Pergamon

Neuroscience and Biobehavioral Reviews, Vol. 20, No. 3, pp. 537–551, 1996

Printed in Great Britain
0149-7634/96 $32.00 + .00

0149-7634(95)00020-8

Astrocyte–Neuron Interaction During One-trial Aversive Learning in the Neonate Chick*

LEIF HERTZ,†[1] MARIE E. GIBBS,‡ BRONA S. O'DOWD,‡ GARRY L. SEDMAN,‡ STEPHEN R. ROBINSON,§ EVA SYKOVÁ,¶ IVAN HAJEK,¶ ELNA HERTZ,† LIANG PENG,† RONG HUANG† AND KIM T. NG‡

†Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N SE5, Canada, ‡School of Psychology, La Trobe University, Bundoora, Victoria 3083, Australia, §Vision, Touch and Hearing Research Centre, The University of Queensland, Qld, 4072, Australia, and ¶Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

HERTZ, L., M.E. GIBBS, B.S. O'DOWD, G.L. SEDMAN, S.R. ROBINSON, E. SYKOVÁ, I. HAJEK, E. HERTZ, L. PENG, R. HUANG AND K.T. NG. *Astrocyte–neuron interaction during one-trial aversive learning in the neonate chick.* NEUROSCI BIOBEHAV REV **20**(3)537–551, 1996.—During two specific stages of the Gibbs–Ng model of one-trial aversive learning in the neonate chick, we have recently found unequivocal evidence for a crucial involvement of astrocytes. This evidence is metabolic (utilization of the astrocyte-specific energy store, glycogen, during normal learning and inhibition of memory formation by the astrocyte specific metabolic inhibitors, fluoroacetate and methionine sulfoximine) as well as physiological (abolition of memory formation in the presence of ethacrynic acid, an astrocyte-specific inhibitor of cellular reaccumulation of potassium ions). These findings are discussed in the present review in the framework of a more comprehensive description of metabolic and physiological neuronal–astrocytic interactions across an interstitial (extracellular) space bounded by minute processes from either cell type. Copyright © 1996 Elsevier Science Ltd.

Astrocyte Energy metabolism Fluoroacetate Glial cell Glutamate Glutamine Glycogen Glycolysis Learning Memory Methionine sulfoximine Neuropil Noradrenaline Potassium ion

INTRODUCTION

MEMORY REFERS to the cognitive ability to recall a past experience, and an experience associated with learning commonly results in a change of behavior. It is generally assumed that the brain processes associated with behavior occur exclusively in nerve cells, and there is no a priori reason to believe that this might not be the case. However, the unique biochemical and biophysical properties of glial cells in the central nervous system (CNS) that have started to unfold in recent years (2,25–28,30,44,72,74,94) have led to repeated claims that disruption of functional and metabolic interactions between neurons and astrocytes may be implicated in several pathological conditions involving behavioral dysfunction. Similarly, there is increasing evidence of an interaction between neurons and glia during information processing by the brain (29,70). We have recently found that glycogenolysis and glutamine synthesis processes (13,52,57), which in normal brains are restricted to astrocytes, play a crucial role in CNS consolidation of memory in the one-trial passive avoidance learning model developed by Gibbs and Ng (11,12,49–51); so does a cellular cotransport system for uptake of potassium (K^+), sodium (Na^+) and chloride (Cl^-) ions which in the brain may be restricted to non-neuronal cells, and specifically occurs in astrocytes (4,38).

In order to attempt to set the stage for an interpretation of the observations suggesting the involvement of neuronal–astrocytic interactions during memory processing in the brain, we will in this paper initially

*These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference, Clearwater Beach, Florida, USA, 22–25 April 1993.

[1]To whom correspondence should be addressed, at: Department of Pharmacology, Health Sciences Building, 107 Wiggins Road, University of Saskatchewan, Saskatoon, Saskatchewan, S7N E5E, Canada. Phone: 1 306 966 6296; Fax: 1-613 474 0538.

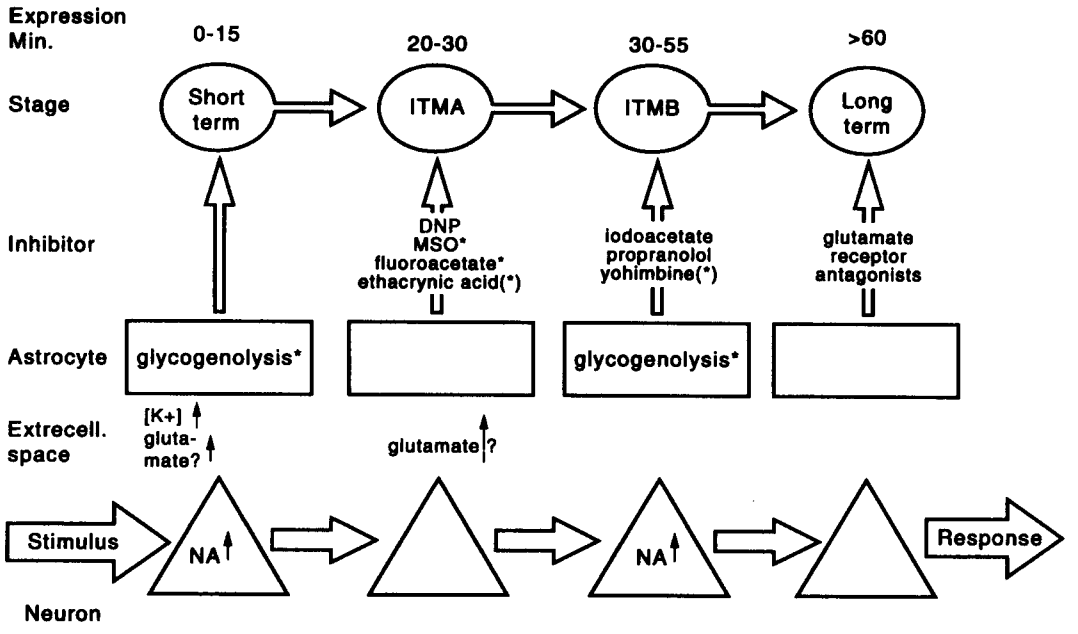

FIG. 1. Schematic representation of the Gibbs–Ng model for one-trial passive avoidance learning in the neonate chick. The different stages are indicated together with the time after learning at which each stage is expressed, selected inhibitors of each stage, the stage at which glycogen (an astrocyte-specific carbohydrate energy store) is decreased on account of glycogenolysis, the stage at which the extracellular potassium concentration ($[K^+]$) is known to be elevated by sensory stimulation, the stages at which noradrenaline (NA) is increased, and the stages at which indirect evidence is found that the extracellular glutamate concentration probably is increased. Events marked with an asterisk are astrocyte specific [*], or probably exerted on astrocytes [(*)]. DNP: dinitrophenol; MSO: methionine sulfoximine.

review the Gibbs–Ng model for a one-trial aversive learning and proceed to describe briefly the histological complexity of the central nervous system (CNS) with emphasis on neuronal–glial interactions occurring around an interstitial (extracellular) space, which is protected by the blood–brain barrier from equilibration with the main extracellular fluid of the body (8). The manner in which events occurring in glial cells may contribute to brain function, including consolidation of memory in the Gibbs–Ng model of one-trial passive avoidance learning, will be discussed on the basis of known astrocytic characteristics and functions. Given the experimental evidence for the importance of astrocytic glycogenolysis and glutamine formation, both of which are restricted to glial cells, especially astrocytes, and of astrocytic potassium ion uptake by the cotransport system during learning, we will focus on metabolic processes, including metabolic interactions between neurons and astrocytes, and on the role of astrocytes in regulation of potassium homeostasis at the cellular level. We will also describe selected effects of the monoamine transmitters noradrenaline and serotonin on neurons and astrocytes (25,26,28,30,43,48), which appear to be of relevance in connection with the role of these monoamines in consolidation of memory (7,11,49–51).

Most of the studies of astrocytic characteristics, neuronal characteristics and neuronal–astrocytic interactions quoted in this paper were obtained from neural tissues other than those directly involved in the one-trial aversive learning task in the chick. This is simply because much more information is available in these structures. Many findings originate from cell culture studies. The cultures used are primary cultures (i.e., cultures obtained from the animal, generally at late fetal or immature stages) which generally, to a much greater extent than cell lines, express the same characteristics as their non-cultured counterparts. As indicated in the text, observations in intact tissue have in virtually every case supported the conclusions drawn from studies of cultured cells.

THE GIBBS–NG MODEL

The Gibbs–Ng model is based on a passive avoidance learning task which capitalizes on the fact that the 1-day-old chick at birth is equipped with a well-developed CNS which enables the newly hatched bird to learn quickly about critical aspects in its environment (e.g., the chick needs to peck while exploring the environment for edible food). The learning task entails pretraining chicks to peck at water-coated red and blue beads. If one of these beads (typically the red) is thereafter coated with the chemical aversant methylanthranilate (MeA) and presented to the chicks for pecking, the chicks peck once and display a strong disgust reaction. Normally, about 75% of the chicks subsequently avoid the red bead, and the number of pecks at the blue bead in a population of birds will be approximately 10 times higher than that at the red bead (discrimination ratio 0.9). However, administration of a drug after learning may extinguish a specific stage and all subsequent stages of memory, resulting in a discrimination ratio closer to 0.5, which indicates no color preference (11,12,49–52).

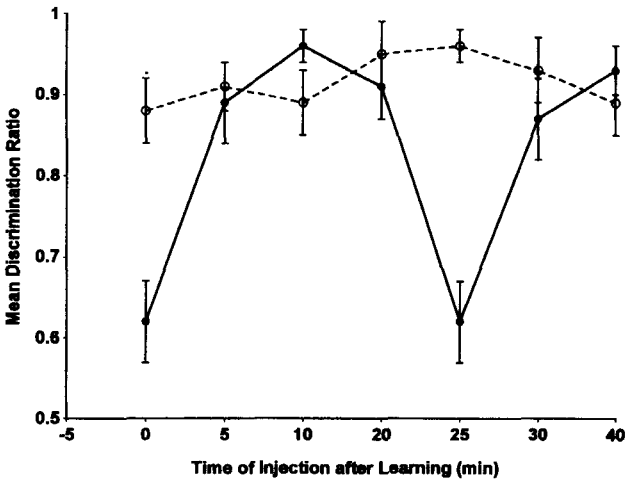

FIG. 2. Mean discrimination ratios at 3 h post-learning after injection of saline (○) or 1 mM iodoacetate (●) at different time periods after learning. A discrimination ratio of 0.9 indicates normal learning and a discrimination ratio of 0.5 indicates no learning at all. Note that iodoacetate administered either immediately after learning or after 25 min abolishes learning (which in this experiment was measured after 3 h), whereas administration of iodoacetate 5–20 min after learning has no effect. Regardless of whether iodoacetate is administered immediately after learning or after 25 min, memory disappears during ITM(A), as indicated in Fig. 1. Each point shows the means ± SEM in a population of 16–20 birds. From Ref. (57).

