Molecular Neurobiology

ISSN0893-7648/92/5/2-3-4/333–350/$3.60

Molecular Mechanisms of Memory Formation

K. T. Ng,[1] M. E. Gibbs,[1] S. F. Crowe,[1] G. L.. Sedman,[1] F. Hua,[1] W. Zhao,[1] B. O'Dowd,[1] N. Rickard,[1] C. L. Gibbs,[2] E. Syková,[3] J. Svoboda,[3] and P. Jendelová [3]*

[1]*Department of Psychology, La Trobe University, Australia;* [2]*Department of Physiology, Monash University, Australia; and* [3]*Laboratory of Cellular Neurophysiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

Contents

Abstract
Introduction
Model of Memory Formation
Mechanisms Underlying Memory Stages
Mechanisms Triggering Long-Term Consolidation
Possible Involvement of Astroglia
Conclusion
Acknowledgments
References

Abstract

Studies with neonate chicks, trained on a passive avoidance task, suggest that at least two shorter-term memory stages precede long-term, protein synthesis-dependent memory consolidation. Posttetanic neuronal hyperpolarization arising from two distinct mechanisms is postulated to underlie formation of these two early memory stages. Maintenance of the second of these stages may involve a prolonged period of hyperpolarization brought about by phosphorylation of particular proteins. A triggering mechanism for long-term consolidation is postulated to occur at a specific time during the second stage, and may involve reinforcement-contingent release of neuronal noradrenaline stimulating cAMP-dependent intracellular processes. The possibility that astroglia may have a critical role to play in these early stages of memory processing is raised.

Index Entries: Memory stages; neuronal hyperpolarization; noradrenaline; cAMP; neuroglia; phosphorylation; 2-deoxyglucose; day-old chicks; passive avoidance learning; triggering of long-term consolidation.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

The view that memory must involve some kind of relatively permanent change in synaptic efficacy, expressed nearly a century ago by Cajal (1911; *see also* Hebb, 1949; Eccles, 1953), is one on which there is considerable consensus. There has been no shortage of hypotheses about what this change may be and the mechanisms underlying it. There appears to be no doubt that protein synthesis processes are involved (*see* Dunn, 1980), but just what these processes are remains uncertain. Both phosphoproteins and glycoproteins have been implicated, the former in shorter-term and the latter in longer-term memory stages (Routtenberg, 1979,1986). Neurotransmitter and neurohormonal activation of second messenger systems, such as cAMP (Kandel and Schwartz, 1982; Dash et al., 1990), impacting on membrane structures and functions via the activity of protein kinases (Acousta-Uriquidè et al., 1984) and proteases (Lynch and Baudry, 1984), has been observed within the context of learning and memory. However, the extent to which they are specifically learning-induced events associated with memory consolidation *per se*, and not simply part of general neuronal processes following any neural input, remains to be convincingly demonstrated. In recent years, the discovery (Bliss and Lomo, 1973) and elaboration (*see* Nicoll et al., 1988) of the phenomena of long-term potentiation and long-term depression have been received with justified enthusiasm, representing as they do evidence that relatively longer-term alterations in the response capabilities of synapses may follow repeated afferent inputs of the sort that might be reasonably expected to occur during a learning experience. The fact that the phenomena appear to be restricted to specific areas of the brain, some of which, like the mammalian hippocampus (Reyman et al., 1988), have been directly associated with memory processing, is particularly encouraging. Nonetheless, instances of the phenomena so far isolated show that both long-term potentiation and long-term depression do decay with time. The synaptic modifications associated with them cannot, therefore, by themselves be substrates for permanent memory consolidation.

The development of an integrated picture of the cellular events underlying formation of memory is daunting and a long way off, given the present kaleidoscope of findings. The task is made more difficult by the variety of animal species and learning tasks used. A systematic study of one species and one task, such as attempted by Kandel and his coworkers (Goelet et al., 1986) suffers from problems of valid cross-species and crosstask generalizations, but, nevertheless, provides a more stable framework within which research findings can be interpreted and integrated. In this article, we present a summary of observations and conclusions derived from behavioral, anatomical, pharmacological, neurochemical, and neurophysiological studies carried out over a period of some 15 years on young (1–3-d-old) domestic chicks trained on a single-trial, passive avoidance task. On the basis of these studies, we have developed a model of memory processing with particular emphasis on metabolic and physiological events in the vertebrate central nervous system that may be correlated with stages and phases postulated to underlie memory formation (Gibbs and Ng, 1977; Ng and Gibbs, 1991). The model and our behavioral paradigm have provided the framework for some of the work carried out by Rosenzweig and his colleagues (*see*, for example, Patterson et al., 1986) and by Rose and his coworkers (*see*, for example, Rose, 1991; Rose and Jork, 1987). In earlier work, the chicks were trained to avoid a small chrome bead made aversive with the chemical aversant methyl anthranilate (MeA), a task devised by Cherkin (1971). We subsequently developed a discrimination task in which a small colored glass bead (usually red) is made aversive, and discriminated against a nonaversive bead (usually blue) (Gibbs and Ng, 1977). Consequently, retention measures vary across studies (*see* Ng and Gibbs, 1991).

Model of Memory Formation

Behavioral observations and pharmacological manipulations (administered intracranially, unless otherwise stated) yield a convergence of results, suggesting at least three behaviorally significant, sequentially dependent stages in the development of a more or less permanent memory trace. This is represented schematically in Fig. 1 (Gibbs and Ng, 1984).

A long-term, antibiotic-sensitive and presumably protein synthesis-dependent stage (LTM) is preceded by two shorter-term stages: intermediate memory (ITM) and short-term memory (STM). This broad picture has been replicated by Patterson et al. (1986). The ITM stage appears to consist of two phases: ITM (A) and ITM (B). We have reason to believe that neuronal events occurring in the transition from ITM (A) to ITM (B) trigger the final sequence of cellular activities that give rise to LTM (Gibbs and Ng, 1984). Our interest in this article is focused on the mechanisms and processes leading up to the formation of LTM.

Mechanisms Underlying Memory Stages

Although the long-term memory stage is inhibited by antibiotics, such as cycloheximide (CXM) (Fig. 2) and anisomycin, these drugs leave memory intact for some 50 min after learning. The cardiac glycoside ouabain induces severe retention deficits after 10 min postlearning, the deficits persisting for up to at least 24 hours. The residual STM stage is blocked by low-dose (4 m*M*) monosodium glutamate and other pharmacological treatments sharing the common action of depolarizing neurons. Interestingly, we have found GABA to have no effect, whereas 1 m*M* bicuculline also abolishes STM and all subsequent stages. We have hypothesized that ITM formation may involve a phase of neuronal hyperpolarization brought about by the activities of an electrogenic sodium pump, whereas STM formation entails an earlier phase of neuronal hyperpolarization arising from an activity-induced increase in K^+ permeability (Gibbs et al., 1978).

The contribution of these two distinct mechanisms to posttetanic hyperpolarization has been demonstrated in the leech ganglia (Jansen and Nicholls, 1973), and, in our case, is consistent with our findings that (1) the inhibitory effects of ouabain on ITM are prevented by a concentration of diphenylhydantoin demonstrated to stimulate Na^+/K^+ ATP/ase activity (Gibbs and Ng, 1977), and (2) STM is susceptible to blockade by the calcium-channel blocker, lanthanum chloride, and is enhanced in a dose-dependent manner by increases in extracellular Ca^{2+} levels in the presence of ouabain (Fig. 3; Gibbs et al., 1979).

A reasonably prolonged period of neuronal hyperpolarization, such as that postulated here, may insulate the memory-relevant neuronal network from further disruptive activation by inputs too different from the learning-related stimuli and allow the relatively protracted structural changes underlying longer-term consolidation to be carried out.

Of particular relevance is the observation that KCl in the concentration range 0.5 to about 5 m*M* induces a significant reduction in retention levels measured 180 min following learning (Fig. 4; Gibbs et al., 1978), with concentrations lower than 0.5 m*M* and a concentration of 7 m*M* showing no effect. The small, but consistent difference between 140 m*M* and 154 m*M* NaCl vehicles is maintained at all levels of KCl, suggesting that Na^+ and K^+ concentration effects are independent. Concentrations of 2.5–4 m*M* KCl appear to abolish ITM (Fig. 5) leaving STM intact, whereas 1.0–2 m*M* blocks STM formation as well, with 0.5 m*M* showing a much less pronounced effect (Fig. 6).

