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The Molecular Neurobiology of Early Learning, Development, and Sensitive Periods, with Emphasis on the Avian Brain

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Abstract

The subcellular processes that correlate with early learning and memory formation in the chick and sensitive periods for this learning are discussed. Imprinting and passive avoidance learning are followed by a number of cellular processes, each of which persists for a characteristic time in certain brain regions, and may culminate in synaptic structure modification. In the chick brain, the NMDA subtype of glutamate receptor appears to play an important role in both memory formation and sensitive periods during development, similar to its demonstrated role in neural plasticity in the mammalian brain. Two important findings have emerged from the studies using chickens. First, memory formation appears to occur at multiple sites in the forebrain and, most importantly, it appears to "flow" from one site to another, leaving neurochemical traces in each as it moves on. Second, the memory is laid down either in different sites or in different subcellular events in the left and right forebrain hemispheres. Hence, we are alerted to the possibility of similar asymmetrical processes occurring in memory consolidation in the mammalian brain. The similarities between early memory formation and experience-dependent plasticity of the brain during development are discussed.

Index Entries: Early learning; memory; sensitive periods; glutamate receptors; brain asymmetry; chicken; imprinting; passive avoidance; neural plasticity.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate; APV, D,L-2-amino-5-phosphovaleric acid; AS/LPO, archistriatum/lobus parolfactorius; DSP4, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzyl-amine hydrochloride; HA, hyperstriatum accessorium; IMHV, intermediate medial hyperstriatum ventrale; LPO, lobus parolfactorius; LTP, long-term potentiation; NMA, *N*-methyl aspartate; NMDA, *N*-methyl-D-aspartate; PA, paleostriatum augmentatum; PPR, persistent potentiation of responses.

Introduction

Over recent years the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor has been thrown into prominence for its important role in learning and neural plasticity in both developing and adult brains. The main body of research in support of a critical role for NMDA receptors in synaptic modification has been concerned with the effects of visual experience, but NMDA receptors may well have a role of similar importance in the development of other sensory modalities. The involvement of NMDA receptors in visual plasticity has been well reviewed by Rauschecker (1991), but the focus of this review was entirely on mammals, cats in particular. The emerging evidence for glutamate-sensitive receptors in learning/memory and early development in the chicken brain was not mentioned, even though the studies using the chicken almost certainly apply to all vertebrate neural systems and they so adequately complement the work with mammals. This review fills the gap by concentrating on the data demonstrating a role for glutamate-sensitive receptors in neural plasticity and learning in the chicken. Other neurobiological events related to neural plasticity and learning will also be discussed. One outstanding feature of the studies of learning and developmental processes in the chick brain is the laterality of many of the molecular and cellular events involved. This aspect has not yet been considered in the studies examining development in mammalian species.

In dealing with the chicken brain, this review is confined to molecular events that are crucial to early development and learning, but it is recognized that many of these processes also occur in adult brains, even if to a lesser degree (Cotman and Iversen, 1987). Indeed, a recent study has demonstrated that there is an increase in the release of glutamate from synapses in the olfactory bulbs of ewes after they have given birth and in response to the smell of the lamb (Kendrick et al., 1992), a change that may be part of neural plasticity processes in an adult brain.

The developing chicken brain is an excellent model system in which to study sensitive periods, influences on early development, and learning and memory. The embryo is easily accessible in the egg, which is beneficial for anatomical and biochemical studies, and before and after hatching the brain development passes through a series of precisely timed phases, that can more easily be coordinated with subcellular processes than can those of the slower developing mammalian species. Moreover, chicks are precocious animals that form powerful and stable memories in early life, possibly allowing the molecular correlates of memory formation to be studied more easily (Andrew, 1991). These early memories are formed very rapidly, and a precise, brief learning period has advantages in experiments that attempt to link learning and memory to molecular changes. Indeed, it is the study of molecular correlates of memory formation in the young chick that has considerably advanced our understanding of this area in recent years.