The Gibbs–Ng model comprises three sequential stages (Fig. 1): a short-term (STM) stage, available for 10 min following learning; an intermediate (ITM) stage, operating between 20 and 50 min post-learning; and a long-term (LTM) stage formed by 60 min after learning (11,12,49–51). Behaviorally, the three pharmacologically distinct stages are separated by transient retention deficits occurring around 15 and 55 min post-learning, and the formation of each stage is critically dependent upon the successful establishment of all previous stages. Memory formation during each of these stages must depend upon different biological processes as indicated by distinct, clearly stage-specific variability in susceptibility to pharmacological agents, some of which are indicated in Fig. 1.

The ITM stage is divided into two phases, ITM(A) and ITM(B), pharmacologically distinguished by the sensitivity of ITM(A), but not ITM(B), to the uncoupler of oxidative phosphorylation, 2-4 dinitrophenol (DNP). In the present context it is especially important that the glia-specific toxins, fluoroacetate, ethacrynic acid and methionine sulfoximine (MSO), all abolish memory during ITM(A) (11,13). However, utilization of glycogen, an energy store with a glial specific localization, occurs towards the end of ITM(B) (56). As illustrated in Fig. 2, differences in drug susceptibility at different time periods in relation to the learning procedure also apply to the stage at which a given drug must be administered (time of administration) in order to be an effective inhibitor (57). In this context it should be kept in mind that administered drugs will be diluted and finally eliminated from the site of injection by diffusion in the tissue. From differences in time of administration and in time of memory extinction, a picture of neuronal–glial interactions starts to emerge which will be discussed in the following, but first the histological, metabolic and functional complexity of brain tissue at the cellular level will be briefly reviewed.

HISTOLOGICAL, METABOLIC AND FUNCTIONAL COMPLEXITY OF BRAIN AT THE CELLULAR LEVEL

Histological Complexity

The central nervous system is made up of nerve cells (neurons) and non-neuronal, i.e., glial, cells separated by an extracellular space which at many locations is narrow enough to allow interactions between adjacent cells of similar or different types, i.e., functions as a communication channel. It is separated from the systemic circulation by the blood–brain barrier.

In most nerve cells, the cell body accounts only for a small part of the total cell volume, with much larger volumes being occupied by cell processes, especially the input-receiving dendrites (64). Neurons are often classified according to the transmitter they release during synaptic transmission (e.g., glutamatergic neurons, GABAergic neurons, noradrenergic neurons, peptidergic neurons). Some individual neurons release more than one type of transmitter. Functionally, the character of the receptors expressed by neurons is as important as the type(s) of transmitter released by the neuron. Most, but not all, receptors are located on the dendrites (Fig. 3). Receptor activation following synaptic release of an excitatory transmitter causes a postsynaptic depolarization, which in many cases spreads electrotonically along the dendrite, but will be too weak to generate an action potential. It is the spatial and/or temporal summation of such postsynaptic depolarizations which eventually may lead to generation of an action potential, generally initiated at the axonal hillock, and facilitated by a high sodium channel density at this location. However, it seems to be well established now that classical action potentials also occur in dendrites, at least in some types of neurons (36,80). Receptors for the inhibitory, hyperpolarizing transmitters are generally located much closer to the cell body than the receptors for the excitatory transmitters and are thus able to block incoming activity (Fig. 3).

The most important general characteristic of all nerve cells is their excitability. A propagating action potential triggers "downhill" transport of monovalent ions along their electrochemical gradients through ion channels which open in response to the depolarization (initially sodium channels and slightly later potassium channels). These passive fluxes of sodium and potassium ions constitute the biophysical basis of the action potential (1). They are not energy dependent, whereas the subsequent restoration of resting ion concentrations (e.g., a high intracellular and a low extracellular K^+ concentration and a low intracellular Na^+ concentration) requires active transport, catalyzed by the ouabain-sensitive Na^+,K^+-ATPase. This restoration of pre-activity ion distribution across cell membranes accounts for by far the major part of the increase in

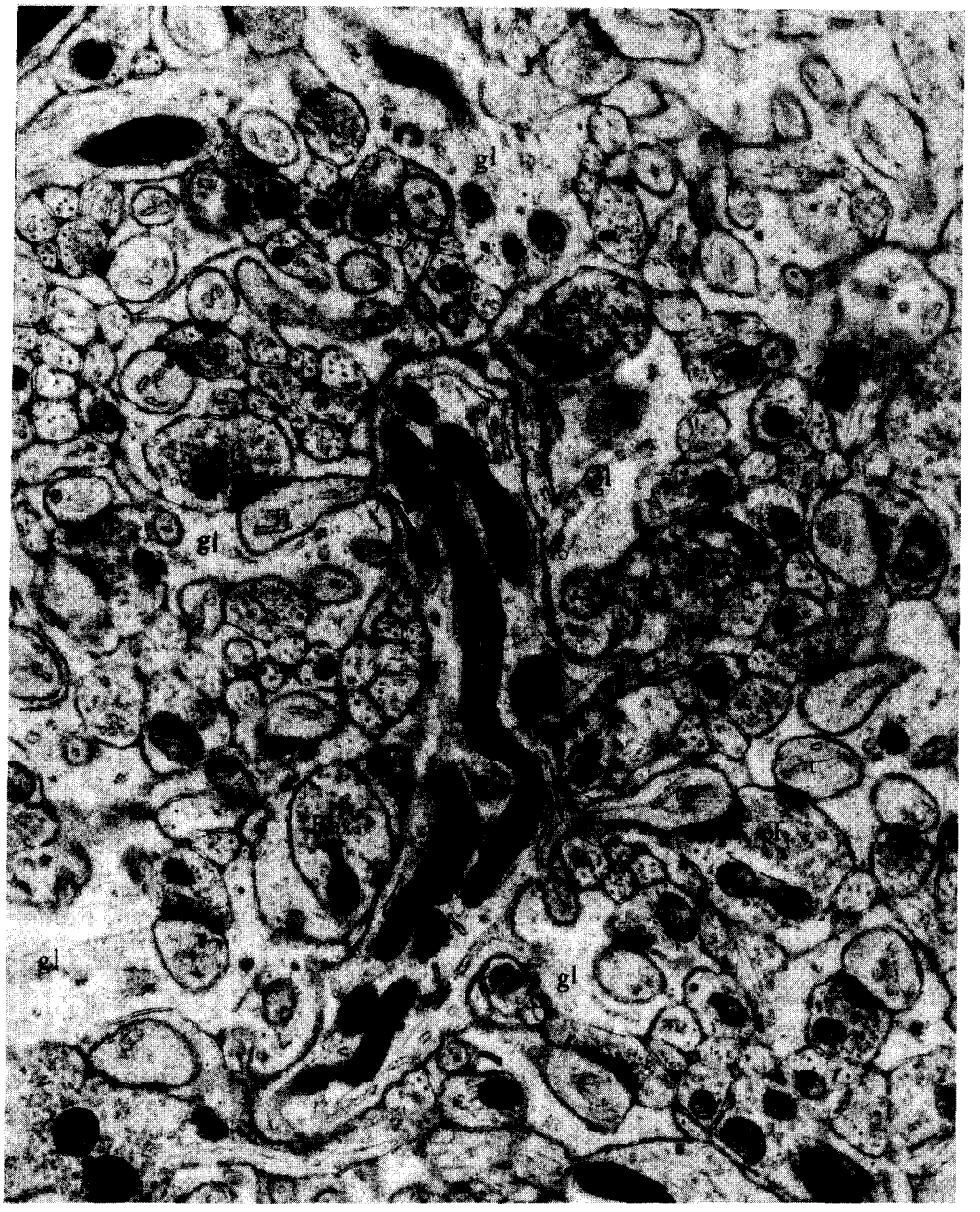

FIG. 3. Electron micrograph of neuropil in the molecular region of the cerebellum. In the center is part of a Purkinje cell dendrite, containing elongated mitochondria (mit). A dendritic spine (t_1), extending from the upper left part of the dendrite, is in synaptic contact with a parallel fiber axon from the excitatory, glutamatergic granule cells (pf_2). This synapse is completely surrounded by glial cells (gl). An axon from the inhibitory, GABAergic basket cells (Bax) synapses more centrally with the Purkinje cell dendrite. Note the multitude of minute cell processes, many of which are astrocytic. From Ref. (64).

energy consumption in the CNS associated with functional activity (76).

Glial cells in the CNS are traditionally divided into protoplasmic and fibrous astrocytes, oligodendrocytes and microglia (64). In large vertebrates, astrocytes and oligodendrocytes together outnumber nerve cells by a factor of 5–10 and astrocytes are more abundant than oligodendrocytes. In humans, astrocytes may account for 25–30% of the total cellular volume in brain cortex (65). In gray matter, the most abundant glial cell is the protoplasmic astrocyte, whereas the fibrous astrocyte is found mainly in white matter. A special type of astrocyte is the perinodal astrocyte, ensheathing the nodes of Ranvier of myelinated nerves. In this context it should be remembered that axons of individual neurons are quite often myelinated, whereas dendrites are virtually never covered by myelin (64). Myelin in the CNS is produced by oligodendrocytes in an interaction with the axon. Most oligodendrocytes are found in white matter, but these cells are also occasionally (although not nearly as often as was previously believed) found close to neurons in gray matter (64).

Myelination increases conduction speed and may facilitate usage of specific pathways, a process which may be important for the development of conditioned reflexes (29,70). Microglial cells are involved in immunological and inflammatory reactions.

Astrocytes are located at strategic positions, e.g., around synapses (Fig. 3) and abutting microvessels, as capillary endfeet. Most astrocytes extend a large number of finely branched processes and extremely thin veils (59) and these cells have a very large surface: volume ratio (93). Glycogen stores, which are found in astrocytes but normally not in neurons, have been demonstrated in even the thinnest processes (64).

