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CHAPTER 9

Ion involvement in memory formation: the potential role of astrocytes

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Introduction

In recent years, we have suggested that neuronal hyperpolarization as a consequence of K^+ conductance change (Jansen and Nicholls, 1973) may be involved in memory processing (Gibbs and Ng, 1977; Ng and Gibbs, 1988). Our hypotheses represented an explicit attempt to link memory processes with ionic activities in neuronal assemblies (Gibbs et al., 1977, 1979). Of particular interest to us is the possibility that astroglia may play a role in these processes.

Increases in K^+ conductance of activated neurones and primary afferent fibres can lead to elevation in $[K^+]_e$ (Nicholson, 1980; Syková, 1983). $[K^+]_e$ changes, as measured by K^+-sensitive microeletrodes, provide a good quantitative index of stimulation-evoked activation of neuronal populations. Such variances in $[K^+]_e$ will lead to depolarization of neurones and glia, and can produce long-term changes in neuronal excitability, synaptic transmission and glia cell function by altering neuronal and glial cell membrane potentials and transmitter release (Orkand, 1977; Nicholson, 1980; Syková, 1983, 1987), as well as triggering cell swelling and shrinkage of the extracellular space (Trubatch et al., 1977; Dietzel et al., 1980; 1982; Syková, 1987).

The ability of glial cells to regulate the extracellular environment of neurones is well-recognized. These cells are in a significant position to not only modulate neuronal excitability per se, but also to supervise and modulate synaptic transmission and neuronal connectivity (Somjen, 1975; Hertz, 1989; Hansson and Rönnbäck, 1990). It is reasonable to suppose, therefore, that glia may play a role in neuronal processing of information associated with learning and memory. As yet there is no convincing direct evidence linking glial activity to learning and memory. In this paper we present some previously published (Gibbs et al., 1977, 1979) findings from pharmaco-behavioural studies, the explanation of which may require the postulation of a role for the glia. The viability of our hypotheses, but not their validity, rests on at least one demonstration that adequate stimuli associated with our learning task give rise to alterations in the ionic state surrounding cells in brain areas shown to be activated specifically during memory processing. We present some recently reported (Syková et al., 1990) and some unpublished data relevant to this issue.

Results and discussion

One to two-day-old domestic chicks trained on a single trial passive avoidance task exhibit at least

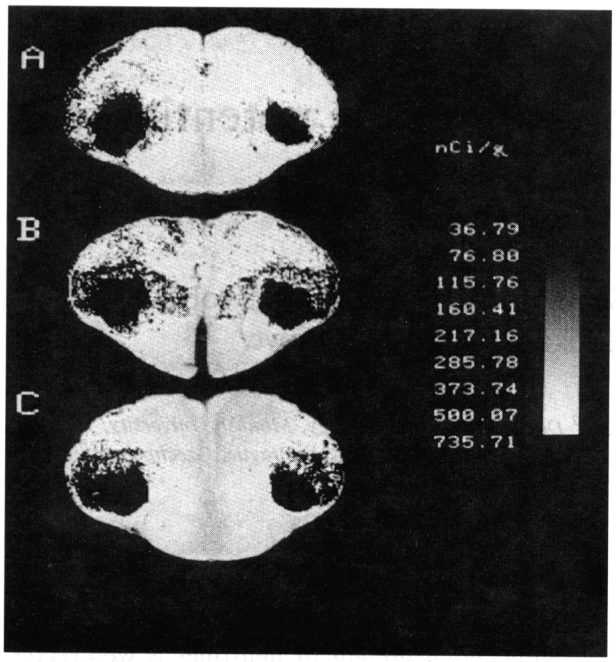

Fig. 1. Normalized images of [^{14}C]2-deoxyglucose autoradiographs of chick brain sections. *A*. Section from a chick that received monocular visual stimulation. Note that the area of greatest metabolic activity corresponds to the ectostriatum contralateral to the eye stimulated. *B,C*. Representative brain sections from a trained and untrained chick, respectively. The bar to the right of the images provides calibration for all three images. (From Syková et al., 1990).