One early learning task used in memory studies is passive avoidance learning of an aversive tasting bead. The chick pecks at the bead once and thereafter avoids it. This task has been used extensively by several research groups investigating the neurobiological correlates of memory formation (*see* Rose, 1991a,b). Indeed, the effect of a variety of pharmacological agents on memory formation of the passive avoidance bead task has been used to develop a model of memory formation (recently reviewed by Ng et al., 1992). Another form of learning that occurs soon after hatching and has long-lasting consequences on behavior is that known as imprinting (for review *see* Bolhuis, 1991). In the natural environment, imprinting involves attachment of the young to the mother, the imprinting stimulus. In the

laboratory the imprinting stimulus is frequently a flashing light (red or yellow) or a rotating, stuffed hen. The optimal age for imprinting is 15–24 h after hatching. The time of learning is much longer than that for passive avoidance learning, but imprinting is exceptionally powerful learning that forms a stable memory trace, and this is considered advantageous in studies of the neurobiology of memory formation. Imprinting is also confined to a sensitive period and may have much in common with the plastic events of early development if, indeed, it is separate from them.

Other forms of learning, such as visual learning to discriminate food grains from a background of small pebbles, are better performed after the first week of the chicken's life, and are also being used to study the neurobiology of learning and neural plasticity (*see* Rogers, 1986; Anokhin and Rose, 1991). In fact, the earliest studies that indicated an important role for glutamate, acting as a neurotransmitter, in brain development of the chicken showed that treatment of the developing chick with glutamate during the first week led to a long-lasting slowing of visual discrimination learning ability tested in the "pebble floor task," as well as to changes in other behaviors (Hambley and Rogers, 1979). We will begin by discussing these effects of glutamate on the developing brain.

Effects of Elevating Brain Glutamate Levels on Behavioral Development

High levels of glutamate in the extracellular fluid of the brain are known to be neurotoxic in chicks (Snapir et al., 1973), as they are also in mammals (Olney, 1974, 1978). The glutamate is believed to cause neuronal death by acting on its receptors, both NMDA-sensitive and -insensitive receptors, to cause an influx of calcium ions followed by a cascade of cellular events (Manev et al., 1990) and ultimately to the release of calcium from intracellular stores. The latter is now believed to be the final step in causing cell death (Llinás and Sugimori, 1990). Hence, glutamate is considered to be an excitotoxin. It is believed to act as such at up to one third of central nervous system synapses (Manev et al., 1990), but the degree of excitotoxic damage brought about by a given dose of glutamate varies in different brain regions, possibly owing in part to interactions with glial cells that have uptake mechanisms for glutamate (McGeer, 1991).

Administration of glutamate to the developing chick brain at doses well below those that are known to cause destruction of neurons influences brain development and subsequent learning ability. For example, intracranial injection of 0.04–12.5 μmol of glutamate on d 2 posthatching causes the chick to be retarded in visual learning tasks when tested in the second week of life (Hambley and Rogers, 1979; Rogers, 1982a). After treatment of each hemisphere with as little as 0.04 μmol of glutamate on d 2, the chicks fail to learn to discriminate grains of food from a background of small pebbles. Many-fold higher doses injected on d 11 have no effect (Fig. 1). Also, injection of 0.25 μmol of glutamate into each forebrain hemisphere on d 2 posthatching is sufficient to retard visual habituation learning on d 9, but injection of as much as 1.25 μmol on d 11 posthatching has no subsequent effect on the rate of visual habituation. Hence, there is a sensitive period for susceptibility to glutamate in early life.

Later experiments showed that the sensitive period is a result of underdevelopment of uptake mechanisms for glutamate (Rogers and Hambley, 1982). Chicks in their first and second weeks of life were injected intracranially with large loading doses of glutamate (4 or 10 μmol) and the levels of free glutamate were measured in forebrain homogenates at various times after the injection. The free glutamate levels remained elevated for much longer in the younger brains, suggesting that uptake mechanisms for glutamate develop over the first week of life, as an aspect of the developing blood-brain barrier systems. It is only free glutamate in the extracellular fluid that can gain access to its receptors and, presumably, cause the long-lasting effects on behavior. Thus, it is only in the first week of the chick's life that the injected glutamate may remain in the extracellular compartment long enough to disrupt development.