In contrast to neurons, astrocytes are not excitable cells (2,23). This difference has the functionally important consequence that CNS excitation will lead to an entry of sodium ions into neurons but not into astrocytes. That does not mean that astrocytes do not perform active ion transport and that energy-requiring processes in astrocytes are not involved in re-establishing the resting extracellular potassium ion concentration. However, as will be discussed below, astrocytic Na^+,K^+-ATPase activity appears to be triggered by activation of an extracellular potassium-sensitive site. Astrocytes and oligodendrocytes resemble neurons in expressing receptors for a multitude of transmitters (43,48). It came as a surprise to many neuroscientists that glial cells displayed such "neuronal characteristics". However, it should be kept in mind that receptor expression is not a neuronal prerogative but is shared by a multitude of cell types, e.g., muscle cells, and epithelial cells, in which receptor activation regulates functions such as energy metabolism and ion transport.

After the volumes occupied by nerve cell and glial cell bodies and their easily recognizable larger processes have been added up, the major fraction of the tissue volume remains to be accounted for. In gray matter most of this fraction is made up by the neuropil. As illustrated in Fig. 3, the neuropil is composed of an intricately interwoven network of minute neuronal and glial processes (64). Although dendrites on neurons may generally constitute a large fraction of the neuropil, both nerve endings and glial cell processes also account for a considerable volume, and the glial, mainly astrocytic contribution has been estimated to 40–50% of the total volume (14). The neuropil is the part of the tissue where functional interactions may most easily occur between neurons and glial cells. Since dendrites are not covered by myelin, whereas many axons are, such interactions may be especially pronounced between glial cells and dendritic elements. It is a reflection of the immense surface area and the intensity of the active, energy-requiring ion movements required for re-establishment of resting intracellular and extracellular concentrations of sodium and potassium ions after neuronal excitation, that it is the energy metabolism in the neuropil, not in the nerve cell bodies, which is increased during functional activity (39,76).

The interstitial or extracellular space shared by neurons and glial cells is, as already mentioned, of critical importance during functional activity in the brain. This space is isolated from the rest of the body by the blood–brain barrier and is mainly localized in the narrow extracellular clefts penetrating the neuropil. At many locations the extracellular space is compartmentalized by astrocytic veils isolating groups of cells (59,64).

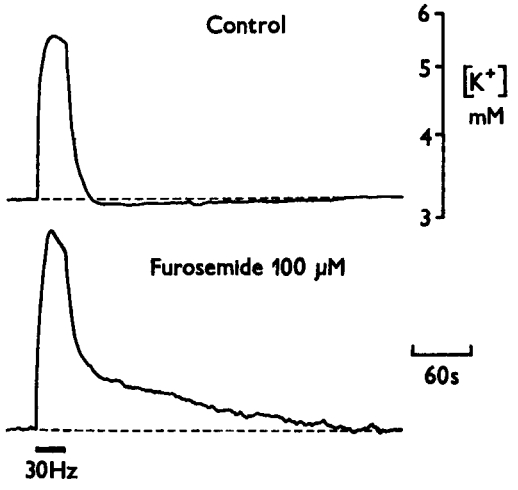

FIG. 4. Increase in extracellular potassium ion concentration (K^+ in the isolated rat spinal cord after sensory stimulation (30 Hz for 60 s) under control conditions and in the presence of 100 µM furosemide. Extracellular K^+ increases as a result of the stimulation, probably mainly or exclusively as a result of K^+ release from excited neurons. It subsequently decreases to and even below ("undershoots") resting levels owing to active uptake processes in both neurons and astrocytes (and maybe also redistribution of extracellular K^+). Note the slowing of K^+ clearance from the extracellular space and the abolition of the undershoot in the presence of furosemide, an inhibitor of the cotransport system for uptake of K^+ which appears to be confined to glial cells. Unpublished results by E. Syková.

The importance of alterations in the composition of the neuronal-glial microenvironment has only been realized during the last 20 years. Aided by newly developed microelectrodes that can be inserted into the extracellular space, it was shown that the extracellular potassium ion concentration rises dramatically during functional activity or pathological conditions: from a few millimolar (e.g., from 3 to 4 mM) during normal nerve cell activity, including sensory stimulation (Fig. 4) during the initiation of the learning process (Fig. 1), to a ceiling level of 10–12 mM during seizures, and to exceedingly high levels (more than 50 mM) during energy failure and during the peculiar electrophysiological phenomenon of "spreading depression" (85,86). In many other tissues such alterations in extracellular ion concentrations would be greatly reduced and rapidly abolished by diffusion into the vascular system. This does not occur in the CNS because of the presence of the blood–brain barrier. Therefore, the extracellular concentrations of potassium ions and other neuroactive compounds are regulated by (i) release and (ii) subsequent uptake into adjacent cells. There is now overwhelming evidence that a large part of the immediate uptake of excess extracellular potassium occurs into glial cells (89).

Another important methodological advance was when it became possible to estimate extracellular

concentrations of glutamate (and of other transmitters, e.g., noradrenaline) with the aid of microdialysis (3,95). Extracellular glutamate content increases during stimulation of glutamatergic neurons, and elevated concentrations of potassium ions cause glutamate release from both neurons and astrocytes (31). Although no direct measurements of extracellular glutamate concentrations have been made during the Gibbs–Ng learning trial, it can be deduced that the extracellular glutamate concentration must become elevated at least twice during the learning experience, since an antagonist of one glutamate receptor (the NMDA receptor) must be administered no later than at the time of learning to prevent establishment of long-term memory, whereas an antagonist of non-NMDA glutamate receptors must be administered between 10 and 25 min after learning (52,68,69), indicating that glutamate receptors are activated by extracellular glutamate initially and at some time after 10 min and before ~25 min, possibly during the entire ITM(A). Experimental verification of an increase in extra cellular glutamate would be important. A large part of the reuptake of glutamate occurs into astrocytes, depleting neurons of a neurotransmitter (32,72), and it has become evident that glial cells are also involved in metabolic processes that replenish neurons with precursors for transmitter glutamate. In order to understand these metabolic interactions and metabolic processes occurring during ITM(A) and ITM(B) of the Gibbs–Ng model of memory for a one-trial aversive learning task, it is essential to discuss CNS metabolism at the cellular level in some detail.

Metabolic Complexity

An intense glucose transport across the blood–brain barrier by the aid of a specific glucose carrier is essential for the supply of CNS with a metabolic substrate, and in the absence of glucose all CNS function is abolished within minutes. However, not all cells or subcellular cell constituents necessarily metabolize glucose completely to carbon dioxide and water. As illustrated in Fig. 5, glucose metabolism is initiated by glycolysis, leading to the formation of pyruvate which, under anaerobic conditions (evoked either by lack of oxygen supply to the tissue or because local utilization of oxygen exceeds the rate at which oxygen can be supplied locally) can be reduced to lactate. In astrocytes, pyruvate is also transaminated to alanine (77,94). Pyruvate, lactate and alanine all readily exit across cell membranes, and it is unknown to what extent each of these compounds is further metabolized in the cell in which pyruvate was produced, or in adjacent cells, which could be of any type. This is of special interest on account of a very high rate of glycolysis in astrocytes, although neurons also are capable of metabolizing glucose to pyruvate and lactate (27,63).

Glycolysis does lead to production of energy (in the form of ATP), although to a quantitatively much smaller extent per molecule of glucose than oxidative metabolism. However, glycolytically derived energy is essential to carry out certain energy-requiring processes in astrocytes, including active uptake of

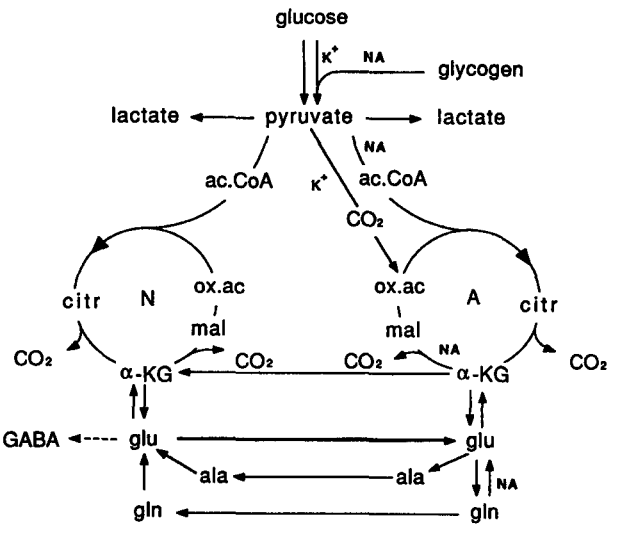

FIG. 5. Glucose and glycogen are metabolized to pyruvate–lactate (during glycolysis) and via acetyl co-enzyme A (ac.CoA), to carbon dioxide (CO_2) and water in the citric acid cycle (during oxidative metabolism) in both neurons (N) and astrocytes (A). Only astrocytes have pyruvate carboxylase activity, allowing a net synthesis of axaloacetate (ox.ac.) and other citric acid cycle constituents, e.g., α-ketoglutarate (α-KG) from glucose, via pyruvate. Glutamate (Glu) is released from glutamatergic neurons and partly accumulated into astrocytes (heavy arrow). Synthesis of glutamine (Gln), an astrocyte-specific process, release of Gln from astrocytes, its reaccumulation into neurons and hydrolysis to form Glu and GABA contribute to replenish neuronal transmitters (the "glutamate–glutamine cycle"). However, astrocytic Glu is also metabolized to CO_2, and its amino group is partly transaminated into other amino acids, e.g., alanine (Ala), which is formed by transamination with pyruvate. Additional Glu precursors must, therefore, exist in glutamatergic neurons. α-KG and Gln are synthesized in astrocytes from ox.ac. formed by pyruvate carboxylation, released, accumulated into neurons and used to form the carbon skeleton of Glu and GABA. Incorporation of amino nitrogen from Ala, formed from pyruvate, mainly in astrocytes, and released to the medium (and/or other amino acids) supplies the amino group. Stimulation of glycogenolysis by noradrenaline is indicated by NA. Glycolysis is inhibited by iodoacetate in both neurons and astrocytes, citric acid cycle activity (degradation of citrate) by fluoroacetate in astrocytes but not in neurons, and oxidative phosphorylation (production of CO_2) by dinitrophenol in either cell type. Modified from Ref. (25).

glutamate which is inhibited in the absence of glucose but not during anoxia (35,41). Astrocytic potassium uptake may also be supported by glycolysis, whereas potassium uptake into neurons may depend upon oxidative metabolism (41,71). In this context it should be recalled that glycogen granules can be observed in even the thinnest astrocytic processes. This is not the case for mitochondria, owing to their relatively large size (see Fig. 3). In addition to glucose, glycogen, a high molecular weight carbohydrate that can be synthesized from glucose and degraded to glucose (glycogenolysis) in astrocytes, but not in neurons, is a suitable substrate for glycolysis (18,64). Glycogenolysis occurs very rapidly in astrocytes (5) and can therefore give rise to a large amount of energy within a short time, in spite of the relatively low energy yield per glucose equivalent. The pyruvate and lactate formed by astrocytic glycogenolysis may be available for further metabolism not only in the astrocytes

themselves but also in adjacent neurons, but the glycolytically generated energy can only be used by the astrocytes themselves. However, pyruvate may also be used for resynthesis of glycogen (9,18,34). The metabolic inhibitor iodoacetate blocks glycolysis (and therefore also glycogenolysis) in both neurons and astrocytes. Under conditions when only glucose and glycogen are being metabolized, inhibition of glycolysis will also abolish oxidative metabolism (see below) owing to impairment of the production of pyruvate. However, in the intact brain, other substrates, e.g., certain amino acids such as glutamate, are also available which do not require glycolytic degradation. It is therefore possible to inhibit glycolysis selectively by the administration of iodoacetate.