three sequentially dependent stages in the formation of memory for this learned experience (Gibbs and Ng, 1977). The chicks are trained to avoid a small chrome or coloured glass bead by coating the bead with a chemical adversant, methylanthranilate (MeA). Using labelled 2-deoxyglucose autoradiography we are able to identify those regions of the brain which show enhanced metabolic activity during the memory formation process. As exemplified in Fig. 1, areas showing significant increases in labelling are circumscribed by the neostriatal-hyperstriatal complex of the chick forebrain (see also Kossut and Rose, 1984). In untrained anaesthetized chicks, stimulation of the beak with MeA results in a substantial rise in $[K_+]_e$ in this region of the brain (Fig. 2; Syková et al., 1990). 2-DG is to some extent incorporated into glycogen (Pentreath, 1982). In rat and mouse brain, glycogen and glycogenolysis appear to be localized primarily in the glia; in rat, mouse, chick and several other species, brain glycogenolysis is activated by neuronally released NA, stimulating β-receptors, with the effect possibly mediated by cAMP (see Stone and Ariano, 1989). If, as is suspected (Hertz, 1989; Stone and Ariano, 1989), β-receptors and cAMP responses are also preferentially localized in glia, then these cells may play some role in underwriting the high energy demand in areas of the brain associated with memory processing.

Intracranial administration, in the above chick forebrain regions, of pharmacological agents known to inhibit or enhance selected brain cell functions yields the picture of memory organization depicted in Fig. 3 (see Gibbs and Ng, 1977; Ng and Gibbs, 1988). Behavioural studies have confirmed this picture (Gibbs and Ng, 1979). Three sequential-

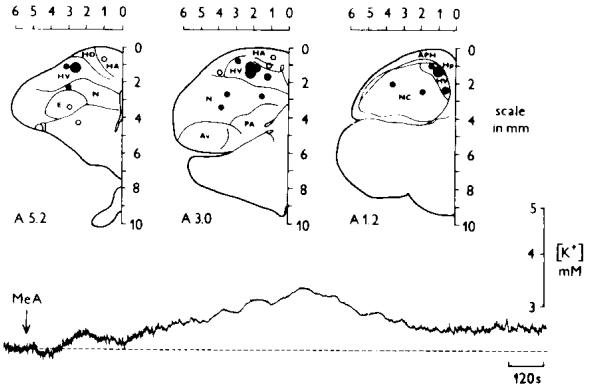

Fig. 2. Increase in $[K^+]_e$ evoked by 0.1 ml of methylanthranilate applied onto the tongue. Recordings are from 22 chicks at the areas marked either by open circles (no response) or by dots (increase in $[K^+]_e$) at about 1.2, 3.0 and 5.2 mm proximally from the ear bar axis. Largest dots mark the increase in $[K^+]_e$ by 0.70 – 1.25 mmol/l, medium-sized dots the changes by 0.35 – 0.50 mmol/l and smallest dots by 0.15 – 0.30 mmol/l K^+. Bottom record: a typical increase in $[K^+]_e$ after MeA in the Hyperstriatum ventrale (HV). Note the spontaneous variations in $[K^+]_e$ and their disappearance during a rise in $[K^+]_e$. HD, hyperstriatum dorsale; HA, hyperstriatum accessorium; N, neostriatum; E, ectostriatum; NC, neostriatum caudale; PA, paleostriatum accessorium; Av, archistriatum pars ventralis. (From Syková et al., 1990.)