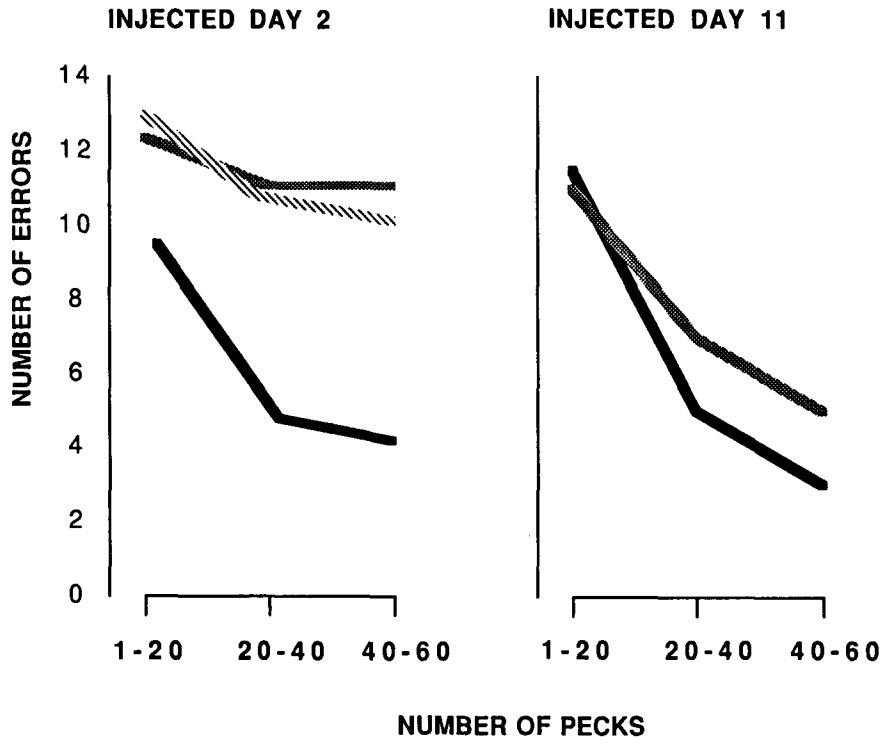

Fig. 1. Learning curves for performance of the visual discrimination learning task requiring search for food scattered on a background of pebbles that have been adhered to the floor. The mean number of pecks at pebbles (errors) has been plotted for each block of 20 pecks allowed. The gray lines represent the data for chicks injected with 1.25 μmol monosodium glutamate into each hemisphere of the forebrain on either d 2 or d 11 posthatching. The hatched line in the graph on the left represents chicks injected with 0.04 μmol monosodium glutamate on d 2. The black lines represent the data for controls injected with 25 μL saline at the same ages. N = 8–14/group. The testing occurred on d 10 and 13, respectively. Note the retarded learning in chicks treated with either dose of glutamate on d 2. Even the higher dose had no effect on d 11. The data is adapted from Hambley and Rogers, 1979; and Rogers, 1982a.

By injecting analogs of glutamate it has been possible to ascertain that the glutamate does indeed have its effect by acting in its capacity as a neurotransmitter on one or more of the subtypes of glutamate receptors. Both kainic acid and *N*-methy-DL-aspartate were found to cause retarded visual discrimination learning, whereas α-methyl-DL-aspartate did not (Rogers and Hambley, 1982). The first two analogs act on subtypes of the glutamate receptor, whereas the latter substance has no action on receptors, mimicking instead the metabolic properties of glutamate.

As already mentioned, on d 2 posthatching administration of as little as 0.04 μmol of glutamate is sufficient to cause retarded visual discrimination learning (Rogers, 1982a), and at this dose the glutamate is most unlikely to be causing any neuronal death. Certainly, no lesions were apparent. Therefore, it is likely that this dose of glutamate is modifying neural connections (possibly by promoting the formation of inappropriate synaptic connections), in such a way that the brain becomes less efficient at learning. Indeed, iontophoretic application of glutamate to neurons is now known to facilitate synaptic modification in response to environmental stimulation (Greuel et al., 1988). A similar interaction between glutamate stimulation and environmental stimulation appears to occur in the young

chick brains treated with glutamate, because the glutamate is effective in changing behavior only if the chick receives certain visual stimulation during the action of the glutamate (Sdraulig et al., 1980). If the 2-d-old chick is injected intracranially with glutamate and placed immediately into a white bucket for 3 h, the treatment has no effect. Visual discrimination learning and visual habituation are not retarded (Table 1). Similarly, if the chick views stationary or moving parallel lines for 3 h after treatment, no retardation results from the treatment. On the other hand, if the chick views intersecting lines in the form of a grid pattern or triangles, retarded learning results from the treatment. Thus, it would seem that glutamate is acting on higher order neurons in the forebrain that respond to angles. It is probable that, as in cats, during the early posthatching stages of visual experience neuronal connectivity at this level of organization is being modified in response to visual experience, and the glutamate treatment disrupts this process to leave long-lasting effects on the subsequent processing of visual information.