Both posttetanic hyperpolarization and Na^+/K^+ ATPase activity (McDougal and Osbom, 1976), as well as ouabain-sensitive Na^+ flux (Baker, et al., 1969), have been shown to be sigmoidally dependent on extracellular K^+ concentration

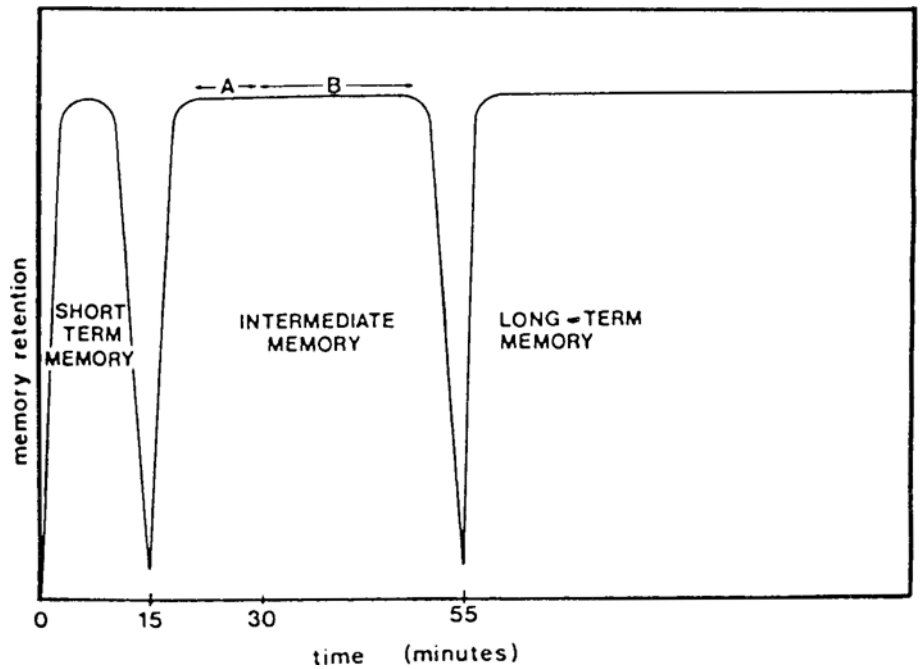

Fig. 1. A schematic model of stages in memory formation based on single-trial passive avoidance learning in neonate chicks (from Gibbs and Ng, 1984).

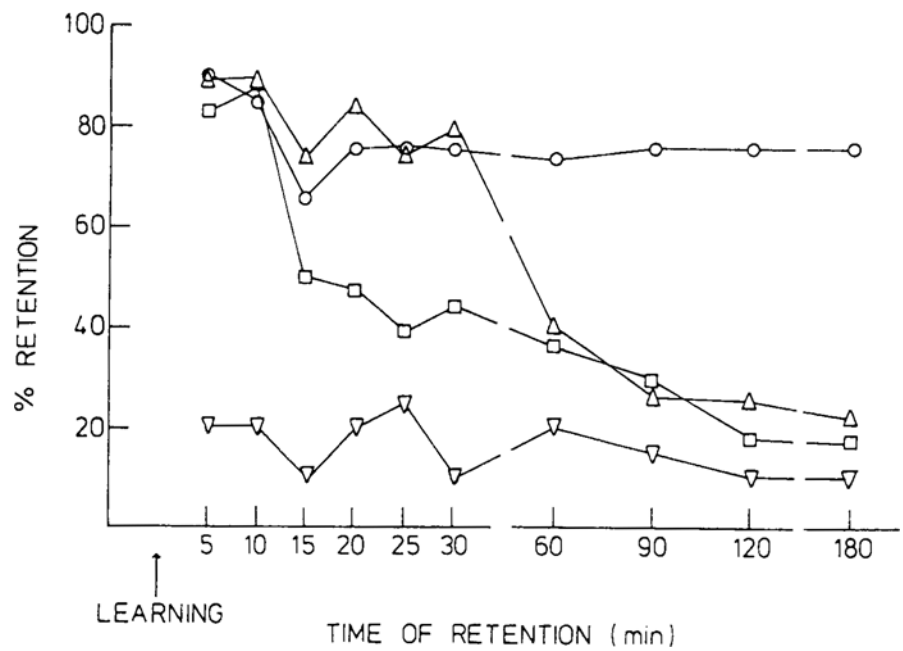

Fig. 2. Effects of various amnesia-inducing pharma-cological agents yielding differential retention functions, suggesting three distinct stages in memory formation (from Gibbs and Ng, 1977). ○ Saline; Δ CXM; ∇ Glutamate; □ Ouabain.

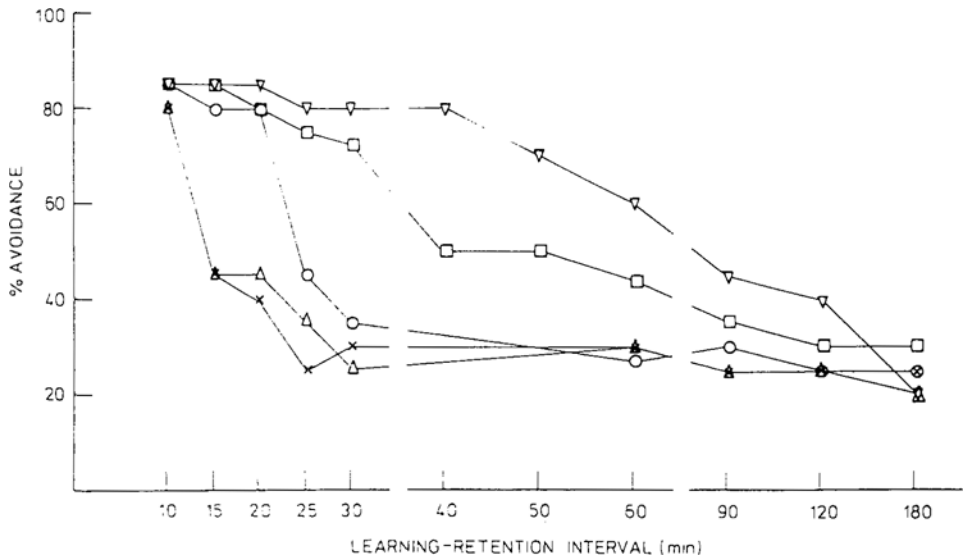

Fig. 3. Increases in extracellular Ca^{2+} increases the duration of short-term memory in chicks pretreated with ouabain to abolish intermediate and long-term memor. (from Gibbs et al., 1979). Calcium concentration (mm): ∇:20; □:10; O:5; △:2.5; ×:0.

($[K^+]_e$). Administration of 1.0–5 m*M* KCl may well retard or abolish Na^+/K^+ ATPase activity and, hence, the ITM stage.

Neuronal activation leads to local accumulation of extracellular K^+ in the intercellular cleft adjacent to recently active neurons. Equalization of extracellular K^+ across the intercellular cleft system is attributed to the combined action of the neuronal sodium pump and astroglia, the latter operating through a number of possible mechanisms, including passive "spatial buffering" via a glial syncytium and active transport via an electrogenic sodium pump or a Cl^- cotransport system (Somjen, 1975; Hansson and Rönnbäck, 1990). Increases in $[K^+]_e$ accompanying tetanus may depolarize glia (Orkand et al., 1966; *see also* Somjen, 1975), leading to changes in glia K^+ conductance, in active K^+ transport mechanisms, or in both (Hertz, 1989). The part played by glia in the uptake and deactivation of glutamate may be relevant here. With $[K^+]_e$ as low as 1 m*M*, the glia acts as an efficient K^+ electrode and hyperpolarizes with $[K^+]_e$ as low as 0.3 m*M* (Kuffler et al., 1966). It is possible, therefore, that administration of 1–2 m*M* KCl results in $[K^+]_e$ levels that effectively inactivate the glia, resulting in a local increase in $[K^+]_e$ around recently activated neurons.

With KCl concentrations below 1 m*M*, both STM and ITM remain relatively intact. This is somewhat puzzling, and no totally satisfactory explanation is available. However, the neuronal resting membrane potential has been shown to be insensitive to changes in $[K^+]_e$ below 1–2 m*M* (Huxley and Stämpfli, 1951). It is possible that, under these conditions, a burst of afferent input may still lead to an increase in K^+ conductance to produce the period of hyperpolarization that we hypothesize to characterize STM. At these $[K^+]_e$ levels, too, the sodium pump may not be electrically disadvantaged, leaving ITM unaffected.

That very low concentrations of exogenous KCl can markedly alter $[K^+]_e$ is supported by electrophysiological data. We have shown, with autoradiographic localization of metabolic activity associated with memory formation, using 2-deoxyglucose (2-DG), that substantial changes in

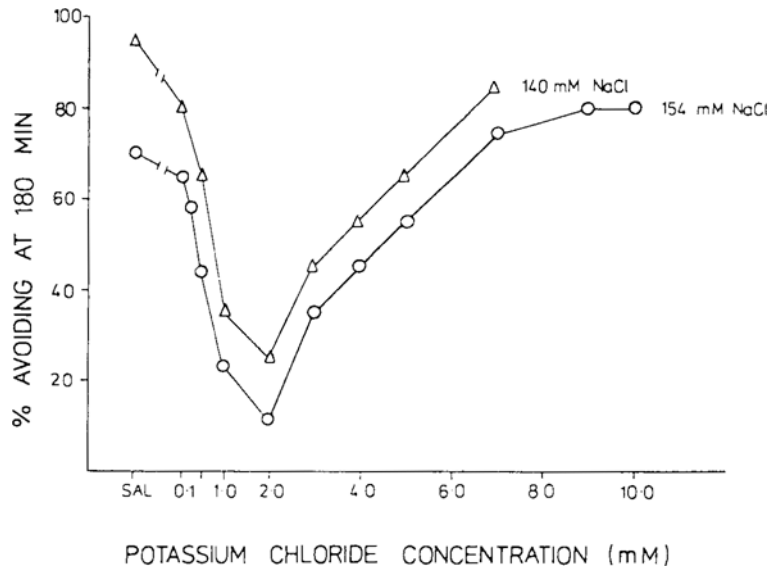

Fig. 4. Dose-dependent effects of low concentration of KCl on retention levels measured 180 min postlearning using either a 140- or 154-m*M* NaCl vehicle (from Gibbs et al., 1978).