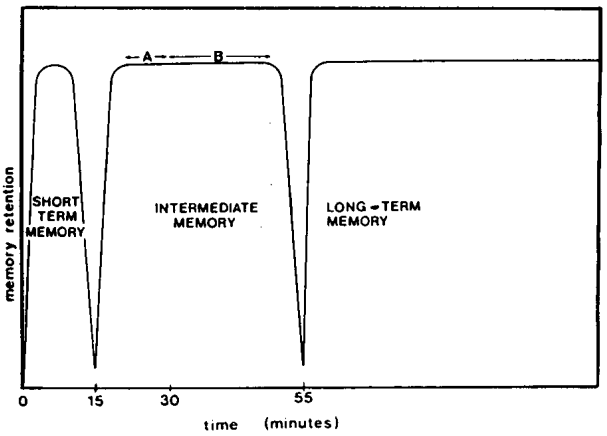

Fig. 3. A schematic model of stages in memory formation. (From Gibbs and Ng, 1984.)

ly dependent stages are postulated: short-term (STM), intermediate (ITM), and long-term (LTM). ITM itself appears to consist of two phases: *A* and *B* (Fig. 3). The STM and ITM stages are relevant here. STM formation is blocked by 4 mM monosodium glutamate (Fig. 4), and by the calcium-channel blocker, lanthanum chloride; it is enhanced in a dose-dependent manner by increases in extracellular Ca^{2+} (Fig. 5). ITM formation, on the other hand, is prevented by the cardiac glycoside, ouabain, and ethacrynic acid, both known sodium pump inhibitors (Fig. 4), leaving STM intact. It is enhanced by pump stimulators, including diphenylhydantion and NA. LTM formation is abolished by protein synthesis inhibitors such as the antibiotic cycloheximide (CXM). We have suggested (Gibbs et al., 1977, 1979) that formation of both STM and ITM involves neuronal hyper-

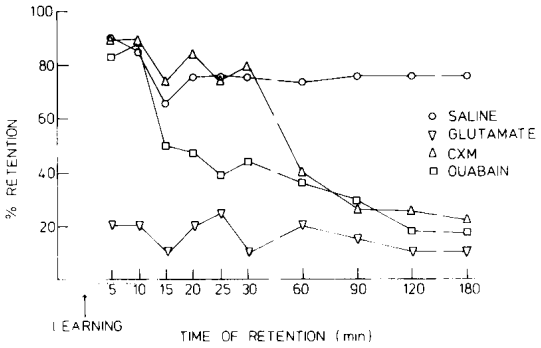

Fig. 4. Retention functions for chicks trained with a single trial passive avoidance task and treated intracranially with various pharmacological agents 5 min before training trial. (From Gibbs and Ng, 1977.)

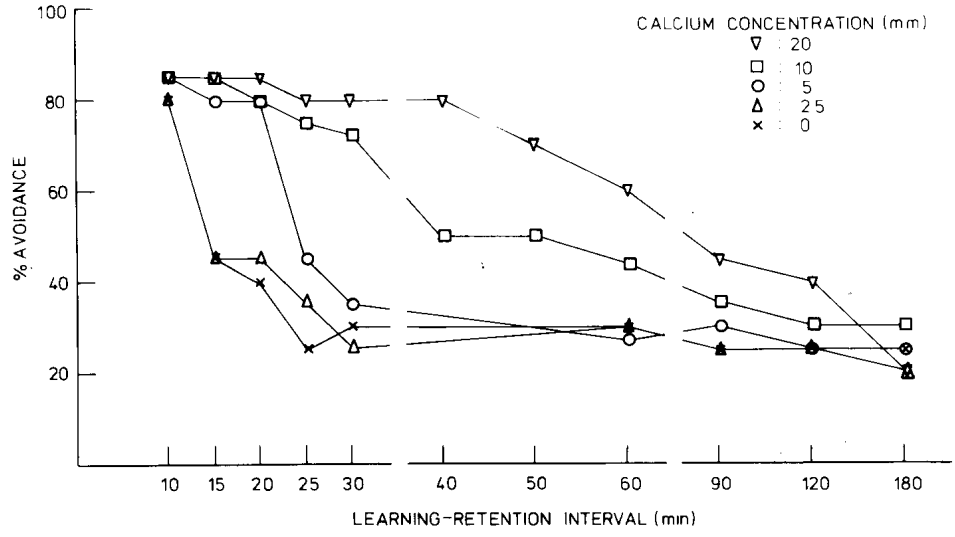

Fig. 5. Effect on retention functions of varying concentrations of CaCl administered intracranially immediately after the training trial to chicks pretreated with an intracranial injection of ouabain. (From Gibbs et al., 1979.)

polarization, the former arising from an activity-induced increase in K^+ permeability, and the latter from activity of an electrogenic sodium pump. These two mechanisms underlying post-tetanic hyperpolarization have been identified in leech neurones (Jansen and Nicholls, 1973). Whether they exist in the chick is yet to be established.