The data for chicks is highly reminiscent of the modification of ocular dominance columns (Wiesel and Hubel, 1963) and orientation selective units in the kitten visual cortex (Blakemore and Cooper, 1970). During sensitive periods soon after eye opening, the ocular dominance columns of the visual cortex of the cat are modified by monocularity or strabismus, and the orientation selective units in the visual cortex are modified by experience of lines oriented in one direction only (*see* Rauschecker, 1991). The visual cortical cells show plasticity: They tune into the dominant input received during the sensitive period. Glutamate-sensitive NMDA receptors appear to be involved in these plastic changes of the visual cortical neurons, since systemic application of ketamine, a noncompetitive antagonist of NMDA receptors, with xylazine after monocular exposure prevents the shift in ocular dominance columns (Rauschecker and Hahn, 1987). The ketamine–xylazine treatment also prevents normal orientation tuning for the exposed eye. The experiments with chicks in which intracerebral glutamate levels are raised, of course, involved mechanisms opposite to those operating in the cats treated with ketamine, but both treatments could be modulating normal developmental processes that depend on NMDA receptors. Recently, in fact, ketamine–xylazine treatment of chickens on d 2 posthatching has been shown to alter the normal processes of brain development by prolonging the imprinting period into the second week of life (Parsons and Rogers, 1992). This study will be discussed in some detail along with the other data on imprinting. It implicates a role of NMDA receptors in the developmental changes that lead to closure of the imprinting period, and therefore, as in cats, NMDA receptors play a role in central nervous system plasticity in the chick.

Table 1
Effect of Intracranial Glutamate on Visual Discrimination Learning and Visual Habituation[a]

Exposure		Visual discrimination	Visual habituation
A	Saline controls	15.1 (0.5)	16 (2)
B	Whitebucket	15.7 (1.1)	14 (4)
	Stationary stripes	16.0 (1.0)	13 (2)
	Moving stripes	16.6 (0.5)	19 (1)
C	Grid pattern	8.6 (1.4)	29 (4)
	Triangles	11.2 (1.3)	27 (3)

[a]The effect on different forms of visual experience following injection of glutamate into the forebrain hemispheres of the chick on d 2 posthatching. The chicks were exposed to various visual patterns for 3 h after treatment. They were tested on d 8 and 9 on the visual discrimination learning task (*see* Fig. 1) and a visual habituation task. Means (with standard errors in brackets) are given. For the visual discrimination task, the number of pecks at grain in the last 20 pecks of the test are an indication of learning rate. For the visual habituation test the period of fixation (in s) of the visual stimulus on its fourth presentation indicates rate of learning: Less fixation time means faster learning. The controls (A) received saline treatment only. Category B lists the patterns that prevented glutamate from causing retarded learning. Category C lists the patterns that interacted with the glutamate treatment to cause retarded learning. The scores in B did not differ from those of A (the controls), whereas those in C differed significantly from both A and B. (Adapted from Sdraulig et al., 1980).

One of the other important features of the effect of intracranial glutamate treatment on the developing chicken brain is that it is lateralized. If the glutamate is injected on d 2 into either the right or left hemisphere of the forebrain, and the other hemisphere is treated with saline, retarded visual learning results only from treatment of the left hemisphere, and not the right (Howard et al., 1980). This result indicates that the neural circuits disrupted by the glutamate are located in the left hemisphere. It was the discovery of lateralized effects of the action of pharmacological agents in the chick forebrain that alerted neurobiologists to the fact that the left or right hemispheres could not be treated as being equivalent. As will be discussed later, this recognition has considerably advanced progress in the study of memory in chicks.

Lateralized neurochemistry of the developing brain might well be explored in mammalian species, since they also have lateralization of the brain (*see* Glick, 1985; Bradshaw and Rogers, 1992). For example, cats are known to have structural asymmetries of the brain and there is some evidence that they have lateralized paw use (Webster, 1972; Webster and Webster, 1981). A large amount of evidence demonstrates lateralized function in the rat brain (Denenberg, 1981; Bianki, 1988), and, moreover, early experience, such as handling, modifies this laterality (Denenberg et al., 1980).