In the presence of oxygen, pyruvate formed during glycolysis can be further metabolized via acetyl coenzyme A in the mitochondrial citric acid cycle (Fig. 5), eventually to be completely oxidized to carbon dioxide and water with the concomitant production of a large amount of energy (ATP) by oxidative phosphorylation, but without giving rise to net synthesis of citric acid cycle constituents (34). The metabolic inhibitor fluoroacetate inhibits citric acid cycle activity in astrocytes at concentrations that have no effect on oxidative metabolism in neurons (20,82).

In order to achieve a net synthesis of a citric acid cycle constituent in the CNS, pyruvate must be condensed with carbon dioxide (bicarbonate) to form oxaloacetate (Fig. 5), catalyzed by pyruvate carboxylase. This reaction occurs in astrocytes but not in neurons (18,28,34,40,77) and it is of special interest because glutamate, the major excitatory transmitter, is formed from the citric acid cycle intermediate α-ketoglutarate, and GABA, the major inhibitory transmitter, is formed by further decarboxylation of glutamate. Thus, net synthesis of any of these two amino acid transmitters requires both net synthesis of oxaloacetate and oxidative metabolism in the astrocytic citric acid cycle to form α-ketoglutarate, the direct precursor of glutamate. It is remarkable that neurons, according to all available evidence, lack pyruvate carboxylase activity and cannot on their own carry out net synthesis of citric acid cycle constituents and their metabolites, including glutamate and GABA, from pyruvate to replenish released transmitters.

Glutamate which has been re-accumulated into glutamatergic neurons may be available for continued use as a transmitter, and glutamate which has been accumulated into astrocytes (in general probably the larger part) is partly converted to glutamine, by glutamine synthetase, a glial-specific enzyme (53), the activity of which is inhibited by methionine sulfoximine (MSO) (10). Unfortunately it is presently not possible selectively to inhibit glutamate uptake into either neurons or astrocytes (67). The preferential uptake of extracellular glutamate into astrocytes, together with the demonstration that high glutamine synthetase activity is present in astrocytes but not in neurons led to the concept of a "glutamate–glutamine cycle" (Fig. 5) in which astrocytic glutamine (which has no transmitter properties), after its synthesis from glutamate, is released to the extracellular space and partly re-accumulated into glutamatergic neurons, where it is used as a glutamate precursor, and into GABAergic neurons which produce GABA from glutamate. There is no doubt that glutamine is an excellent precursor for synthesis of transmitter glutamate and GABA in neurons (32). This is illustrated by the recent demonstration by Pow and Robinson (66) that the glutamatergic bipolar and ganglion cells in the isolated and incubated rabbit retina rapidly lose all cellular glutamate during exposure to MSO. This is accompanied by impairment of glutamatergic transmission between the retina and the brain (45).

Although astrocytically generated glutamine is a suitable precursor for neuronal glutamate, there must be additional precursors. This conclusion is based upon the experimental finding that a substantial fraction of the glutamate which has been taken up into astrocytes is not converted to glutamine but used as a metabolic fuel, i.e., oxidized to carbon dioxide and water (27,32,72,77). This obviously means that there is a net diversion of glutamate from the glutamate–glutamine cycle (Fig. 5). Since glutamate does not easily cross the blood–brain barrier, net synthesis of glutamate within the CNS must occur from glucose. As previously discussed, astrocytes (but not neurons) can readily synthesize the citric acid cycle intermediate oxaloacetate which can be further oxidatively metabolized in the citric acid cycle to α-ketoglutarate (Fig. 5). α-Ketoglutarate is one of the citric acid cycle intermediates that are released from cultured astrocytes to the incubation medium (90) and cultured glutamatergic cerebellar granule cell neurons can utilize exogenous α-ketoglutarate for net synthesis of the carbon skeleton of transmitter glutamate (32,62,72). In addition, they need a donor for the amino group in glutamate, which can be supplied by transamination with, for example, alanine (Fig. 5), an amino acid formed in astrocytes in large amounts from pyruvate (94) and released to the medium (91). The glia-specific inhibitor of the citric acid cycle, fluoroacetate, inhibits astrocytic formation of glutamine via glutamate and α-ketoglutarate in concentrations which have little, if any, effect on ATP content (20,82), but abolishes glutamatergic transmission in brain slices (42). In contrast to astrocytes, neurons do not rapidly oxidize glutamate (or glutamine) as metabolic fuel (27,32).

The neuronal–astrocytic interactions in glutamate/glutamine metabolism sketched above are presently the best known metabolic interactions between neurons and astrocytes. The effects on learning of interrupting these interactions by either MSO or fluoroacetate have also been studied in detail, as will be discussed later.

Functional Complexity

As already mentioned, CNS stimulation leads to an increase in potassium ion concentration in the interstitial space (85,86,89) and in intracellular sodium concentration in neurons, but not in astrocytes which are non-excitable cells. This is indicated schematically in Fig. 6. Owing to the low extracellular concentration of potassium ions and the relatively small volume of

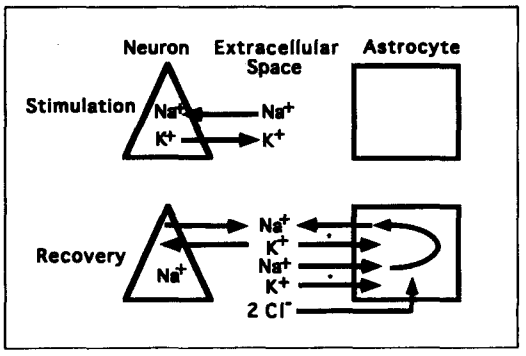

FIG. 6. Diagram of sodium (Na^+), potassium (K^+) and chloride (Cl^-) ion fluxes during neuronal stimulation (passive Na^+ uptake and K^+ release in neurons; no ion movements in astrocytes) and subsequent recovery by active transport mechanisms. The latter comprise active uptake of K^+ and extrusion of Na^+ in both neurons and astrocytes, catalyzed by the Na^+,K^+-ATPase, and co-accumulation of Na^+, K^+ and Cl^- by the cotransport system, which is energetically driven by the Na^+ gradient and is present in astrocytes but appears to be absent in neurons. The joint operation of the Na^+,K^+-ATPase and the cotransport system leads to a reaccumulation of K^+, together with Cl, but without any concomitant extrusion of Na^+, because accumulated Na^+ is extruded by stimulation of the intracellular, Na^+-sensitive site of the Na^+,K^+-ATPase. If intracellular Na^+ is still elevated in neurons (as a result of the Na^+ entry during the previous stimulation) after complete clearance of excess K^+ from the extracellular space, neuronal Na^+,K^+-ATPase activity may remain activated and lead to an "undershoot" in the extracellular concentration of K^+. From Ref. (28).

the extracellular space, compared to a much larger intracellular space, the relative alteration will be especially marked in extracellular potassium ion concentration. Moreover, the magnitude of the extracellular potassium concentration has profound effects on neuronal excitability (85,89), and a rise in extracellular potassium concentration may lead to a conductance block in neurons. An energy requiring subsequent restoration of the previous balance between intracellular and extracellular concentrations of potassium and sodium ions is therefore essential to maintain membrane polarization and excitability and accounts for a very major fraction of the total energy demand in the CNS (76). The finding that the increase in energy metabolism resulting from excitation occurs in the neuropil reflects the fact that most of the active transport occurs in this structure (39,76), but it does not give any information about the extent of the energy expenditure in, respectively, astrocytic processes, axons and dendrites.

Na^+,K^+-ATPase activity is present in both neurons and astrocytes (83,84). In both cell types the enzyme activity increases as a function of the extracellular potassium concentration up to the normal extracellular potassium concentration; in astrocytes, but not in neurons, there is a further stimulation when the extracellular potassium concentration is increased above its normal level (15,16,22,34,47). This is reflected by a ouabain-sensitive increase in glucose utilization (60,63) and by a steep increase in intracellular potassium content in cultured astrocytes (92), but not in neurons, when the extracellular potassium concentration is increased from 4 to 10 mM, a concentration that does not cause a sufficient depolarization to evoke an action potential and ensuing entry of sodium ions in neurons. Thus, the small increase in interstitial potassium concentration resulting from neuronal excitation will per se lead to an enhanced uptake of potassium ions into astrocytes but not into neurons. In neurons, stimulation of Na^+,K^+-ATPase activity by an excitation-induced elevation of the intracellular concentration of sodium at much more highly elevated concentrations of potassium ions may be the reason for a considerable, ouabain-inhibitable increase in rate of oxidative metabolism of glucose which has been demonstrated in cultured cerebellar granule cell neurons (34,61,63). This response may occur especially in dendrites, as indicated by the finding that little or no stimulation was seen in corresponding cells that had been cultured in a manner causing massive degeneration of dendrites with normal morphology of other subcellular constituents (61).

Many cell types, including astrocytes but probably not neurons, express a cotransport system, accumulating in conjunction with one potassium ion, one sodium ion and two chloride ions (Fig. 6). This transport system is inhibited by furosemide, ethacrynic acid or bumetanide and is stimulated by elevated extracellular potassium ion concentrations (38,85,88). Administration of furosemide delays potassium ion clearance from the extracellular space (Fig. 4), indicating the functional importance of this system. It is an electroneutral uptake system but, provided the cell membrane is permeable to water, it will for osmotic reasons lead to a concomitant uptake of water, i.e., cell swelling (33). Once inside the cell, the accumulated sodium ions stimulate Na^+,K^+-ATPase activity at the intracellular, sodium-sensitive site of the enzyme, leading to an active, energy-dependent extrusion of sodium ions and a concomitant uptake of potassium ions (88). Therefore, the functional implication of the joint operation of the cotransport system and the Na^+,K^+-ATPase in astrocytes is an uptake of potassium ions together with chloride ions, and a corresponding removal of interstitial potassium (and chloride) ions without any net movement of sodium ions (Fig. 6).