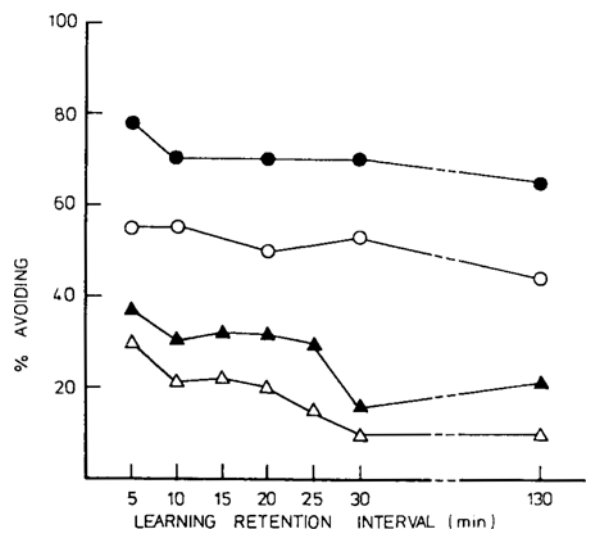

Fig. 5. KCl at concentrations of 2.5–4 m*M* abolishes ITM, leaving STM intact (from Gibbs et al., 1978). ● 0.1 m*M* KCl; ▲ 1.0 m*M* KCl; ○ 0.5 m*M* KCl; △ 2.0 m*M* KCl.

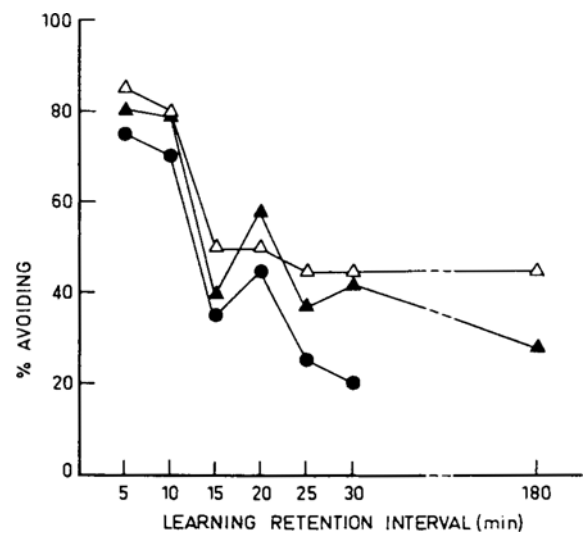

Fig. 6. KCl at concentrations of 1–2 m*M* abolishes STM (from Gibbs et al., 1978). ● 2.5 m*M* KCl; △ 4.0 m*M* KCl; ▲ 3.0 m*M* KCl.

labeled 2-DG uptake occur in the neostriatal–hyperstriatal complex, significantly so in the medial neostriatum (Syková et al., 1990; *see also* Kossut and Rose, 1984). Details of these changes are presented by Sedman et al. in the poster paper *Brain Metabolic Activity Associated with Long-Term Memory Consolidation*. A sample is presented in Fig. 7.

Untrained, anesthesized chicks, stimulated with MeA placed on the beak, yield significant increases in $[K^+]_e$ in the hyperstriatum ventrale in particular

and part of the neostriatum (Fig. 8; Syková et al., 1990), confirming the view that the neostriatal–hyperstriatal complex in the chick is implicated in the learning experience used in our experiments, at least to the extent that it is responsive to part of the sensory input associated specifically with the learning task. Microelectrode application of 0–7 m*M* KCl in 1–2 µL vol into the neostriatal–hyperstriatal complex of these untrained, anesthesized chicks yields substantial increases in $[K^+]_e$ (Fig. 9), as does 4 m*M* of monosodium glutamate (Fig. 10). The notable exception is 3 m*M* KCl, which is close to the resting $[K^+]_e$ level of 3–4 m*M* observed in 1–3-d-old chicks (Syková et al., 1990). The precise reconciliation of these findings to the behavioral consequences of applying these concentrations of KCl immediately before or after training is yet to be achieved. The observation that both saline and 20 m*M* KCl yielded changes in $[K^+]_e$ similar to those observed with 1–2 m*M* KCl is disturbing. Although the effects of 20 m*M* on memory have not been explored by us, saline certainly does not induce a loss of STM. Our hypothesis that neuronal hyperpolarization arising from K^+ conductance changes following neuronal activation may need reconsideration. However, neither the investigation of the effects of MeA nor that of the effects of KCl on $[K^+]_e$ was intended to test the validity of our hypothesis regarding the cellular mechanisms of STM, only its feasibility. The effects of administration of these concentrations of KCl in the presence of any training-induced changes in the ionic environment are yet to be established and are presently under investigation. The consequences of using anesthesia on memory processing are also relevant, although we have preliminary evidence that memory remains intact with anesthesia applied at various times after training. Finally, differences in placement and volume (10 µL/hemisphere in the behavioral studies) of injections will also have to be taken into account.

With the above qualifications in mind, our pharmacological findings still suggest a possible role for astrocytes in the early stages of memory formation. Neuroglia have been demonstrated to be present in neonatal chicks (Curtis et al., 1989). In a somewhat gross analysis, these authors estimated the glial cell population to decrease from 0.11/100 μm^2 at 16 d *in ovo* to 9/100 μm^2 at 19 d *in ovo*, and then to increase to 10/100 μm^2 at 9 d post hatch. However, the course of development and maturation of glia cells, particularly astrocytes and oligodendrocytes, in the chick awaits more experimental work. In rat spinal cord (Jendelová and Sykova, 1991) and rat optic nerve (Foster et al., 1982; Ransom et al.,1985), proliferation of astrocytes, oligodendrocytes, and myelination occurs postnatally with a time-course that correlates well with the observed development of an acid shift in the context of stimulation-evoked extracellular pH changes ($[pH]_e$). The enzyme carbonic anhydrase is thought to play an important role in $[pH]_e$ regulation and is mainly located in glial cells (*see* Jendelová and Syková, 1991). It is of interest, therefore, to examine the development and maturation of glial cells in the brain of neonatal chicks via alkaline–acid shifts following stimulation. In a preliminary series of studies, we have shown that neither light nor MeA stimulation of untrained, anesthesized chicks yielded reliable evidence of acid shifts until after 10 d posthatch (Figs. 11 and 12). It is possible, therefore, that at least with respect to pH responses, the glia may be functionally immature in the 1–3 d-old chicks that we used in our memory experiments. The fact that the chicks were untrained again needs to be borne in mind. The possibility that the glia may be mature in other ways, or more interestingly, that glial maturation in these brain regions may be stimulated by the learning experience itself merits investigation, especially given that chicks are precocially prepared for the learning task used. Accumulating evidence of experience-induced early gene activation is not inconsistent with this possibility (Anokhin et al., 1991).

Although the formation of ITM is inhibited by sodium pump blockers given 5 min or earlier after learning, maintenance of ITM after formation is susceptible to inhibition by the uncoupler of oxidative phosphorylation, 2, 4-dinitrophenol

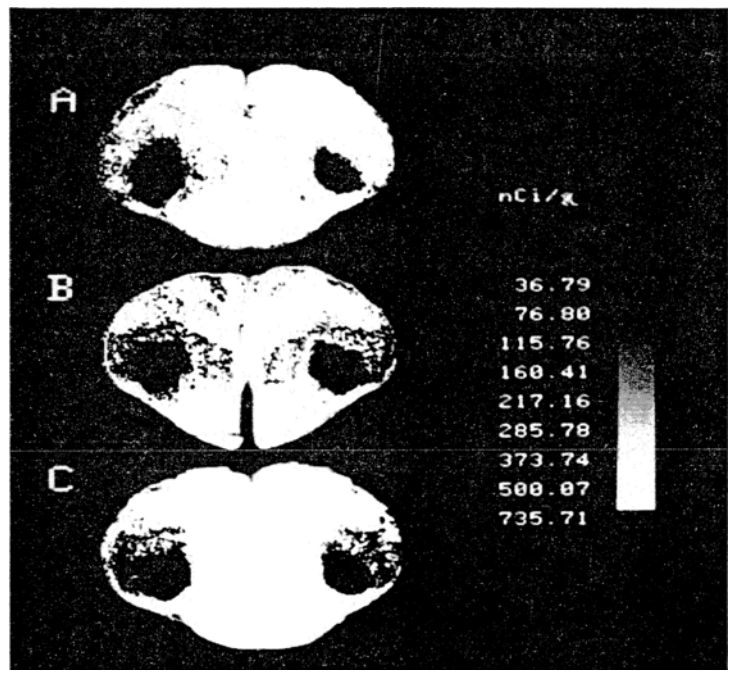

Fig. 7. Normalized images of [^{14}C] 2-deoxyglucose autoradiographs of chick brain sections. A: Section from a chick that received monocular visual stimulation. Note that the area of greatest metabolic activity corresponds to the ectostriatum contralateral to the eye stimulated. B and C: Representative 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).