In the chick lateralized neurobiological processes change with age. Cycloheximide, which is known to exert its effect via accumulation of glutamate and aspartate (Hambley and Rogers, 1979), causes retarded visual learning when it is administered to the left hemisphere, and not the right, at any time between d 2 and 5 posthatching, but on d 10 or 11 it has the same effect when administered to the right hemisphere (*see* Rogers, 1991). Unilateral treatment with glutamate is likely to follow the same time-course of changing susceptibilities. Possibly the earlier susceptibility of the left hemisphere followed by the right reflects a pattern of development in which the left hemisphere develops in advance of the right.

The glutamate treatment of the left hemisphere has broader effects on behavior than simply on visual discrimination learning. Auditory habituation learning is also slowed and, surprisingly, attack and copulation levels become elevated, just as if the chicks had been treated with the sex hormone testosterone (Howard et al., 1980; Bullock and Rogers, 1986). The effect of glutamate treatment of the left hemisphere on attack and copulation is, as for the retarded visual discrimination learning, mimicked by kainic acid or *N*-methyl-D,L-aspartate treatment of the left hemisphere yet it is qualitatively different from the effect on visual learning, since it occurs irrespective of the visual input received by the chick immediately after treatment (Rogers and Hambley, 1982). If the treated chick is placed in the white bucket for 3 h following the glutamate treatment, the elevation of copulation and attack still occurs. This may suggest that the effect of glutamate on attack and copulation performance is a more direct toxic action, not necessarily involving the modulation of synaptic connections in response to stimulation, although forms of stimulation other than that tested experimentally may be involved.

Taken together these studies of the effect of glutamate on brain development indicate a role for glutamate receptors in neuroplasticity in the chick brain. Over the same period of time the developing brain is forming important memories that also involve glutamate receptors.

Imprinting

The method of imprinting that has proved most useful in study of the neurobiological basis of learning and memory was developed by Bateson and Wainwright (1972), and it has been used extensively by Horn, Bateson, and coworkers at Cambridge University (*see* Horn, 1985). With slight modifications, the method has been used in other laboratories. The chick, previously held in the dark, except perhaps for a brief period of priming in the light (exposure to diffuse light), is placed in a running wheel and exposed to the imprinting stimulus. The latter is presented approx 50 cm from the wheel. The imprinting stimulus might be a rotating box (with two opposite faces painted, say, red and the other

two black or a similar box illuminated from inside) or a rotating stuffed hen. The exposure time varies, but is often around 140 min. The chick is then returned to the dark until testing occurs after an optional delay period. Testing involves giving the chick a choice between the imprinting stimulus and a novel stimulus. The latter might be a differently colored box or differently colored hen. The two stimuli are placed on either side of the running wheel, into which the chick is placed. For a 5 min period the number of rotations made by the wheel in the clockwise and counterclockwise directions is counted. These rotations are generated by the chick as it attempts to approach either the imprinting stimulus or the novel stimulus. A percentage preference score is calculated as the number of rotations toward the imprinting stimulus divided by the total number of rotations in either direction. This score excludes any contribution that could be made by differences in activity between individuals. A high percentage (usually in the region of 70–80%) indicates a preference to approach the training stimulus and hence memory recall. An untrained chick shows no preference, scoring around 50% imprinting. Alternatively, the imprinting (A) and nonimprinting (B) stimuli are presented sequentially, usually in the order ABBA for 2–4 min each, and the percentage imprinting calculated accordingly. Either of these methods gives a measurement of the amount of imprinting for each animal, which is beneficial for correlating with the size of the neurochemical changes measured to occur as a consequence of imprinting.

The earliest investigation of the neurobiology of imprinting demonstrated a significant increase in the incorporation of radioactive lysine into protein and radioactive uracil incorporation into ribonucleic acid (RNA) molecules in the roof of the forebrain, and not in the base of the forebrain or the midbrain (Bateson et al., 1972). The increase in RNA synthesis was shown to correlate with learning *per se*, rather than simply resulting from exposure to the stimulus. Chicks were exposed to the imprinting stimulus for varying periods of time on d 1 (20, 60,120, and 240 min) followed on d 2 by measuring incorporation of radioactive uracil into RNA as a consequence of a fixed period (60 min) of reexposure to the imprinting stimulus (Bateson et al., 1973). Less exposure on d 1 should lead to more learning on d 2 and therefore incorporation of a greater amount of uracil into RNA. This was, in fact, the result obtained: The incorporation of radioactive uracil into RNA on d 2 was negatively correlated with length of training on d 1. Since the chicks in each group received the same period of exposure to the imprinting stimulus on d 2 following the injection of radioactive uracil, the varying levels of incorporation reflected the amount of learning performed on d 1 and not simply exposure to the imprinting stimulus on d 2. A further experiment (Bateson et al., 1975) demonstrated that the amount of incorporation of radioactive uracil into RNA correlated with the strength of preference for the imprinting stimulus (i.e., percentage imprinting) scored in the choice test. That is, the chicks that learned more during the imprinting training synthesized more RNA in the roof of the forebrain.