Substantial and patterned effects on these two stages of memory occur with changes to ex-

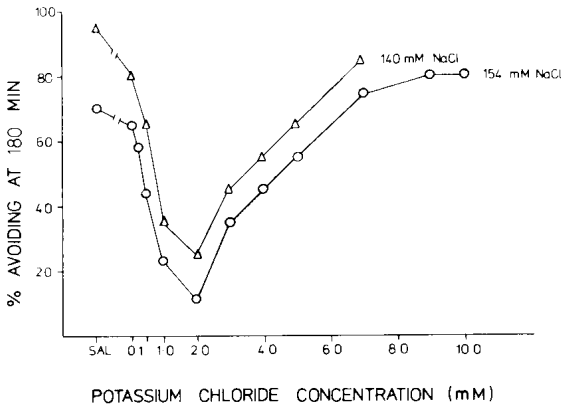

Fig. 6. Effect of various concentrations of KCl administered intracranially 5 min before training on retention levels tested 180 min after training. The KCl was prepared in either 140 mM NaCl or 154 mM NaCl solutions. (From Gibbs et al., 1977.)

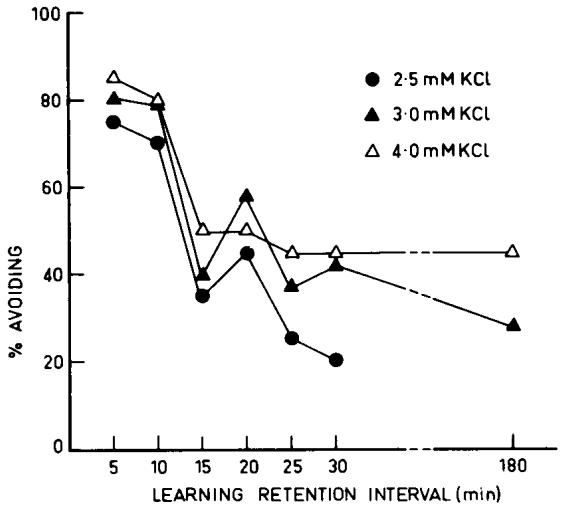

Fig. 7. Retention functions following 2.5 mM, 3.0 mM or 4.0 mM KCl administered intracranially 5 min before the learning trial. (From Gibbs et al., 1977.)

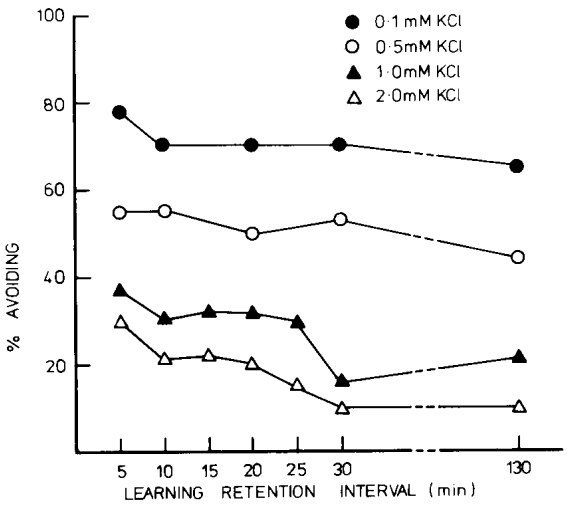

Fig. 8. Effects on retention functions of various concentrations (0.1 – 2.0 mM) of KCl administered intracranially 5 min before the learning trial. (From Gibbs et al., 1977.)

tracellular K^+ concentrations, brought about by intracranial administration of relatively low concentrations of KCl in 10 μl volumes (Gibbs et al., 1977). In the range 0.1 – 10 mM KCl, an inverted U-shaped dose – response function is observed in retention levels measured 180 min after learning (Fig. 6). Thus, KCl concentrations of 1 – 5 mM clearly induce amnesia, while 7 mM KCl and concentrations below 1 mM have little effect.