(DNP), given up to 30 min postlearning, during the ITM phase A (Fig. 13; *see* Fig. 1). Of interest is evidence from our laboratories that purified anti-chick Thy-1 IgG abolishes memory after 20 min postlearning, with the effect achieved with Fab and $F_{(ab')2}$, but not Fc fragments (Fig. 14). This contrasts markedly with our previous reports that both polyclonal and monoclonal antichick Thy-1 ascites fluids abolish only LTM, with memory loss occurring 60 min after learning (Bernard et al., 1983; Lappuke et al., 1987). Paradoxically, this purified IgG was found to bind not only to the Thy-1 molecule, but also to a 75-kDa molecule of chick forebrain homogenate. A similar amnesic effect is observed with normal mouse IgG, which also binds to a 75-kDa protein molecule. Initial evidence also suggests that phosphorylation of a molecule of approx the same molecular weight occurs at this time after learning. The identification of this molecule is yet to be achieved, with synapsin I being a strong candidate. Further-more, we have evidence to show that 2-deoxygalactose induces amnesia with a retention function similar to that observed with the purified IgG. Memory loss occurs after 20 min post-learning (cf Rose and Jork, 1987). It may be speculated that phos-phorylation of a 75-kDa protein molecule may underlie the maintenance of ITM phase A, whereas the Thy-1 glycoprotein is more specifically involved in long-term memory consolidation.

Mechanisms Triggering Long-Term Consolidation

ITM (B) is insensitive to DNP (Gibbs and Ng, 1984). However, this phase of ITM appears to be associated with the level of arousal contingent on the learning experience. Chicks trained with a diluted solution of MeA (10–20% in absolute

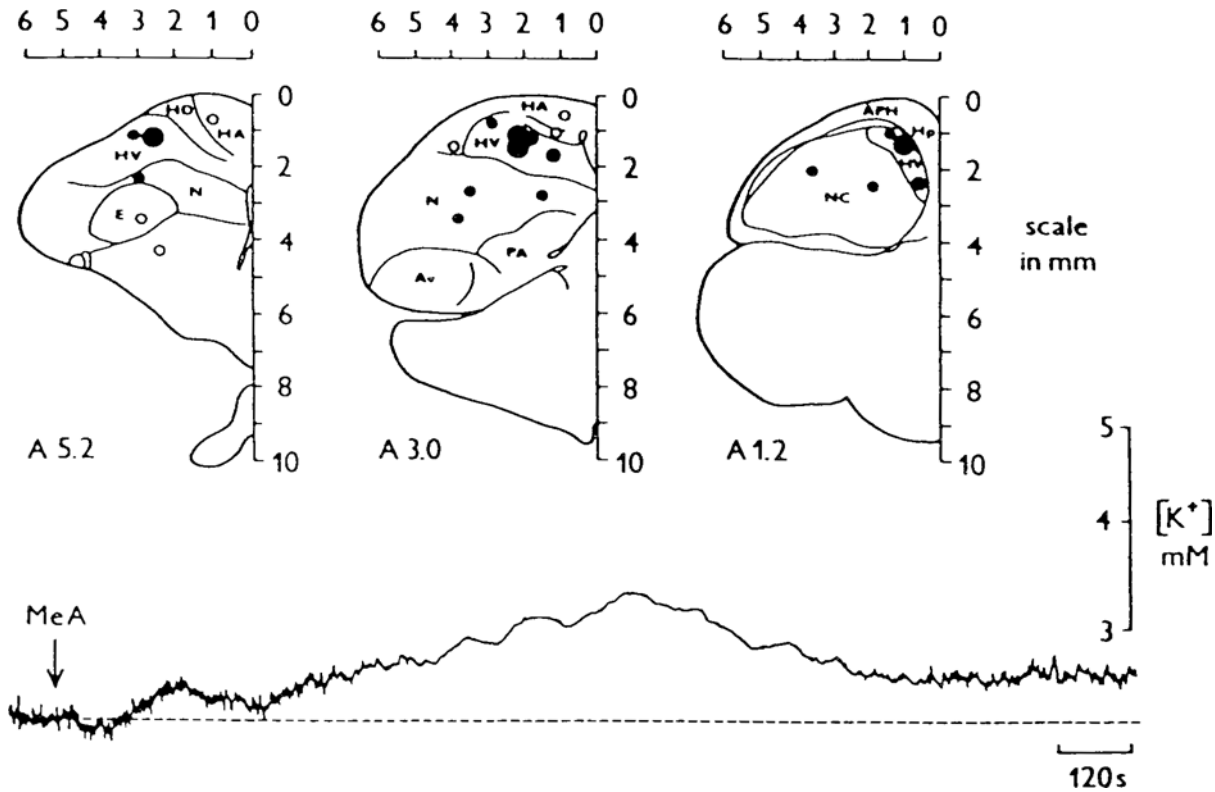

Fig. 8. Effects of MeA placed on the beaks of anesthesized, untrained chicks on $[K^+]$ in various regions of the chick forebrain. The size of filled in circles indicates the extent of increase in $[K^+]_e$ (from Syková et al., 1990).

alcohol) exhibit normal memory until 30 min after training (Fig. 15; Crowe et al., 1989a). Immediate posttraining administration of appropriate doses of noradrenaline (NA) or $ACTH_{1-24}$ recovers ITM (B) and LTM (Crowe et al., 1990), as do repeated trials (Crowe et al., 1989b). Thus, induction of ITM (B) appears necessary for LTM formation.

The level of whole forebrain NA varies with the strength of the learning experience (Fig. 16; Crowe et al., 1991), as indexed by the concentration of the aversant or represented through the use of repeated training trials. For approx 10 min after the training trial, all experimental groups demonstrated a substantial increase in NA levels over baseline. This increase is undifferentiated with respect to the nature of the training trial itself and would appear to reflect, therefore, a response to nonspecific arousal. From 15–25 min posttraining, during ITM phase A, NA levels for all groups returned to baseline. Given the absence of a differentiated NA response prior to this phase of memory processing, it is not unreasonable to conclude that NA plays no specific role in memory processing during phase A of ITM. The differential NA response is first observed at 30 min after learning, in the temporal juncture between ITM (A) and ITM (B). At this time, too, an increase in whole forebrain cAMP has been observed (Brown, 1987). This increase is abolished by the antibiotic cycloheximide administered close to the learning trial and attributed to CXM inhibition of NA synthesis (Gibbs and Ng, 1984). This effect of CXM on retention is overcome by phosphodiesterase inhibitors and dibutryl-cAMP. Both the phosphodiesterase inhibitors, including theophylline, and db-cAMP extend the duration of the interme-

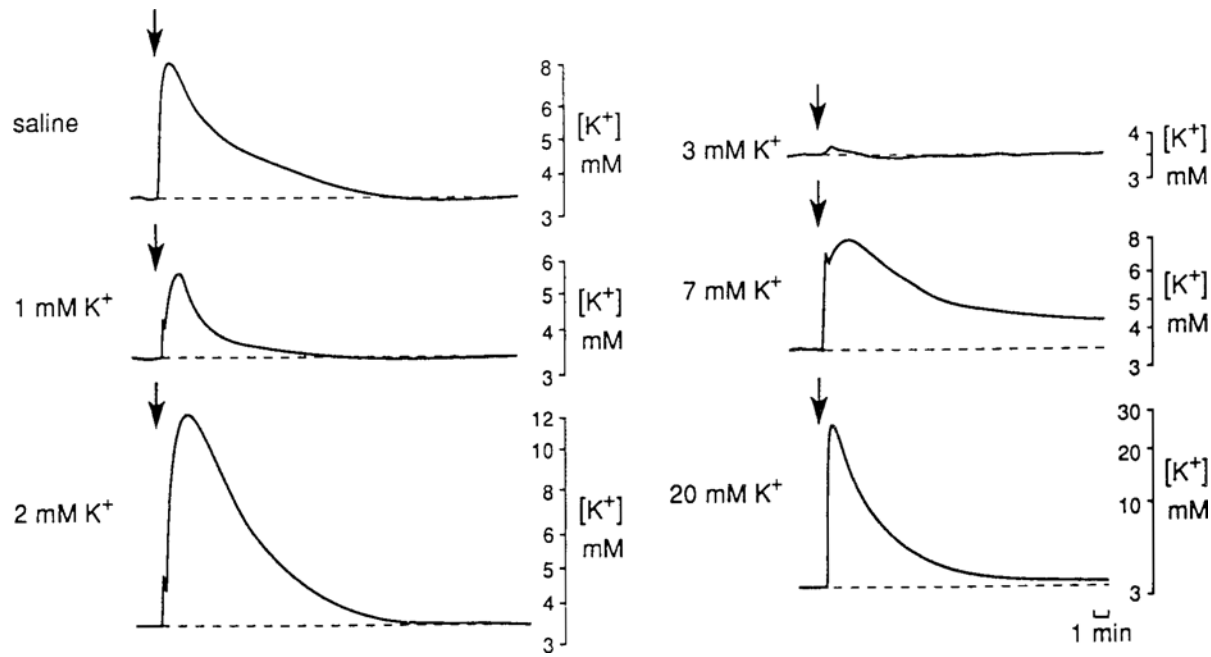

Fig. 9. Changes in $[K^+]_e$ following microelectrode application of low concentrations of KCl to the neostriatal–hyperstriatal complex of untrained, anesthesized chicks.