Owing to the intensity of the potassium transport mechanisms operating across the astrocytic membrane and the lack of any net movement of sodium ions during the operation of the system described above, the interstitial potassium ion concentration may return to its normal resting level before the interstitial sodium concentration has been normalized, i.e., before excess intraneuronal sodium ions resulting from entry during the excitation have been quantitatively extruded. This has been demonstrated experimentally during spreading depression in rat hippocampus (24). Continuous stimulation of neuronal Na^+,K^+-ATPase activity after this point by the elevated intraneuronal sodium concentration can be expected to lead not only to sodium extrusion from neurons but also to a coupled active uptake of potassium ions from the extracellular space below the resting potassium concentration, i.e., to an "undershoot" of extracellular potassium concentration (Fig. 4). In turn, an undershoot of the extracellular

potassium concentration would lead to a neuronal hyperpolarization, a phenomenon which may be essential in learning (29,50,51,70). An undershoot in extracellular potassium concentration established in this manner would be a direct consequence of a transport process in neurons, but the ability of this transport process to cause a lowering of the extracellular potassium concentration below its resting level is critically dependent upon the joint operation of the cotransport system and the Na^+,K^+-ATPase in astrocytes. A different potential mechanism for establishment of an undershoot in extracellular potassium concentration is stimulation of Na^+,K^+-ATPase activity (in neurons or astrocytes or both) by transmitters like noradrenaline (see below).

Another complex functional interaction between neurons and astrocytes is the effect of neuronally released autonomic nervous system transmitters such as the monoamines noradrenaline and serotonin and some neuropeptides on glial cells (25,26,30,43,48). In the periphery, the autonomic nervous system regulates bodily functions, e.g., muscle contraction, glandular secretion, energy metabolism and vascular perfusion, according to functional demands. It has, for many years, been a dogma that, in contrast to all other organ systems, the CNS does not possess an autonomic nervous system. The main reason for this belief is probably the presence of the blood–brain barrier, which makes receptors located on the abluminal side of the endothelial cells, as well as on other target cells within the CNS, inaccessible to most systemically administered receptor agonists. Moreover, specific nuclei of origin for noradrenergic, serotonergic, and cholinergic fibers to the CNS have only been discovered relatively recently, and even less information is available about intracerebral peptidergic pathways.

Neurons, astrocytes and microvessels all express noradrenergic receptors. In the rodent brain, most β-adrenergic receptors appear to be located on astrocytes and microvessels, with only a sparse neuronal receptor expression (79). α-Adrenergic receptors are more evenly distributed between neurons and astrocytes, although there is a distinct expression of α_2-adrenergic receptors on target cells, especially on astrocytes; serotonergic (5-HT) receptors are also found on both cell types, but there might be cell-specific expression of specific subtypes (43). Dopaminergic receptors have been observed on striatal astrocytes (21). Astrocytes also express receptors for many peptide transmitters (43,48). Less is known about receptor expression on oligodendrocytes, but these glial cells also express receptors for at least some autonomic transmitters.

Both serotonin and noradrenaline stimulate glycogenolysis in mouse astrocytes (5,30,78,81). Noradrenaline has been shown to exert such an action in chick astrocytes of an age corresponding to the 1-day-old chick (55). It is of special interest that the α_2-adrenergic agonist clonidine causes a stimulation of glycogenolysis, i.e., acts like noradrenaline (81). This is in contrast to effects of α_2-adrenergic agonists on neurons where they act presynaptically to reduce noradrenaline release and thus have an opposite effect to the application of noradrenaline itself. Noradrenaline also increases uptake of glutamate into rat astrocytes in primary cultures (19), whereas serotonin inhibits potassium-stimulated glutamate release from neurons as well as astrocytes (31,46). Noradrenaline stimulated Na^+,K^+-ATPase activity in both astrocytes and neurons; the β-adrenergic agonist isoproterenol has a similar effect in astrocytes, but is inactive in neurons (16), probably reflecting the scarcity of β-adrenergic receptors on neurons. Administration of noradrenaline to the spinal cord *in vivo* causes a reduction in the potassium increase seen after sensory stimulation, perhaps reflecting the enhancement of Na^+,K^+-ATPase activity and removal of potassium ions (Syková and Chvatal, unpublished experiment). It is unknown whether noradrenaline has any effect on the cotransport system, but from other cell types it is known that this ion uptake carrier can be modulated by different second messengers (58).

It seems of special interest that the effect of noradrenaline on Na^+,K^+-ATPase activity is dependent upon the extracellular potassium concentration. Thus, the stimulatory effect of nordrenaline on astrocytic Na^+,K^+-ATPase activity is seen at normal and possibly also at slightly elevated potassium concentrations (but not at subnormal potassium concentrations), and neuronal Na^+,K^+-ATPase activity is reduced by noradrenaline in the presence of either elevated or reduced potassium concentrations (16). Release of noradrenaline in the brain is global since the entire brain is innervated by fibers extending from locus coeruleus (25,26), but the modulation of noradrenergic effects by alterations in extracellular potassium concentrations, which are local, reflecting ongoing or previous excitation, means that the effect of noradrenaline differs according to its site of release. This may be of importance during establishment of memory, including the Gibbs–Ng model of one-trial aversive learning in the 1-day-old chick, where evidence is found of local increases in potassium concentration and release of noradrenaline (see below). The differential effect of an increased extracellular potassium concentration on Na^+,K^+-ATPase activity in neurons and astrocytes also means that noradrenaline will cause a relative increase in potassium accumulation in astrocytes during functional activity.

EXPERIMENTAL EVIDENCE FOR THE INVOLVEMENT OF NEURONAL–ASTROCYTIC INTERACTIONS DURING THE GIBBS–NG MODEL OF MEMORY FORMATION

Extracellular Communication Channel

Because the extracellular space functions as a communication channel between cells of similar and dissimilar types in the CNS, exact knowledge about alterations in extracellular concentrations of neuro active compounds during different stages of the Gibbs–Ng model of aversive learning would be extremely useful. Unfortunately such information is presently not available. It is known that the sensory events associated with the mere exposure to MeA cause an increase in the extracellular potassium ion

concentration (87) in anaesthetized and immobilized animals (procedures which are necessary in order to perform the measurements) but it is not possible to determine whether subsequent stages of the memory consolidation are accompanied by renewed increase(s) in extracellular potassium concentration in awake, freely moving animals. Nor have extracellular concentrations of glutamate been directly measured; but the findings that two different types of glutamate receptor antagonist must be administered at different time periods (at 0 and between 10 and 25 min, respectively) (52,68,69) is a strong indication that the extracellular glutamate concentration is elevated at these two time points.

Extracellular concentrations of noradrenaline have not been measured during the establishment of memory in the Gibbs–Ng model, but it has been reported that there is an increase in total (extracellular plus intracellular) noradrenaline immediately after learning and another increase at 25–30 min (7). A substantial portion of this noradrenaline is likely to be released to the extracellular space.

Glutamate–Glutamine Metabolism

The concept of an increase in extracellular glutamate concentration immediately after the aversive experience and a second increase in extracellular glutamate concentration between 10 and 25 min post-learning is strengthened by the finding that MSO must be administered before learning, whereas fluoroacetate can be given slightly later (13,52,57). MSO inhibits conversion of previously released, astrocytically accumulated glutamate to glutamine, and fluoroacetate inhibits astrocytic de novo formation of glutamine from glucose in astrocytes, and either compound alone is capable of extinguishing memory during ITM(A) (Fig. 7). Therefore, both astrocytic conversion of accumulated glutamate to glutamine and astrocytic de novo synthesis of glutamate from glucose must be essential for maintaining memory through ITM(A), probably because both processes are required for the neuronal supply of sufficient transmitter glutamate to be released during the hypothesized second stage of increased extracellular glutamate during this phase.

If the theory outlined above is correct, it should be possible to challenge the retention deficits induced by MSO by administration of extracellular glutamine—the product of the inhibited enzymatic reaction. That this is indeed the case can be seen from Fig. 7, which shows that the decreased discrimination ratio seen in the presence of MSO can be brought back to normal by intracranial administration of glutamine (52). However, facilitation of neuronal glutamate synthesis by administration of α-ketoglutarate plus alanine, an alternative precursor of neuronal glutamate, has a similar effect, as does glutamate itself (13). In contrast, neither α-ketoglutarate nor alanine alone has such an action. Along similar lines, the inhibition of memory formation brought about by fluoroacetate can be challenged by glutamine (Fig. 7). It is likely that this, again, is due to the supply of an alternative precursor for neuronal glutamate, but it cannot be excluded that

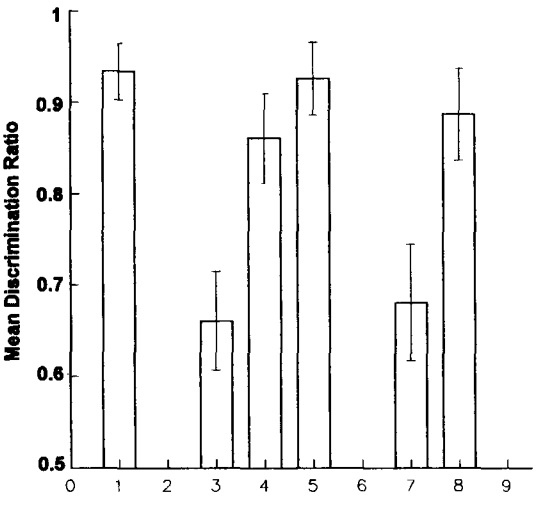

FIG. 7. Mean discrimination ratios at 2 h post-learning after injection of saline (column 1); MSO (column 3): MSO + glutamine (column 4); MSO + α-ketoglutarate plus alanine (column 5); fluoroacetate (column 7); and fluoroacetate + glutamine (column 8). Each group shows the means in a population of 15–19 birds. Discrimination ratios around 0.9 indicate normal learning and a ratio of 0.5 no learning at all. Note that both MSO and fluoroacetate impair learning and that learning can be re-established by administration of either glutamine or α-ketoglutarate plus alanine. Modified from results in Ref. (13). Vertical bars indicate SD.

glutamine, by acting as a metabolic substrate for astrocytes, partly overcomes a fluoroacetate-induced inhibition of energy production in astrocytes. However, it is against the latter interpretation that both Fonnum and co-workers and Swanson found that fluoroacetate toxicity is mainly evoked by its inhibition of synthesis of precursors for glutamate/glutamine, not by interference with astrocytic energy production (20,82). Thus, inhibition of either de novo synthesis of glutamate precursors in astrocytes or glutamine synthesis from accumulated glutamate in astrocytes is sufficient to inhibit memory formation, whereas exogenous supply of any glutamate precursor re-establishes memory in the Gibbs–Ng model. It is understandable that glutamine synthesis alone cannot supply sufficient glutamate precursor since it is only glutamate that previously has been released which is accumulated, and part of the glutamate accumulated into astrocytes is utilized as a metabolic substrate rather than converted to glutamine. The reason that de novo synthesis of α-ketoglutarate from glucose alone cannot supply sufficient glutamate may be the relatively slow production of neuronal glutamate from this precursor (62). Administration of a glutamate uptake inhibitor also abolishes memory during ITM(A) (unpublished experiments), but it is unknown whether this is due primarily to inhibition of neuronal or astrocytic glutamate uptake.