Using autoradiographic techniques, the exact region of the roof of the forebrain in which these biochemical changes occur was narrowed down to the intermediate medial hyperstriatum ventrale (IMHV). Imprinting has been shown to correlate with a number of structural and neurochemical changes in this region of the forebrain (Horn, 1985, 1990, 1991).

Lesioning studies have revealed that the intermediate medial hyperstriatal ventrale, IMHV, region of the left hemisphere is involved in both the early and later phases of storage of imprinting memory, but the IMHV region of the right hemisphere is involved in the early events of memory storage only (Cipolla-Neto et al., 1982). If a chick is imprinted on d 1 and then the right IMHV is lesioned 3 h later, followed by lesioning of the left IMHV some 26 h later, no memory is present on retest. By contrast, if the left IMHV is lesioned first and then the right, the chicks can recall the memory on retest (*see* Horn, 1985, 1990). These results suggest that the left IMHV is involved in both the long-term and short-term processes of memory formation, whereas the right IMHV has a transient role in the initial (short-

term) phase only, so that when it is lesioned first, soon after training, the memory is lost, whereas when it is lesioned later the memory trace is unaffected. In the right hemisphere the memory appears to be shunted away from the IMHV to a store somewhere else in the hemisphere (Horn, 1985). This site is not yet known, although there is suggestion that it might be the lobus parolfactorius (LPO) or the archistriatum (Rose and Csillag, 1985; Salzen et al., 1975; and *see later).*

Thus, the right IMHV appears to act as a buffer store for memory, before the memory is moved to another region that may have a larger capacity for storage and perhaps an ability to modify and extend the memory through subsequent experience. The latter would give the memory more flexibility for use in a variety of contexts (Horn, 1990). A similar a role in memory formation is now assigned to the mammalian hippocampus (McNaughton et al., 1986). Horn and Johnson (1989) have suggested that, as is the case for the mammalian hippocampus, the right IMHV may add to the "depth" of processing by contributing contextual information during learning. In this context, it is worth noting that there is structural asymmetry of the mammalian hippocampus (Diamond, 1985), and also that a wealth of studies have implicated NMDA receptors in the mammalian hippocampus in long-term potentiation (LTP) and memory formation (e.g., Lynch et al., 1982; Melan et al., 1991; Kudo et al., 1991).

Lateralized involvement of the IMHV regions in imprinting learning is also apparent at the subcellular, electrophysiological, and neurochemical levels. After imprinting there are asymmetrical changes in the frequency of multiple-unit discharges recorded from the left and right IMHV regions (Davey and Horn, 1991). In dark-reared control chicks, the right IMHV has a slight but significantly higher level of discharge. In imprinted chicks the asymmetry is lost immediately after training, and 6 h after training it returns to an enhanced degree. Bursting activity is reduced in both left and right IMHV regions at 6 h after training. Imprinting learning leads to changes in the synaptic structure in the left IMHV, but no significant changes occur in the right IMHV (Bradley et al., 1981). In untrained control chicks the length of the postsynaptic density of the synaptic apposition zones is greater in the right IMHV than in the left. Training removes this difference by increasing the length of the postsynaptic density in the left IMHV. The increase in postsynaptic density profile is confined to axospinous synapses in the left IMHV (Horn et al., 1985), and there is some evidence that some axospinous synapses in the mammalian brain contain receptors for glutamate (*see* McCabe and Horn, 1991).