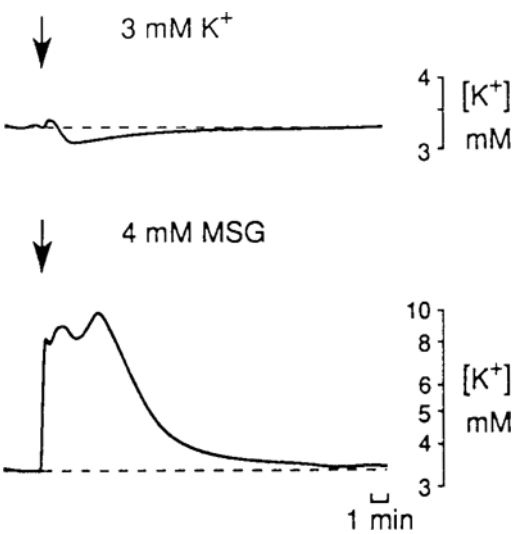

Fig. 10. Effects of 3.0 m*M* KCl and 4 m*M* monosodium glutamate, applied to the neostriatal–hyperstriatal complex of untrained, anesthesized chicks, on $[K^+]_e$.

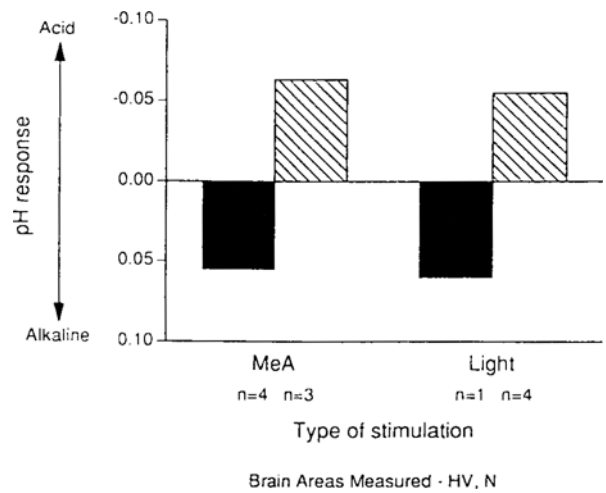

Fig. 11. Age-related mean pH changes following stimulation with light or with MeA placed on the beak of anesthesized, untrained chicks. In all cases, increases in $[K^+]_e$ were detected (not shown). Age of chick: ■ 2–4 d; ▨ 11–13 d.

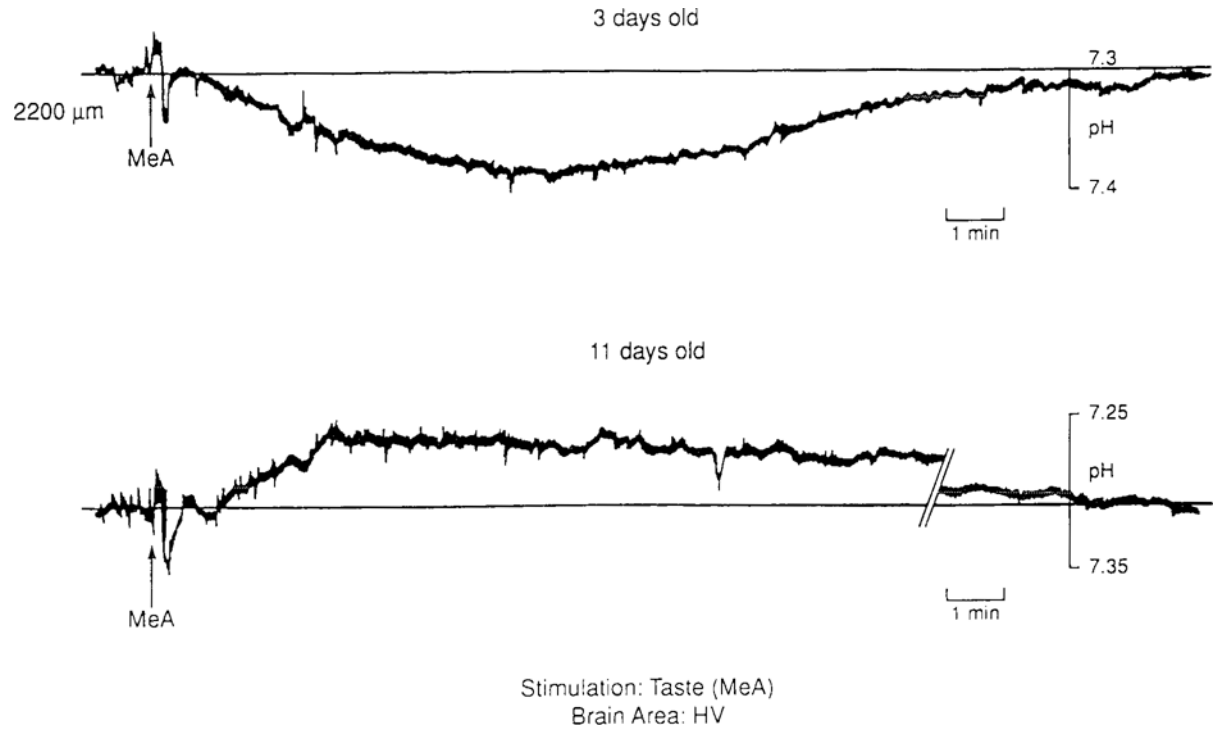

Fig. 12. Age-related pH responses to MeA stimulation in a 3-d-old and an 11-d-old anesthesized, untrained chick.

diate memory stage (Fig. 17), an effect shown by DNP studies to be owing entirely to extension of ITM (B). Similar findings were obtained with db-cAMP. Finally, the β-blockers propranolol (Crowe et al., 1991) and sotalol (Stephenson and Andrew, 1981) produce significant retention deficits beginning some 30 min after learning, whereas the α–blocker, yohimbine has no effect. These results suggest that NA, released in a manner contingent on the level of reinforcement associated with the learning experience, is crucial to the appearance of ITM (B) and subsequent memory consolidation.

Possible Involvement of Astroglia

It is relevant to note here that our autoradiographic studies referred to earlier showed that, compared with controls, a substantial increase in 2-DG uptake occurred in the area identified as the medial neostriatum, under the normal version of the learning task. In the weak-reinforcement version of the task, there was no increase in metabolic activity. When ACTH was administered immediately postlearning to weakly trained chicks, an increase in 2-DG uptake equivalent to that observed with normal, strongly reinforced learning resulted. These changes in metabolic activity seem to occur only between 30–60 min following learning. The metabolic activity observed at this time appears to be specifically associated with phase B of ITM and with the triggering of long-term memory consolidation. The fact that 2,4-dinitrophenol had no effect on memory at this time (Gibbs and Ng, 1984) suggests that the changes in metabolic activity indexed by 2-DG uptake may be independent of whatever metabolic processes are associated with phase A of ITM, the latter possibly involving oxidative phosphorylation. It should be pointed out, parenthetically, that the lack of sensitivity of phase B of ITM to 2,4-dinitrophenol, does not necessarily imply independence from metabolic processes during this phase of ITM, since glycolysis would proceed normally.

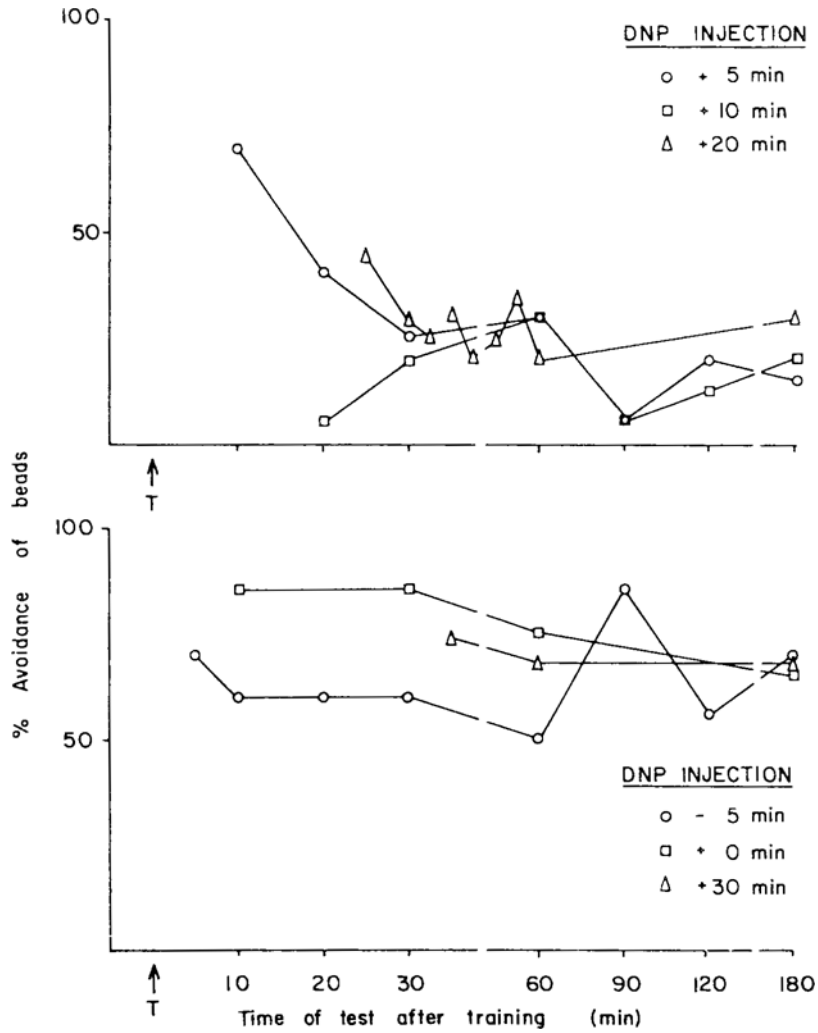

Fig. 13. Effects of the uncoupler of oxidative phosphorylation, 2,4-dinitrophenol, administered at different times on retention measured 5 min after drug injection (from Gibbs and Ng, 1984).