Energy Metabolism

Since glycolysis and oxidative metabolism take place in both neurons and astrocytes (27,63), it is difficult, if

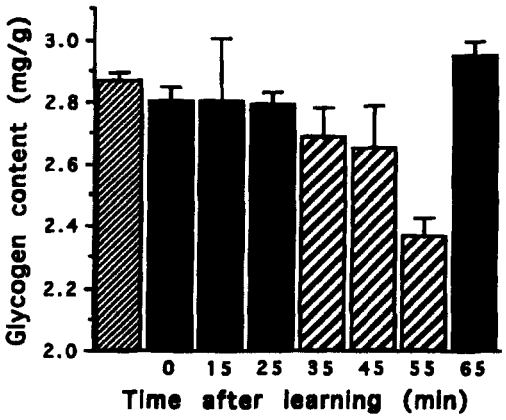

FIG. 8. Contents of glycogen in the forebrains of 1-day-old untrained chicks (▨) or in trained chicks at different time periods after one-trial passive avoidance learning (■ and ▨) in the absence of drug administration. (▨) corresponds to the ITMB stage of the Gibbs–Ng model. Results are the means of three experiments ± SEM. From Ref. (56).

not impossible, to conclude whether an abolition of memory in the presence of dinitrophenol (DNP), an inhibitor of oxidative phosphorylation, which occurs during ITM(A) (Fig. 1), is due primarily to inhibition of neuronal or of glial energy metabolism. However, it is likely that neuronal dysfunction is at least involved (which will be discussed in more detail under *Active Ion Transport*) since astrocytes survive and accumulate glutamate on glycolysis alone (35,75) whereas neurons do not survive. Iodoacetate, an inhibitor of glycolysis in both neurons and astrocytes, has no effect on ITM(A) but abolishes memory retention during ITM(B) (11,57). Correlation of memory retention with a stimulated glycolysis during this stage (73), which is insensitive to DNP (Fig. 1), may suggest that there is an active accumulation of glutamate and of potassium ions specifically or predominantly into astrocytes, which appear capable of actively accumulating these compounds utilizing glycolytically derived energy. Furthermore, the mean forebrain level of glycogen, which is confined to astrocytes, shows a reduction beginning after 30 min post-learning, reaching a nadir by 55 min and recovering rapidly to become identical to untrained levels by 65 min post-learning (Fig. 8) (56). Since glycogen is confined to glial cells, this finding indicates a net stimulation of energy metabolism which at least includes glycolysis in glial cells, although the pyruvate formed during glycogenolysis may be oxidatively degraded in both neurons and glial cells. A corresponding or even larger breakdown of glycogen may also occur immediately after learning (54). Thus, glycogen utilization occurs immediately after the time periods when we have postulated that the extracellular glutamate concentration becomes elevated (Fig. 1).

Iodoacetate extinguishes memory during ITM(B) regardless of whether it is administered immediately after learning or around 25 min post-learning (Fig. 2). These time periods also correspond to those at which indirect evidence was found for an increase in the extracellular glutamate concentration. This is in good agreement with the concept that extracellular glutamate is re-accumulated at the expense of glycolytically derived energy. However, it should be noted that interference with glutamate re-accumulation after iodoacetate administration immediately after learning does not impair memory retention until ITM(B), possibly because establishment of memory at this stage can proceed based on neuronal accumulation of glutamate, which may be energetically driven by oxidative metabolism.

Taken together, the findings discussed so far strongly suggest, but do not prove that (i) the aversive learning experience causes an initial release of glutamate; (ii) this glutamate to a large extent is accumulated into astrocytes shortly thereafter, at the expense of glycolytically derived energy, as well as into neurons; (iii) conversion of astrocytically accumulated glutamate to glutamine and de novo synthesis of glutamate in astrocytes are both essential to replenish neurons, with glutamate released immediately after learning and required again between 10 and 25 min post-learning; and (iv) this glutamate and possibly also potassium ions must again be accumulated using glycolytically derived energy, probably mainly into astrocytes. These processes start immediately after learning and are completed by 60 min post-learning, i.e., at the end of ITM(B). Interference with any of them abolishes memory, although impairment of glutamate synthesis does so at an earlier stage [ITM(A)] than impairment of astrocytic glutamate uptake [ITM(B)]. What remains unexplained is the dependence on oxidative metabolism during ITM(A). Since re-accumulation of potassium ions and re-extrusion of sodium ions account for most of the increase in energy utilization after stimulation and are partly dependent upon oxidative metabolism, probably especially in neurons (71), the possibility will be discussed below that the dependence on oxidative metabolism largely reflects re-establishment of resting ion distribution. Neuronal re-accumulation of glutamate, which occurs to some extent, may also require energy derived from oxidative metabolism.

Active Ion Transport

In the CNS in vivo, ouabain delays potassium clearance from the extracellular space and abolishes the undershoot after neuronal activity (85). Since Na^+,K^+-ATPase activity is present in both neurons and astrocytes, no conclusion about cellular involvement in memory retention can be drawn from the inhibition of memory retention during ITM(A) with ouabain. However, the probable confinement of the cotransport system to astrocytes and the ability of both furosemide (unpublished experiment) and ethacrynic acid (11), a drug with the same mechanism of action as furosemide, to abolish memory from ITM(A) and onwards (11) indicate that the cotransport system may constitute an essential component of learning processes at this stage.

The abolition of memory during ITM(A) by ethacrynic acid or furosemide might be a direct result of the reduced active accumulation of potassium ions

into astrocytes, since the resulting increase in extracellular potassium concentration, especially during high-frequency stimulation, will tend to compromise impulse conduction (85,89). In this context, we would like again to draw attention to the electrophysiological observation that dendritic action potentials occur in at least some types of neurons and also to a morphological finding that the receptors on the glutamatergic cerebellar granule cells in vivo generally are located at the very end of the relatively long dendrites (64). Such a morphological arrangement creates a situation in which electrotonic impulse conduction may not be sufficient to carry impulses to the cell body and, accordingly, there would be a need for dendritic action potentials.

Before reaching their cell bodies, the naked cerebellar granule cell dendrites traverse a neuropil containing a dense network of minute processes originating from astrocytes and from other types of neurons. Propagation of an action potential towards the cell body would be accompanied by massive potassium ion exit and sodium ion entry along the entire length of the dendrite. After transmission of a few impulses, further impulse propagation would be blocked by the resulting elevation of the extracellular potassium concentration, unless extracellular potassium ions are rapidly removed. The rate of accumulation of potassium ions into both the neurons themselves and adjacent cells may, therefore, control impulse propagation through cerebellar granule cell dendrites and thus firing frequency in these cells. The same may apply to other types of neurons. Since the most immediate response to previous stimulation is probably triggered in astrocytes (owing to the rapid elevation of the extracellular potassium concentration), astrocytic components in the neuropil may play a major role in such a regulation of neuronal activity, explaining the extinction of memory in the presence of inhibitors of the cotransport system. Active ion transport in neurons is probably also essential for memory, as reflected by the extinction of memory during ITM(A) when oxidative metabolism is inhibited by DNP. Thus, maintained neuronal impulse conduction appears to be essential for the expression of ITM(A), and this requires neuronal–astrocytic interactions in clearance of excess extracellular potassium. Another reason for the abolition of memory during ITM(A) in the presence of inhibitors of the cotransport system may be that furosemide inhibits the undershoot in the extracellular potassium concentration after neuronal excitation. Such an undershoot, in turn, may be important for the establishment of neuronal hyperpolarization, which is often envisaged to be essential during establishment of memory.

Taste stimulation in newly hatched chicks, i.e., part of the learning experiment in the Gibbs–Ng model, has been found to cause a decrease in whole-brain Na^+,K^+-ATPase activity, apparent after 10 min and lasting for several hours (17). This effect could not be observed in anaesthetized animals. The interpretation of this finding is difficult because it is unknown whether it is astrocytic or neuronal Na^+,K^+-ATPase activity which is reduced (or both), but such a decrease would be consistent with an inhibitory action of noradrenaline on neuronal Na^+,K^+-ATPase activity at elevated potassium concentrations (16).

Transmitters

It is generally accepted that noradrenaline plays a key role in the establishment of memory, and increases in noradrenaline levels were found at specific stages of the Gibbs–Ng model (7). Administration of propranolol, a β-adrenergic blocker, leads to extinction of memory during ITM(B) (11). This could be due to the abolition of a multitude of β-adrenergic effects, including stimulation of protein phosphorylation and of GAP43 phosphorylation (96) and induction of glycogenolysis. The temporal correlation with glycogenolysis makes it likely that a reduction in noradrenaline-induced glycogenolysis is a key factor, and preliminary experiments have indicated that the glycogenolysis normally occurring during ITM(B) is abolished in the presence of propranolol, a β-adrenergic receptor blocker (54).

Although β-adrenergic receptors appear mainly to be found on astrocytes, α-adrenergic noradrenaline effects could be exerted on both neurons and astrocytes. It is therefore of interest that yohimbine, an α_2-antagonist, disrupts memory during ITM(B), i.e., behaves in a similar manner to propranolol (unpublished experiments). Since most neuronal α_2 receptors are presynaptic and act by restricting noradrenaline release, yohimbine would be expected to act like an adrenergic agonist, not an antagonist, if it acted primarily on neurons. Its abolition of memory, therefore, suggests that it acts primarily on astrocytes, expressing α_2 receptors on the target cells, stimulation of which may lead to glycogenolysis.