Indeed, imprinting has also been shown to correlate with a lateralized change in the number of receptors for glutamate and specifically of the NMDA receptors in the IMHV region. A significant increase in the level of glutamate binding occurs 7–8 h after imprinting in the left IMHV, but not in the right. McCabe and Horn (1988) measured ^{3}H-glutamate binding in chicks imprinted on a rotating red box at 8 h after they had been trained by exposure to the imprinting stimulus for 140 min. Controls were held in the dark, and not exposed to the imprinting stimulus. McCabe and Horn found significantly increased binding in the left IMHV of the imprinted chicks, compared to the controls. They also demonstrated an increased level of NMDA-displaced glutamate binding in the left IMHV, and that the amount of NMDA-binding correlated significantly with percentage imprinting scores. That is, the chicks that imprinted more strongly showed a greater increase in NMDA-receptor binding in the left IMHV, a result that strongly implicates the glutamate-sensitive NMDA receptors in imprinting memory formation, rather than some side-effect of the imprinting procedure.

McCabe and Horn (1988) attributed the increased binding levels of ^{3}H-glutamate to an increase in the number of glutamate receptors in the left IMHV, but their study could not definitively attribute increased glutamate binding to an increase in receptor number rather than to an increase in receptor affinity. Receptor number (B_{max}) and receptor affinity (K_d) may covary, and McCabe and Horn (1988) used a fixed concentration of glutamate, calculated an approximate K_d, and then held K_d constant. Without directly

estimating both variables (B_{max} and K_d) it is impossible to say which one is varying. A recently completed study used full Scatchard displacement techniques to determine both B_{max} and K_d in imprinted and control chicks 7–8 h after training (Johnston and Rogers, 1992; Johnston et al., 1993). Two regions of the forebrain were assayed; the hyperstriatum ventrale, which is an area containing the IMHV, and AS/LPO, an area containing the archistriatum and the lobus parolfactorius. ^{3}H-glutamate binding was performed. In the left hyperstriatum ventrale both the number and affinity of glutamate receptors increased (*see* Fig. 2). Control, nonimprinted chicks that were held in the dark had a tendency to have a higher B_{max} value for the right hyperstriatum ventrale compared to the left. Chicks imprinted on a rotating stuffed hen had a significantly higher B_{max} value for the left hyperstriatum ventrale compared to the right. A similar result was found for K_d values obtained for the hyperstriatum ventrale samples. In the AS/LPO sample, imprinting was found to have no effect on B_{max} (glutamate receptor number), but K_d was significantly elevated in samples taken from the left side of the brain of imprinted chicks. Thus, in the left AS/LPO, the affinity, but not the number of glutamate receptors, increases with imprinting. The change in glutamate receptor kinetics is therefore not specifically localized to the IMHV region. It would now be interesting to assay other regions of the forebrain for glutamate receptor changes.

Although the first experiment used by McCabe and Horn (1988) and that of Johnston et al. (1993) used L-glutamate to displace the ^{3}H-glutamate receptor ligand, other experiments of McCabe and Horn (1988, 1991) have used NMDA as the displacer, and so specified the changes in binding to the NMDA subtype of glutamate receptor.

The elevation of NMDA receptor number in the left IMHV appears to be a manifestation of long-term memory formation in the left IMHV since it does not occur prior to 6 h after training (Horn and McCabe, 1990; McCabe and Horn, 1991). Also, no increase in glutamate receptor number occurs in the right IMHV, and, as demonstrated by the lesioning studies, the right IMHV apparently plays no part in long-term storage of imprinting memory. In these experiments no significant change in NMDA-sensitive ^{3}H-glutamate binding was found to occur in either the left or right IMHV at less than half an hour or 3 or 6 h after imprinting. The change in the left IMHV, and not the right, occurred only 6–8.5 h after imprinting. As before, K_d was held constant in this study. It would therefore be of interest to assay for both receptor number and receptor affinity changes at various times after imprinting, and also to assay other regions of the forebrain.

The increase in length of the postsynaptic density in the left IMHV of imprinted chicks is seen at around 3 h after imprinting (Bradley et al., 1981; Horn et al., 1985), which is well in advance of the changes in receptor binding. Although NMDA receptors are associated with the postsynaptic density (Fagg and Matus, 1984), the initial change in the latter does not appear to be directly related to the increase in NMDA receptors. Possibly, new NMDA receptors are inserted into the postsynaptic thickenings after a delay period, although there is as yet no evidence that the increased number of receptors occurs in the same synapses that have undergone an increase in synaptic apposition length.