2-DG has been shown to be in part incorporated into glycogen (Penreath, 1982). Glycogen and glycogenolysis are preferentially localized in the glia (Hertz, 1989; Stone and Ariano, 1989). There is evidence to suggest that brain glycogenolysis is activated by neuronally released NA, stimulating β-receptors, and possibly mediated by cAMP (Stone and Ariano, 1989). It is possible that the glia may play a role in supplying the high-energy demands in those brain areas associated with memory processing in general and the triggering of LTM formation in particular.

The fact that the efficacy of associative learning depends on the level of reinforcement contingent on the learning experience is well documented. The physiological mechanisms underlying this action of reinforcers are not well understood. In our memory model, we speculate that reinforcement-contingent neuronal release of NA in the transition from phase A to phase B of ITM may be the significant event in triggering processes leading to long-term memory consolidation. If reinforcement is expressed neurophysiologically through the release of neurohormones, such as

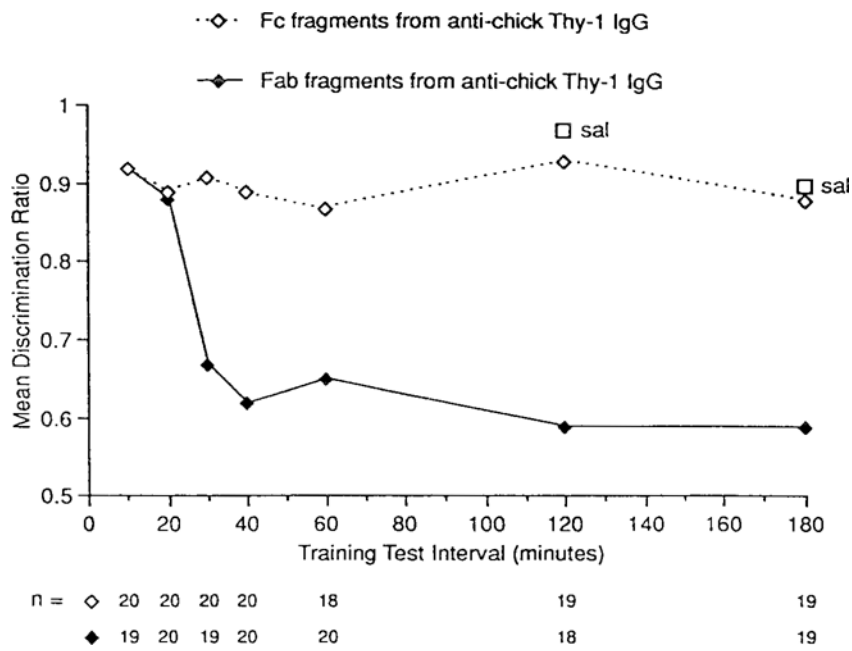

Fig. 14. Retention functions for chicks administered either Fc or F_{ab} fragments from antichick Thy-1 IgG.

NA, then neuroglia may mediate the effects of reinforcement. In this context, we have shown recently that the calcium–calmodulin inhibitor, trifluoroperazine, also produces amnesia after 30 min postlearning, the deficits persisting for up to at least 24 hours.

There is increasing evidence from cultured cell, in vivo neurotoxin and electrophysiological studies (*see* Stone and Ariano, 1989) that, in mammalian forebrain, at least, β-receptors (principally β_1) as well as possibly α_1 and α_2 receptors may be primarily localized in the glia. If this is so and given strong evidence of cAMP responses in forebrain glia, release of neuronal NA following learning may activate glia receptors rather than neuronal receptors. We have already mentioned the possible role of glia in meeting the energy demands of memory processing through glycogenolysis following learning-induced stress or arousal, perhaps via NA activation. Cultured glial cells have also been shown to release nerve growth factor (NGF) in response to β-stimulation of cAMP release (Schwartz, 1988), and NGF has been implicated in associative learning (Van Caulker and Hamprecht, 1980). Together with increasing evidence of experience-induced early gene activation (Anokhin et al., 1990), a platform exists for the development of the sort of neural connectivity that may characterize memory consolidation following associative learning. The possibility that β-receptor stimulation of cultured glial cells leads to release of cAMP (*see* Stone and Ariano, 1989), which may in tum activate neuronal early genes, such as c-*fos*, is consistent with the view that glia, specifically astrocytes, may modulate neuronal signaling processes, such as long-term potentiation. In short, we hypothesize that astrocytes in regions of the brain associated with memory processing may be implicated in the physiological events of motivation and reinforcement.

Conclusion

In sum, we suggest that at least two stages of memory processing precede long-term memory consolidation. A short-term stage (STM), lasting approx 10 min, is followed by an intermediate

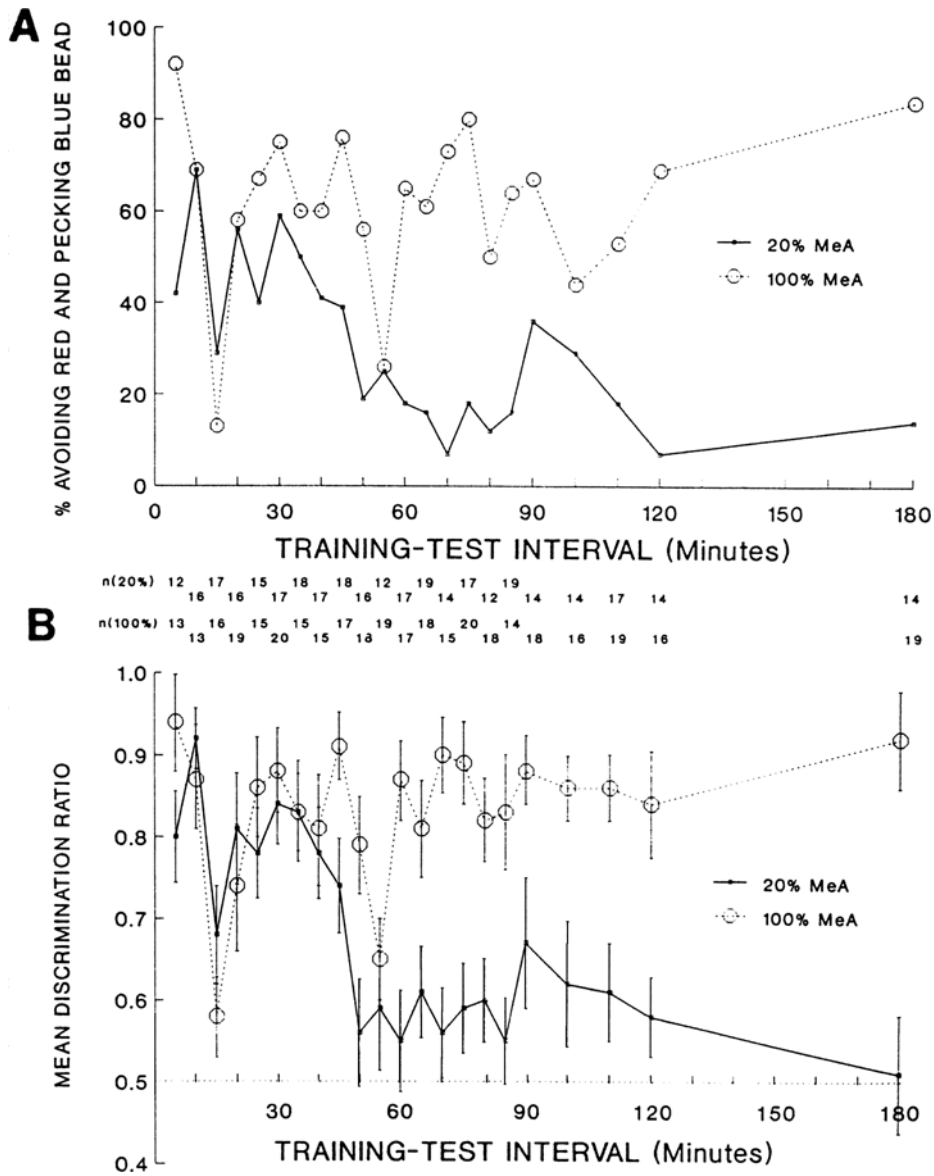

Fig. 15. Retention functions for chicks trained on a strongly reinforced or a weakly reinforced single-trial passive discrimination avoidance task (from Crowe et al., 1989a).