CONCLUDING REMARKS

We believe that the present review has provided compelling evidence for an involvement of glial cells during the establishment of memory in the Gibbs–Ng model of memory formation following aversive learning in the 1-day-old chick. This is a confirmation of the visionary studies by Holger Hyden 30–35 years ago (37). The available information is presently unrefined and fragmentary and no attempt has been made in this paper to review comprehensively the Gibbs–Ng model or to discuss other memory tasks. Apart from the knowledge that sensory activity in the visual and gustatory system at the time of learning must lead to increases in the extracellular potassium concentration in specific pathways, and that the aversive experience is likely to lead to a concomitant arousal, the biological events leading up to the hypothesized activation of glutamatergic transmission during ITM(A) are completely unknown. So too are the cellular mechanisms linking activation of the glutamatergic system at this stage to the establishment of long-term memory. These may be complex and convoluted, as indicated by the finding that memory loss after glutamate receptor blockade does not occur until 90 min post-learning (52,69). These mechanisms may also not be restricted to neurons, since glutamate release from neurons

exerts profound effects also on astrocytes, including the generation of a wave of increased cytosolic calcium concentrations spreading from astrocyte to astrocyte (6,74), and since protein synthesis inhibitors which also inhibit long-term memory (11) might do this equally well by inhibiting protein synthesis in glial cells as in neurons. Further research is required to enhance our understanding of the events in both neurons and astrocytes which form the biological basis for the establishment of memory in the Gibbs–Ng memory model and to establish the general validity of these findings in other systems. In such research endeavours, it will be essential to integrate sophisticated behavioral, ultrastructural, biochemical and biophysical studies.

REFERENCES

1. Albers, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. Molecular biology of the cell, 2nd edn. New York: Garland Publishing; 1989.
2. Barres, B. A. New roles for glia. J. Neurosci. 11:3684–3695; 1991.
3. Benveniste, H.; Drejer, J.; Schousboe, A.; Diemer, N. H. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracranial microdialysis. J. Neurochem. 43:1369–1374; 1984.
4. Chen, Y.; McNeill, J. R.; Hajek, I.; Hertz, L. Effect of vasopressin on brain swelling at the cellular level—Do astrocytes exhibit a furosemide-vasopressin-sensitive mechanism for volume regulation? Can. J. Physiol. Pharmacol. 70:S219–S222; 1992.
5. Chen, Y.; Pent, L.; Zhang, X.; Stolzenburg, J. U.; Hertz, L. Further evidence that fluoxetine interacts with a 5-HT_{1C} receptor in glia cells. Brain Res. Bull. 38:153–159; 1995.
6. Cornell-Bell, A. H.; Finkbeiner, S. M.; Cooper, M. S.; Smith, S. J. Glutamate induces calcium waves in cultured astrocytes: Long-range glial signalling. Science 247:470–473; 1990.
7. Crowe, S. F.; Ng, K. T.; Gibbs, M. E. Forebrain noradrenaline concentration following weakly reinforced training. Pharmacol. Biochem. Behav. 40:173–176; 1991.
8. Cserr, H. F.; Bundgaard, M. The neural microenvironment: A comparative view. Ann. N.Y. Acad. Sci. 481:1–6; 1986.
9. Dringen, R.; Schmoll, D.; Hamprecht, B. Incorporation of radioactivity from [^{14}C] lactate into the glycogen of cultured mouse astroglial cells. Evidence for gluconeogenesis in brain cells. Biol. Chem. Hoppe Sayler, 374:343–347; 1993.
10. Engelsen, B.; Fonnum, F. The effect of methionine sulfoximine, an inhibitor of glutamine synthetase, on the levels of amino acids in the intact and decorticated rat neostriatum. Brain Res. 388:165–168; 1985.
11. Gibbs, M. E. Behavioural and pharmacological unravelling of memory formation. Neurochem. Res. 16:715–736; 1991.
12. Gibbs, M. E.; Ng, K. T. Psychobiology of memory: Towards a model of memory formation. Biobehav. Rev. 1:113–136; 1977.
13. Gibbs, M. E.; O'Dowd, B. S.; Hertz, L.; Robinson, S. R.; Sedman, G. L.; Ng, K. T. Inhibition of glutamine synthetase activity prevents memory consolidation. Brain Res. in press.
14. Grab, D.; Reisert, I.; Pilgrim, C. Volumendichte und spezifische Oberflächen neuronaler und glioser Gewebselemente in verschiedenen Regionen des Rattenhirns. Verhandl. Anat. Gesellsch. 77:255–256; 1983.
15. Grisar, T.; Frere, J.-M.; Franck, G. Effect of K^+ ions on kinetics of the Na^+K^+-ATPase (EC 3.6.1.3) of bulk isolated glial cells, perikarya and synaptosomes from rabbit brain cortex. Brain Res. 165:87–103; 1979.
16. Hajek, I.; Subbarao, K. V.; Hertz, L. Acute and chronic effects of potassium and noradrenaline on Na^+K^+-ATPase activity in cultured mouse neurons and astrocytes. Neurochem. Int. in press.
17. Hajek, I.; Sykova, E.; Sedman, G.; Ng, K. T. Na^+K^+-ATPase activity in young chicks after taste stimulation. Brain Res. Bull. 33:87–91; 1994.
18. Hamprecht, B. Energy metabolism. In: Kettenmann, H.; Ransom, B., eds. Glial cells. in press.
19. Hansson, E.; Ronnback, L. Regulation of glutamate and GABA transport by adrenoceptors in primary astroglial cell cultures. Life Sci. 44:27–34; 1988.
20. Hassel, B.; Sonnewald, U.; Unsgard, G.; Fonnum, F. NMR spectroscopy of cultured astrocytes: Effects of glutamine and the gliotoxin fluorocitrate. J. Neurochem. 62:2187–2194; 1994.
21. Henn, F. A. Separation of neuronal and glial cells and subcellular constituents. In: Fedoroff, S.; Hertz, L., eds. Advances in cellular neurobiology, vol. 1. New York: Academic Press; 1980: 373–403.
22. Henn, F. A.; Haljamae, H.; Hamberger, A. Glial cell function: Active control of extracellular K^+ concentration. Brain Res. 43:437–443; 1972.
23. Herreras, O.; Somjen, G. G. Propagation of spreading depression among dendrites and somata of the same cell population. Brain Res. 610:276–282; 1993.
24. Herreras, O.; Somjen, G. G. Analysis of potential shifts associated with recurrent spreading depression and prolonged unstable spreading depression induced by microdialysis of elevated K^+ in hippocampus of anesthetized rats. Brain Res. 610:283–294; 1993.
25. Hertz, L. Is Alzheimer's disease an anterograde degeneration, originating in the brainstem, and disrupting metabolic interactions between neurons and glial cells? Brain Res. Rev. 14:335–353; 1989.
26. Hertz, L. Autonomic control of neuronal–astrocytic interactions, regulating metabolic activities and ion fluxes in the CNS. Brain Res. Bull. 29:303–313; 1992.
27. Hertz, L.; Drejer, J.; Schousboe, A. Energy metabolism in glutamatergic neurons, GABAergic neurons and astrocytes in primary culture. Neurochem. Res. 13:605–610; 1988.
28. Hertz, L.; Hertz, T. Neuronal–glial interactions. In: Bittar, E. E.; Bittar, N., eds. Principles of medical biology. Greenwich: JAI Press. in press.
29. Hertz, L.; O'Dowd, B.; Ocherashvili, E. Glial cells. In: Ramachandran, V. S., ed. Encyclopedia of human behavior, vol. 2. Orlando, FL: Academic Press; 1994:429–439.
30. Hertz, L.; Peng, L. Effects of monoamine transmitters on neurons and astrocytes: Correlation between energy metabolism and intracellular messengers. Prog. Brain Res. 93:283–301; 1992.
31. Hertz, L.; Peng, L.; Hertz, E.; Juurlink, B. H. J.; Yu, P. H. Development of monoamine axidase activity and monoamine effects on glutamate release in cerebellar neurons and astrocytes. Neurochem. Res. 10:1039–1096; 1989.
32. Hertz, L.; Peng, L.; Westergaard, N.; Yudkoff, M.; Schousboe, A. Neuronal–astrocytic interactions in metabolism of transmitter amino acids of the glutamate family. In: Schousboe, A.; Diemer, N. N.; Kofod, H., eds. Drug research related to neuroactive amino acids. Copenhagen: Munksgaard; 1992:30–50.
33. Hoffmann, E. K. Introduction III: Cell swelling and volume regulation. Can. J. Physiol. Pharmacol. 70:S310–S313; 1992.
34. Huang, R.; Peng, L.; Chen, Y.; Hajek, I.; Zhao, S.; Hertz, L. Signalling effect of elevated potassium concentrations and monoamines on brain energy metabolism at the cellular level. Dev. Neurosci. 16:1–15; 1994.
35. Huang, R.; Shuaib, A.; Hertz, L. Glutamate uptake and glutamate content in primary cultures of mouse astrocytes during anoxia, substrate deprivation and simulated ischemia under normothermic and hypothermic conditions. Brain Res. 618:346–351; 1993.
36. Huguenard, J. R.; Hamill, O. P.; Prince, D. A. Sodium channels in dendrites of rat cortical pyramidal neurons. Proc. Natl Acad. Sci. U.S.A. 86:2475–2477; 1989.
37. Hyden, H. Behavior, neural function and RNA. Prog. Nucl. Acid. Res. 6:187–218; 1967.
38. Johnson, J. H.; Dunn, D. P.; Rosenberg, R. N. Furosemide-sensitive K^+ channel in glioma cells but not neuroblastoma cells in culture. Biochem. Biophys. Res. Commun. 109:100–105; 1982.
39. Kadekaro, M.; Crane, A. M.; Sokoloff, L. Differential effects of electrical stimulation of sciatic nerve on metabolic activity in