Equally significant is the fact that 2.5 – 5 mM KCl abolishes ITM but leaves STM intact (Fig. 7), a result similar in all respects to that observed with ouabain. However, 1 – 2 mM KCl abolishes STM as well (Fig. 8), in like manner to the effects of 4 mM monosodium glutamate. These results are somewhat puzzling, given that we have established $[K^+]_e$ in 1 – 3-day-old chicks to be about 3.5 mM in the regions of the brain being investigated (Syková et al., 1990).

Both post-tetanic hyperpolarization and neuronal Na^+/K^+-ATPase activity have been shown to be sigmoidally dependent on $[K^+]_e$ in garfish olfactory nerve (McDougal and Osborn, 1976). A similar sigmoidal relationship between the ouabain-sensitive Na^+ flux and $[K^+]_e$ in the range of 0 – 6 mM

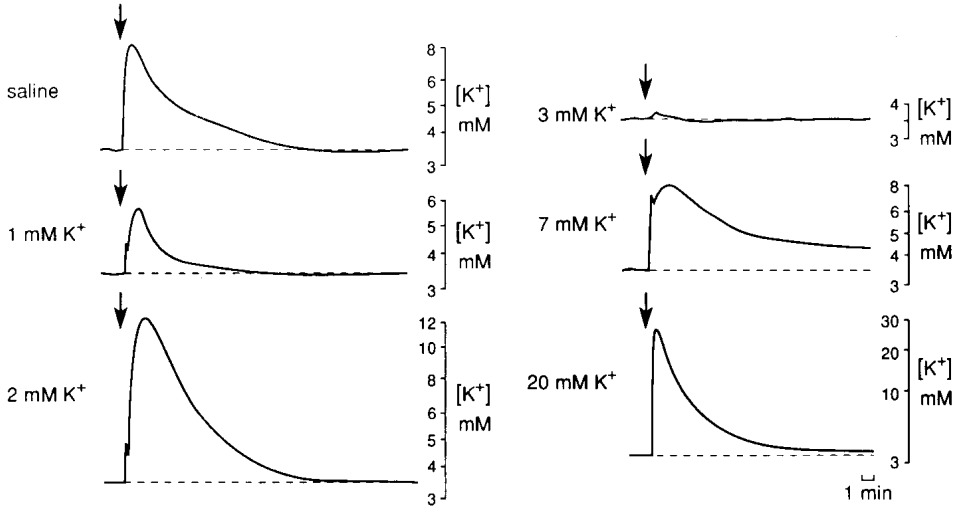

Fig. 9. Extracellular K^+ concentration in the medial neostriatum of untrained anaesthetized 1 – 3 day-old chicks following local application of 0 – 7 mM KCl.

applies to the squid axon (Baker et al., 1969). If such a dependence also occurs in the chick, it is possible that injections of 2.5 – 5 mM KCl may yield $[K^+]_e$ levels which abolish sodium pump activity and hence ITM. At these $[K^+]_e$ levels, however, K^+ conductance-dependent hyperpolarization must remain intact since STM is unaffected.