stage (ITM) of approx 30-min duration and consisting of two phases: ITM (A) and ITM (B). The STM stage and formation of ITM may involve two mechanisms of posttetanic neuronal hyperpolarization, the first attributed to activity-induced increases in neuronal K^+ permeability and the second to the action of a neuronal sodium pump. Astrocytic participation in the molecular events underlying both these hypothesized processes merits consideration. Maintenance of ITM (A) may occur via phosphorylation of particular membrane proteins prolonging the period of hyperpolarization initiated by sodium pump activity. A prolonged period of neuronal hyper-

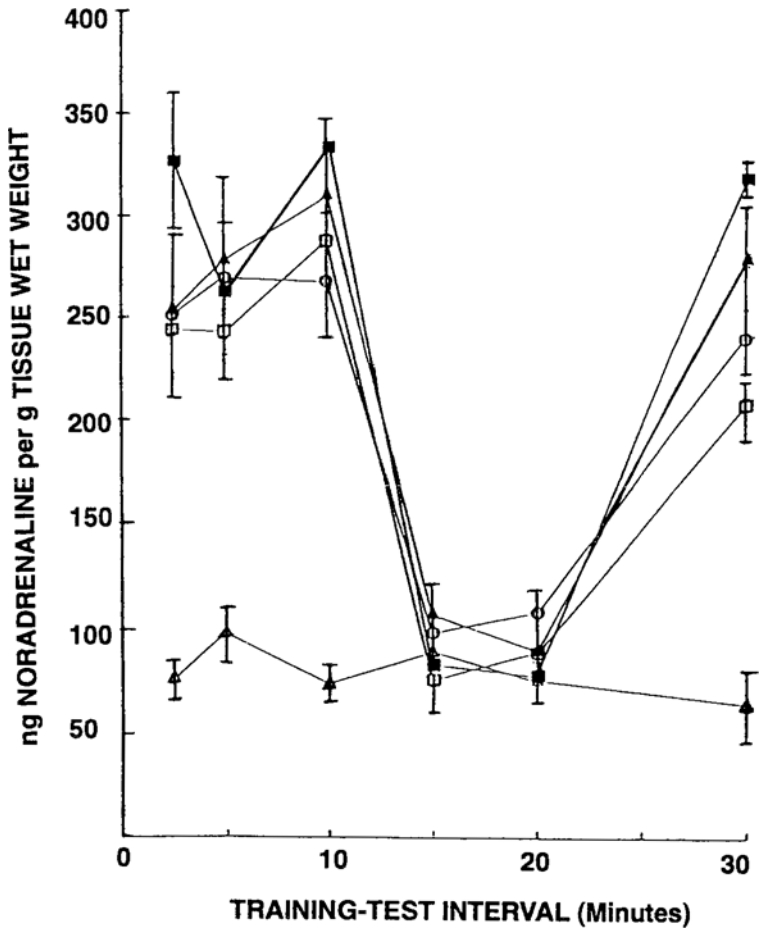

Fig. 16. Levels of whole forebrain NA at various times after training with strongly reinforced learning or weakly reinforced learning augmented by retraining or post-learning administration of ACTH (from Crowe et al., 1991). -△- No training; -□- 20% aversant; -○- 100% aversant; -▲- 20% with ACTH; -■- 2 presentations 20%.

polarization may serve the function of marking neurons for the synaptic modifications generally thought to underlie long-term consolidation and/or restrict the reactivity of the neurons to further stimulation during the consolidation process. The trigger for consolidation appears to occur in the transition between ITM (A) and ITM (B), and entails reinforcement-contingent release of neurohormones, possibly NA, which in turn may stimulate cAMP-dependent intracellular processes. This hypothesis is consistent with the earlier views expressed by Kety (1972) and Shashoua (1971). Astroglia may play a central role in this process. Furthermore, specialization of glia in selected brain regions associated with memory processing is conceivable. Regionally specialized astrocytes with great differences in active uptake of amino acids, including L-glutamate, L-aspartate, and GABA, have been reported (*see* Hansson and Rönnbäck, 1990). A similar glial specialization for visual stimulation has been reported in cat primary visual cortex (Kelly and Van Essen, 1974). However, much of what we know about glial functions is derived from in vitro glial preparations, and the caveat that these may not be adequate models for in vivo glial processes needs to be kept in mind (Barnes et al., 1990). Long-term consolidation seems to implicate *de novo* protein

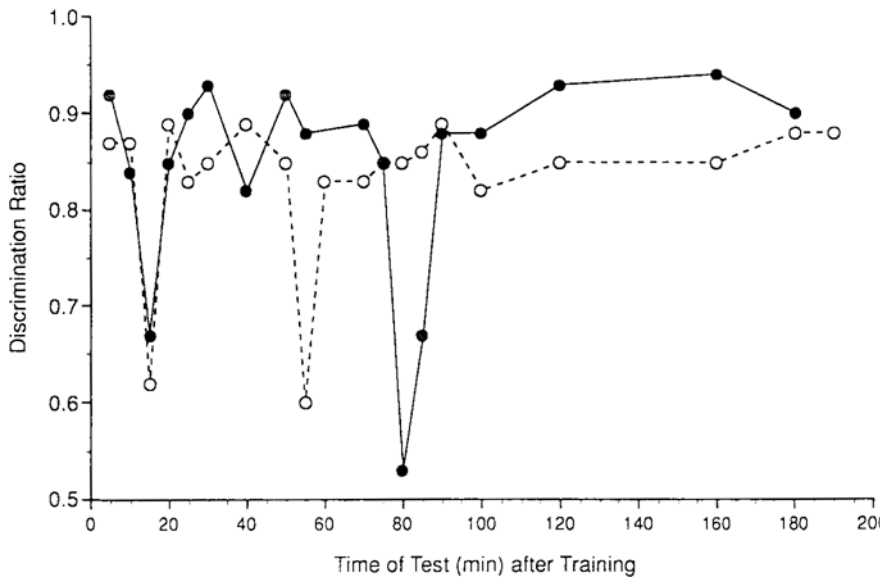

Fig. 17. Effect of the phosphodiesterase inhibitor, theophylline, on the duration of intermediate memory. ITM is extended beyond its normal 50 min through extension of ITM (B).

synthesis. Just what proteins are involved is uncertain, although specific glycoproteins, such as Thy-1 in the chick, may be implicated in the early stages of this process (*see* Routtenberg et al., 1974) to modify such postsynaptic processes as the postsynaptic density (*see* Rose, 1991). The possibility that long-term potentiation may be associated with memory consolidation needs to be reconciled with the hypothesis that neuronal hyperpolarization may be precursor processes to consolidation. Functional anatomical differentiation may render these two aspects of neuronal processing complementary in the formation of memory. Precise anatomical localization of these processes may be critical to achieving a coherent picture.

As indicated at the beginning of this article, an integrated account of the molecular mechanisms of memory formation is yet a long way off. We have attempted here to outline a temporally defined sequence of neuronal events within the framework of a temporally explicit model of memory processing. Within this context, some capacity exists for the interpretation and integration of available and accumulating research findings.

Acknowledgments

This research was supported by grants from the Australian Research Council, formerly Australian Research Grants Committee, from the Brain Behaviour Research Institute, La Trobe University, and from La Trobe University. We wish to acknowledge Jenny Barnett, Christine Papasava, Robyn Hodge, Jenny Redman, Amanda Richdale, Mara Silins, and Julie Barrington for their assistance in the laboratory at various times in the course of the research.

References

Acousta-Uriquidè J., Alkon D. L., and Neary J. T. (1984) Ca^{++} dependent protein kinase injection in a photoreceptor mimics biophysical effects of associative learning. *Science* **224**, 1254–1257.

Anokhin K. V., Mileusnic R. M., Shumakina I. Y. and Rose S. P. R. (1991) Effects of early experience on c-*fos* expression in the chick forebrain. *Brain Res.* **544**, 101–107.

Baker P. F., Blaustein M. P., Keynes R. D., Manil J., Shaw T. J., and Steinhardt R. A. (1969) The ouabain-

sensitive fluxes of sodium and potassium in giant squid axons. *J. Physiol.* **200,** 459–496.

Barnes B. A., Chun L. L. Y., and Corey D. P. (1991) Ion channels in vertebrate glia. *Ann. Rev. Neurosci.* **13,** 441–474.

Bernard C. C. A., Gibbs M. E., Hodge R. J., and Ng K. T. (1983) Inhibition of long-term memory formation in the chicken by anti chick Thy-1 antibody. *Brain Res. Bull.* **11,** 111–136.

Bliss T. V. P. and Lomo T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232,** 331–356.

Brown D. F. (1987) The role of adenosine 3' 5'-cyclic monophosphate in memory formation: A behavioral, pharmacological, and biochemical study. Unpublished Doctoral Dissertation, La Trobe University, Australia.

