receptors: New light on amino acid-mediated synaptic excitation. *Trends in Neurosciences* **3**, 61–64.

WEILER, C. T., NYSTRÖM, B. & HAMBERGER, A. (1979). Characteristics of glutamine vs glutamate transport in isolated glia and synaptosomes. *J. Neurochem.* **32**, 559–565.

WEITSCH-DICK, F., JESSELL, T. M. & KELLY, J. S. (1978). The selective neuronal uptake and release of [^{3}H]DL-2,4-diaminobutyric acid by rat cerebral cortex. *J. Neurochem.* **30**, 799–806.

WHITE, F. P., DUTTON, G. R. & NORENBERG, M. D. (1981). Microvessels Isolated from Rat Brain: Localization of Astrocyte Processes by Immunohistochemical Techniques. *J. Neurochem.* **36**, 328–332.

WHITE, W. P., SNODGRASS, S. R. & DICHTER, M. (1980). Identification of GABA neurons in rat cortical cultures by GABA uptake autoradiography. *Brain Res.* **190**, 139–152.

YOUNG, A. B., OSTER-GRANITE, M. L., HERNDON, R. N. & SNYDER, S. H. (1974). Glutamic acid: selective depletion by viral induced granule cell loss in hamster cerebellum. *Brain Res.* **73**, 1–13.