If K^+ conductance-dependent neuronal hyperpolarization underlies formation of STM, then administration of 1 – 2 mM KCl must somehow abolish this phase of hyperpolarization. In the leech, the glia are an efficient K^+ electrode for $[K^+]_e$ as low as 1 mM and hyperpolarize noticeably to $[K^+]_e$ as low as 0.3 mM (Kuffler et al., 1966). It is possible that administration of KCl at 2 mM concentrations or less may lead to extracellular K^+ concentrations which hyperpolarize the glia. This may prevent the glia from functioning effectively as a potassium sink or from active uptake of K^+ to equalize $[K^+]_e$ across various regions of the intercellular cleft system. Localized accumulation of K^+ in the intercellular space following neural activity may, under these conditions, greatly exceed normal $[K^+]_e$ levels and abolish the neuronal hyperpolarization postulated to underly STM.

Why then do injections of 0 – 0.5 mM KCl not yield loss of either ITM or STM? There is some evidence to suggest that at $[K^+]_e$ below 1 – 2 mM, the resting membrane potential of neurones is insensitive to changes in $[K^+]_e$ (Huxley and Stämpfli, 1951). The resting potential decreases to – 75 mV and further reductions in $[K^+]_e$ are without effect. In these circumstances, a burst of afferent input may lead to an increase in membrane potassium conductance leading to a period of hyperpolarization. Nor, in these conditions, would the neuronal sodium pump be electrically disadvantaged.

The above explanations of our results are highly speculative, as are the hypotheses regarding the role of K^+ conductance and sodium pump-mediated phases of hyperpolarization. We have direct evidence that in the neostriatal region of the brain of untrained, anaesthetized 1 – 3-day-old chicks, local application of 0 – 7 mM KCl in 1 – 2 μl volumes yields significant increases in $[K^+]_e$, with the notable exception of 3 mM KCl (Fig. 9). A similar result is observed with 4 mM monosodium glutamate (Fig. 10). We are as yet unable to reconcile these effects with the behavioural findings. Furthermore, preliminary results from our laboratories suggest that, following adequate stimulation, the glia of chicks do not show significant acid shifts until about

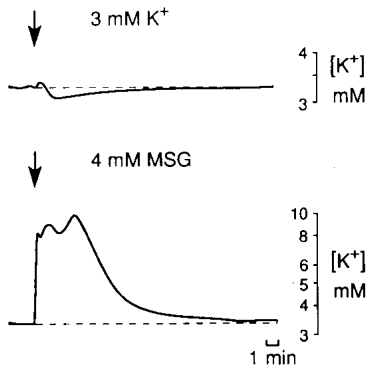

Fig. 10. Extracellular K^+ concentration in the medial neostriatum of untrained anaesthetized 1 – 3 day-old chicks following local administration of 3 mM KCl or 4 mM monosodium glutamate (MSG).

11 days of age. Any attempt to implicate glia-neuron interactions in the early stages of memory processing needs to be cognizant of the fact that the glia may be immature in 1 – 2-day-old chicks, which are nonetheless highly efficient in acquiring and retaining complex associative information of the type required in the learning task used.

Alternatively, it may be that stimulation-evoked ionic changes associated with extracellular alkaline-acid shifts may not be relevant to memory processing. Equally, however, the fact that these animals are precocially prepared for such learning experiences needs to be kept in mind. The possibility that maturation of the glia in brain regions specialized for memory processing may be stimulated by the learning experience itself merits investigation. With respect to the latter possibility, in the primary visual cortex of the cat, glia responses to stimulation show a preference for stimulation by optical edges of a given tilt, consonant with the specialization of neurones in this region (Kelly and Van Essen, 1974). A similar synergism of glial and neuronal responses in memory processing should not, therefore be dismissed.

Finally, we have evidence to suggest that triggering of protein synthesis-dependent long-term memory consolidation occurs during the ITM stage and may be initiated by the release of neuronal NA, with resulting mediation from cAMP (Gibbs and Ng, 1984; Crowe et al., 1990, 1991a,b). Blockade of β-receptors abolishes this aspect of memory processing. If, in the neostriatal-hyperstriatal complex of the chick brain, β-receptors are preferentially localized in glial cells, as appears to be the case in mammalian forebrain, then the glia may play a more direct role in regulating the hormonal effects of reinforcement in learning.

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