Cajal S. R. (1911) Histologie du Systeme Nerveux de l'Homme et des Vertebrès, vol. II, Maloine, Paris.

Cherkin A. (1971) Biphasic time course of performance after one-trial avoidance training in the chick. *Comm. Behav. Biol.* **5,** 379–381.

Crowe S. F., Ng K. T., and Gibbs M. E. (1989a) Memory processing in weakly reinforced learning. *Pharmacol. Biochem. Behav.* **33,** 881–887.

Crowe S. F., Ng K. T., and Gibbs M. E. (1989b) Effect of retraining trials on memory consolidation in weakly reinforced learning. *Pharmacol. Biochem. Behav.* **33,** 889–894.

Crowe S. F., Ng K. T., and Gibbs M. E. (1990) Memory consolidation of weak training experiences by hormonal treatments. *Pharmacol. Biochem. Behav.* **37,** 729–734.

Crowe S. F., Ng K. T., and Gibbs M. E. (1991) Possible noradrenergic involvement in training stimulus intensity. *Pharmacol. Biochem. Behav.* **39,** 717–722.

Crowe S. F., Ng K. T., and Gibbs M. E. (1991) Forebrain noradrenaline concentration following weakly reinforced training. *Pharmacol. Biochem. Behav.* **40,** 173–176.

Curtis E. M., Stewart M. G., and King T. S. (1989) Quantification of synaptic, neuronal and glial development in the intermediate and medial hyperstriatum ventrale (IMHV) of the Chick Gallus domesticus, pre- and post-hatch. *Dev. Brain Res.* **48,** 105–118.

Dash P. K., Hochner B., and Kandel E. R. (1990) Injection of the cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. *Nature* **345,** 718–721.

Dunn A. J. (1980) Neurochemistry of learning and memory. *Ann. Rev. Psychol.* **31,** 343–390.

Eccles J. C. (1953) *The Neurophysiological Basis of Mind. The Principles of Neurophysiology,* Clarendon Press, Oxford.

Foster R. E., Connors B. M., and Waxman S. G. (1982) Rat optic nerve: electrophysiological, pharmacological, and anatomical studies during development. *Dev. Brain Res.* **3,** 371–386.

Gibbs M. E., Gibbs C. L., and Ng K. T. (1978) A possible physiological mechanism for short-term memory. *Physiol. Behav.* **20,** 619–627.

Gibbs M. E., Gibbs C. L., and Ng K. T. (1979) The influence of calcium on short-term memory. *Neurosci. Lett.* **14,** 355–360.

Gibbs M. E. and Ng K. T. (1977) Psychobiology of memory: Towards a model of memory formation. *Biobehav. Rev.* **1,** 113–136.

Gibbs M. E. and Ng K. T. (1984) Dual action of cycloheximide on memory formation in day-old chicks. *Behav. Brain Res.* **12,** 21–27.

Goelet P., Castelucci V. F. Schacter S., and Kandel E.R. (1986) The long and the short of long-term memory—a molecular framework. *Nature* **322,** 419–422.

Hansson E. and Rönnbäck L. (1990) Astrocytes in neurotransmission. *Cell. Mol. Biol.* **36 (5),** 487–496.

Hebb D. O. (1949) *The Organization of Behaviour,* John Wiley & Sons, New York.

Hertz L. (1989) Is Alzheimer's disease an anterograde degeneration, originating in the brainstem, and disrupting metabolic and functional interactions between neurons and glial cells? *Brain Res. Rev.* **14,** 335–353.

Huxley A. F. and Stämpfli R. (1951) Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. *J. Physiol.* **112,** 496–508.

Jansen J. K. S. and Nicholls J. G. (1973) Conductance changes, an electrogenic pump and the hyperpolarization of leech neurons following impulses. *J. Physiol.* **229,** 635–655.

Jendelová P. and Syková, E. (1991) Role of glia in K^+ and pH homeostasis in the neonatal rat spinal cord. *Glia* **4,** 56–63.

Kandel E. R. and Schwartz J. H. (1982), Molecular biology of learning: Modulation of transmitter release. *Science* **218,** 433–443.

Kelly J. P. and Van Essen D. C. (1974) Cell structure and function in the visual cortex of the cat. *J. Physiol.* **238,** 515–547.

Kety S. (1972) Brain catecholamines, affective states and memory. *Chemistry of Mood, Motivation and Memory* McGaugh J. L. ed., Plenum, New York.

Kossut M. and Rose S. P. R. (1984) Differential 2-deoxyglucose uptake into chick brain structures during passive avoidance learning. *Neuroscience* **12 (3),** 971–977.

Kuffler S. W., Nicholls J. G., and Orkand R. K. (1966) Physiological properties of glial cels in the central nervous system of amphibia. *J. Neurophysiol.* **29,** 768–787.

Lappuke R., Bernard C. C. A., Gibbs M. E., Ng K. T., and Bartlett P. F. (1987) Inhibition of memory in the chick using a monoclonal anti-Thy-1 antibody. *J. Neuroimmunol.* **14,** 317–324.

Lynch M. A. and Baudry M. (1984) The biochemistry of memory. A new and specific hypothesis. *Science* **224,** 1057-1063.

McDougal D. B. Jr. and Osborn L. A. (1976) Post-tetanic hyperpolarization, sodium-potassium-activated adenosine triphosphatase and high energy phosphate levels in garfish olfactory nerve. *J. Physiol.* **256,** 41–60.

Ng K. T. and Gibbs M. E. (1991) Stages in memory formation: A review, *Neural and Behavioural Plasticity: The Use of the Domestic Chick as a Model*. Andrew R. J., ed., Oxford University Press, Oxford.

Nicoll R. A., Kaner J. A., and Malenka R. C. (1988) The current excitement in long-term potentiation. *Neuron.* **1,** 97–103.

Orkand R. K., Nicholls J. G., and Kuffler S. W. (1966) Effect of nerve impulses on the membrane potential of glial cells on the central nervous system of amphibia. *J. Neurophysiol.* **29,** 788–806.

Patterson T., Alvarado M. C., Warner I. T., Bennett E. L., and Rosenzweig M. R. (1986) Memory stages and brain asymmetry in chick learning. *Behav. Neurosci.* **100,** 856–865.

Penreath V. W. (1982) Potassium signaling of metabolic interactions between neurons and glial cells. *Trends Neurosci.* **5 (10),** 339–345.

Ransom B. R., Carlini W. G., and Connors B. (1985) Brain extracellular space: Developmental studies in rat optic nerve. *Ann. NY Acad. Sci.* **41,** 87–105.

Reyman K., Frey U., and Matthies H. (1988) A multiphase model of synaptic long-term potentiation in hippocampal CA1 neurons: Protein kinase C activation and protein synthesis are required for maintenance of the trace, *Synaptic Plasticity in the Hippocampus* Haas H. L. and Buzsaki G., eds., Springer, Berlin.

Rose S. P. R. (1991) Biochemical mechanisms involved in memory formation in the chick, *Neural and Behavioral Plasticity: The Use of the Domestic Chick as a Model*, Andrew R. J., ed., Oxford University Press, Oxford.

Rose S. P. R. and Jork R. (1987) Long-term memory formation in chicks is blocked by 2-deoxygalactose, a fucose analogue. *Behav. Neural Biol.* **48,** 246–258.

Routtenberg A. (1979) Anatomical localization of phosphoprotein and glycoprotein substrates in memory. *Prog. Neurobiol.* **12,** 85–113.

Routtenberg A. (1986) Synaptic plasticity and protein kinase C. *Prog. Brain Res.* **69,** 211–234.

Routtenberg A., George D. A., Davis L. G., and Brunngraber E. G. (1974) Memory consolidation and fucosylation of crude synaptosomal glycoproteins resolved by gel electrophoresis. *Behav. Biol.* **12,** 461–475.

Shashoua V. E. (1971) Dibutyryl adenosine cyclic 3':5'-monophosphate effects on goldfish behaviour and brain RNA metabolism. *Proc. Natl. Acad. Sci. USA* **68 (11),** 2835–2838.

Schwartz J. P. (1988) Stimulation of nerve growth factor MRNA in Cb glioma cells by a β-adrenergic receptor and by cyclic AMP. *GLIA* **1,** 282–285.

Somjen G. G. (1975) Electrophysiology of neuroglia. *Ann. Rev. Physiol.* **37,** 163–190.

Stephenson R. M. and Andrew R. J. (1981) Amnesia due to β-antagonists in a passive avoidance task in the chick. *Pharm. Biochem. Behav.* **15,** 597–604.

Stone E. A. and Ariano M. A. (1989) Are glial cells targets of the central nervous system? A review of the evidence. *Brain Res. Rev.* **14,** 297–309.

Syková E., Jendelová P., Svoboda J., Sedman G., and Ng K. T. (1990) Activity-related rise in extracellular potassium concentration in the brain of 1-3-day-old chicks. *Brain Res. Bull.* **24,** 569–575.

Van Caulker D. and Hamprecht B. (1980) Effects of neurohormones on glial cells. *Adv. C.ell. Neurobiol.* **1,** 31–67.