spinal cord and dorsal root ganglion in the rat. Proc. Natl Acad. Sci. U.S.A. 82:6010–6013; 1985.
40. Kaufman, E. E.; Driscoll, B. F. Carbon dioxide fixation in neuronal and astroglial cells in culture. J. Neurochem. 58:258–262; 1992.
41. Kauppinen, R. A.; Enkvist, K.; Holopainen, I.; Akerman, K. E. Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. Neuroscience 26:283–289; 1988.
42. Keyser, D. O.; Pelmar, T. C. Synaptic transmission in the hippocampus: Critical role for glial cells. Glia 10:237–243; 1994.
43. Kimelberg, H. K., ed. Glial cell receptors. New York: Raven Press; 1988.
44. Laming, P. R. Do glia contribute to behaviour? A neuromodulatory review. Comp. Biochem. Physiol. 94A:555–568; 1989.
45. Lee, K. M.; Rosa, M. G. P.; Schmid, L. M.; Robinson, S. R. Ocular dominance shifts in cat visual cortex following selective blockade of glial glutamine in the retina. Proc. Austr. Neurosci. Soc. 5:165; 1994.
46. Maura, G.; Carbone, R.; Guido, M.; Pestarino, M.; Raiteri, M. 5-HT_2 presynaptic receptors mediate inhibition of glutamate release from cerebellar mossy fibre terminals. Eur. J. Pharmacol. 202:185–190; 1991.
47. Mercado, R.; Hernandez, J. Regulatory role of a neurotransmitter (5-HT) on glial Na^+K^+-ATPase in the rat brain. Neurochem. Int. 21:119–127; 1992.
48. Murphy, S.; Pearce, B. Functional receptors for neurotransmitters on glial cells. Neurosci. 22:381–394; 1987.
49. Ng, K. T.; Gibbs, M. E. Stages of memory formation: A review. In: Andrew, R. J., ed. Neural and behavioural plasticity: The use of the domestic chick as a model. Oxford: Oxford University Press; 1991:351–269.
50. Ng, K. T.; Gibbs, M. E.; Crowe, S. F.; Sedman, G. L.; Hua, F.; Zhao, W.; O'Dowd, B.; Rickard, N.; Gibbs, C. L.; Sykov, E.; Svoboda, J.; Jendelova, P. Molecular mechanisms of memory formation. Mol. Neurobiol. 5:333–350; 1992.
51. Ng, K. T.; Gibbs, M. E.; Gibbs, C. L.; Sedman, G.; Svoboda, J.; Jendelova, P.; O'Dowd, B.; Rickard, N.; Crowe, S. F. Ion involvement in memory formation: The potential role of astrocytes. Prog. Brain Res. 94:109–115; 1992.
52. Ng, K. T.; Rickard, N. S.; O'Dowd, B. S.; Zhao, W.-Q.; Gibbs, M. E.; Sedman, G. L.; Robinson, S. R.; Hertz, L. Complex roles of glutamate in the Gibbs–Ng model of one-trial aversive learning in the new-born chick. Brain Res. Bull.; in press.
53. Norenberg, M. D.; Martinez-Hernandez, A. Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res. 161:303–310; 1979.
54. O'Dowd, B. S. Metabolic activity in astrocytes essential for the consolidation of one-trial passive avoidance memory in the day-old chick. Ph.D. Thesis, La Trobe University; 1995.
55. O'Dowd, B. S.; Barrington, J., Ng, K. T., Hertz, E., Hertz, L. Glycogenolytic response of primary chick and mouse cultures of astrocytes to noradrenaline across development. Dev. Brain Res.; 88:220–223; 1995.
56. O'Dowd, B. S.; Gibbs, M. E.; Ng, K. T.; Hertz, E.; Hertz, L. Astrocytic glycogenolysis energizes memory processes in neonate chicks. Dev. Brain Res. 78:137–141; 1994.
57. O'Dowd, B. S.; Gibbs, M. E.; Sedman, G. L.; Ng, K. T. Astrocytes implicated in the energizing of intermediate memory processes in neonate chicks. Cogn. Brain Res. 2:93–102; 1994.
58. O'Grady, S. M.; Palfrey, H. C.; Field, M. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. Cell Physiol. 22:C177–C192; 1987.
59. Palay, S. L.; Chan-Palay, V. Cerebellar cortex: Cytology and organization. New York: Springer; 1974.
60. Peng, L. Metabolic trafficking between neurons and astrocytes. Ph.D. Thesis, University of Saskatchewan; 1994.
61. Peng, L.; Hertz, L. Potassium-induced stimulation of oxidative metabolism of glucose in cultures of normal cerebellar granule cells but not in corresponding cells with dendritic degeneration. Brain Res. 629:331–334; 1993.
62. Peng, L. A.; Schousboe, A.; Hertz, L. Utilization of alpha-ketoglutarate as a precursor for transmitter glutamate in cultured cerebellar granule cells. Neurochem. Res. 16:29–34; 1991.
63. Peng, L.; Zhang, X.; Hertz, L. Elevation of the extracellular K^+ concentration stimulates oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes but has little effect in a GABAergic neuronal culture. Brain Res. 63:168–172; 1994.
64. Peters, A.; Palay, S. L.; Webster, H. deF. The fine structure of the nervous system. Neurons and their supporting cells, 3rd edn. New York: Oxford University Press; 1991:1–494.
65. Pope, A. Neuroglia: Quantitative aspects. In: Schoffeniels, E.; Franck, G.; Hertz, L.; Tower, D. B., eds. Dynamic properties of glia cells. Oxford: Pergamon Press; 1978:13–20.
66. Pow, D. V.; Robinson, S. R. Glutamate in some retinal neurons is derived solely from glia. J. Neurosci. 60:355–366; 1994.
67. Rauen, T.; Jeserich, G.; Danbolt, N. G.; Kanner, B. I. Comparative analysis of sodium-dependent L-glutamate transport of synaptosomal and astroglial membrane vesicles from mouse cortex. FEBS Lett. 312:15–20; 1992.
68. Rickard, N. S. Glutamate receptor-mediated processing in memory consolidation in the day-old chick. Ph.D. Thesis, LaTrobe University; 1995.
69. Rickard, N. S.; Poot, A. C.; Gibbs, M. E.; Ng, K. T. Both non-MNDA and NMDA glutamate receptors are necessary for memory consolidation in the day-old chick. Behav. Neurol. Biol. 62:33–40; 1994.
70. Roitbak, A. I. Glia and its role in nervous activity (in Russian). Moscow: Nauka; 1993:1–392.
71. Rosenthal, K.; Sick, T. J. Glycolytic and oxidative metabolic contributions to potassium ion transport in rat cerebral cortex. Can. J. Physiol. Pharmacol. 70:165–169; 1992.
72. Schousboe, A.; Westergaard, N.; Sonnewald, U.; Petersen, S. B.; Yu, A. C. H.; Hertz, L. Regulatory role of astrocytes for neuronal biosynthesis and homeostasis of glutamate and GABA. Prog. Brain Res. 94:199–212. 1992.
73. Sedman, G.; O'Dowd, B.; Rickard, N.; Gibbs, M. E.; Ng, K. T. Brain metabolic activity associated with long-term memory consolidation. Mol. Neurobiol. 5:351–354; 1992.
74. Smith, S. J. Do astrocytes process neural information? Prog. Brain Res. 94:119–136; 1992.
75. Sochocka, E.; Juurlink, B. H.; Code, W. E.; Hertz, V.; Peng, L.; Hertz, L. Cell death in primary cultures of mouse neurons and astrocytes during exposure to and 'recovery' from hypoxia, substrate deprivation and simulated ischemia. Brain Res. 638:21–28; 1994.
76. Sokoloff, L. The brain as a chemical machine. In: Yu, A. C. H.; Hertz, L.; Norenberg, M. D.; Sykova, E.; Waxman, S. G., eds. Progress in brain research, vol. 94. Amsterdam: Elsevier; 1992:19–33.
77. Sonnewald, U.; Westergaard, N.; Hassel, B.; Muller, T. B.; Unsgard, G.; Fonnum, F.; Hertz, L.; Schousboe, A.; Petersen, S. B. NMR spectroscopic studies of ^{13}C-acetate and ^{13}C-glucose metabolism in neocortical astrocytes: Evidence for mitochondrial heterogeneity. Dev. Neurosci. 15:351–357; 1993.
78. Sorg, O.; Magistretti, P. J. Characterisation of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in cultures of mouse cerebral cortex astrocytes. Brain Res. 563:227–233; 1991.
79. Stone, E. A.; Ariano, M. A. Are glial cells targets of the central noradrenergic system? A review of the evidence. Brain Res. 14:297–309; 1989.
80. Stuart, G. J.; Sakmann, B. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. Nature 367:69–72; 1994.
81. Subbarao, K. V.; Hertz, L. Effects of adrenergic agonists on glycogenolysis in primary cultures of astrocytes. Brain Res. 536:220–226; 1990.
82. Swanson, R. A.; Graham, S. H. Fluorocitrate and fluoroacetate effects on astrocyte metabolism in vitro. Brain Res. 664:94–100; 1994.
83. Sweadner, K. J. Overlapping and diverse distribution of Na-K ATPase isozymes in neurons and glia. Can. J. Physiol. Pharmacol. 70:S255–S259; 1992.
84. Sweadner, K. J. Na^+K^+-ATPase. In: Kettenmann, H.; Ransom, B., eds. Glial cells. In press.
85. Syková, E. Extracellular K^+ accumulation in the central nervous system. Prog. Biophys. Mol. Biol. 42:135–189; 1983.
86. Syková, E. Ionic and volume changes in the microenvironment of nerve and receptor cells. In: Autrum, H.; Ottoson, D.; Perl, E. R.; Schmidt, R. F.; Shimazu, H.; Willis, W. D., eds. Progress in sensory physiology, vol. 13. New York: Springer; 1992:1–162.
87. Syková, E.; Jendelova, P.; Svoboda, J.; Sedman, G.; Ng, K. T.

Activity-related rise in extracellular potassium concentration in the brain of 1–3-day-old chicks. Brain Res. Bull. 24:569–575; 1990.
88. Walz, W. Role of Na/K/Cl cotransport in astrocytes. Can. J. Physiol. Pharmacol. 70:S260–S262; 1992.
89. Walz, W.; Hertz, L. Functional interactions between neurons and astrocytes. II. Potassium homeostasis at the cellular level. Prog. Neurobiol. 20:133–183; 1983.
90. Westergaard, N.; Sonnewald, U.; Schousboe, A. Release of α-KG, malate and succinate from cultured astrocytes: Possible role in amino acid neurotransmitter homeostasis. Neurosci. Lett. 176:105–109; 1994.
91. Westergaard, N.; Varming, T.; Peng, L.; Sonnewald, U.; Hertz, L.; Schousboe, A. Uptake, release, and metabolism of alanine in neurons and astrocytes in primary cultures. J. Neurosci. Res. 35:540–545; 1993.
92. White, H. S.; Chow, S. Y. C.; Yen-Chow, Y. C.; Woodbury, D. M. Effects of potassium on the ion content of mouse astrocytes and neurons. Can. J. Physiol. Pharmacol. 70:S263–S268; 1992.
93. Wolff, J. R. Quantitative aspects of astroglia. VIth Int. Congress Neuropathol. 727–733; 1970.
94. Yudkoff, M.; Nissim, I.; Hertz, L.; Pleasure, D.; Erecinska, M. Nitrogen metabolism: Neuronal–astroglial relationships. Prog. Brain Res. 94:213–224; 1992.
95. Zetterstrom, T.; Vernet, L.; Ungerstedt, U.; Tossman, U.; Johzom, B.; Fredholm, B. B. Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci. Lett. 29:111–115; 1982.
96. Zhao, W.-Q.; Ng, K. T.; Sedman, G. L. Passive avoidance learning induced change in GAP43 phosphorylation in day-old chicks. Brain Res. Bull. 36:11–17; 1994.