To summarize, it would seem that the lateralized change in NMDA receptor number may reflect learning-dependent plasticity changes in the left hemisphere. Changes in glutamate receptor numbers may also occur in other regions apart from IMHV and also elsewhere in the right hemisphere, but this awaits further study. A study by A. N. Johnston in my laboratory has shown that injection of glutamate into the right hemisphere at 1, 3, or 6 h after imprinting training blocks recall of the imprinting memory, whereas similar treatment of the left hemisphere has no effect (Johnston and Rogers, 1992, and Fig. 3). The effect produced by treating the right hemisphere could be motivational, but this seems unlikely since the activity level of the chicks is unchanged. At 9 h after training, glutamate treatment of either hemisphere is ineffective. Therefore, it would seem that temporarily enhancing

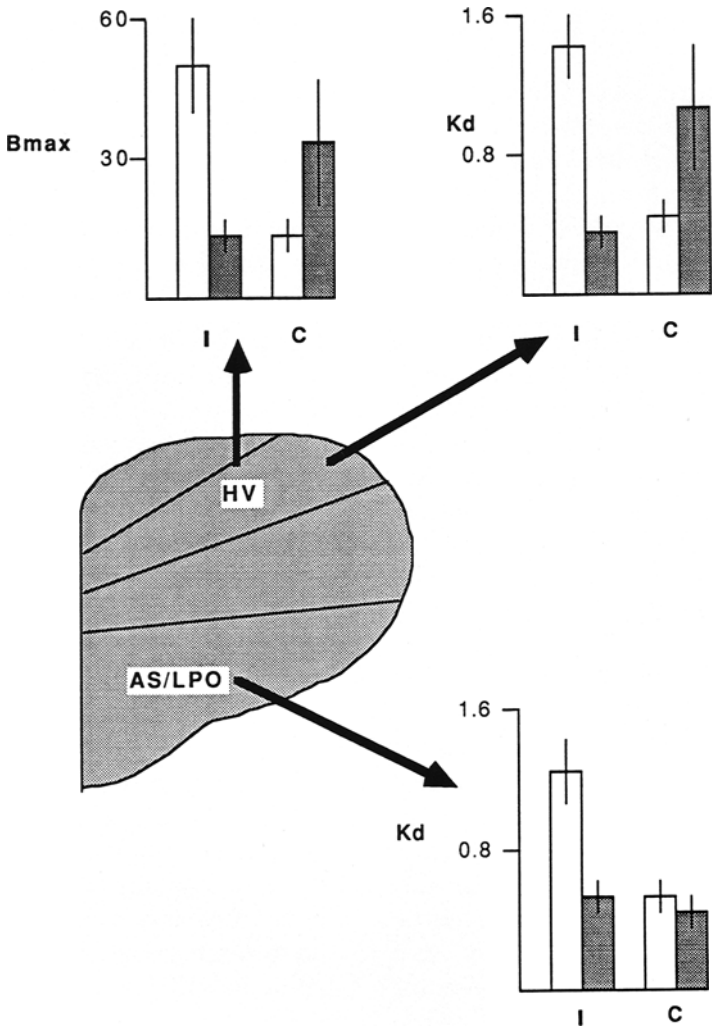

Fig. 2. Mean B_{max} and K_d values (with standard errors) of ^{3}H-glutamate binding in samples taken from the hyperstriatum ventrale (HV) and base of the forebrain (containing archistriatum and lobus parolfactorius, AS/LPO) regions of the left and right sides of the chick forebrain. The diagram indicates the sections made of the forebrain. The histographs present the data for the left (white bars) and right (gray bars) sides of imprinted (I) and control (C) chicks. For the HV, data for B_{max} (glutamate receptor number) and K_d (glutamate receptor affinity) are presented. Imprinting causes a significant increase in both of these values in samples taken from the left hemisphere *(see text)*. For the AS/LPO region the data for K_d only is presented, since no significant changes were scored for B_{max}. In AS/LPO from the left hemisphere imprinting causes an increase in glutamate receptor affinity. Data from Johnston et al., 1993, and reported in Johnston and Rogers, 1992.

glutamatergic mechanisms in the left hemisphere has no significant effect on the encoding of imprinting memory, in terms of glutamate receptor number and affinity increase. This result was unexpected, as also was the effect of glutamate treatment of the right hemisphere on recall. The latter treatment must block memory formation in both hemispheres, or access to the memory from either hemisphere. This suggests coordination between the hemispheres in the initial stages of memory formation. Given that a lesion placed in the right IMHV does not prevent recall *(see earlier)*, any disruptive effect of the glutamate treatment in the right hemisphere is

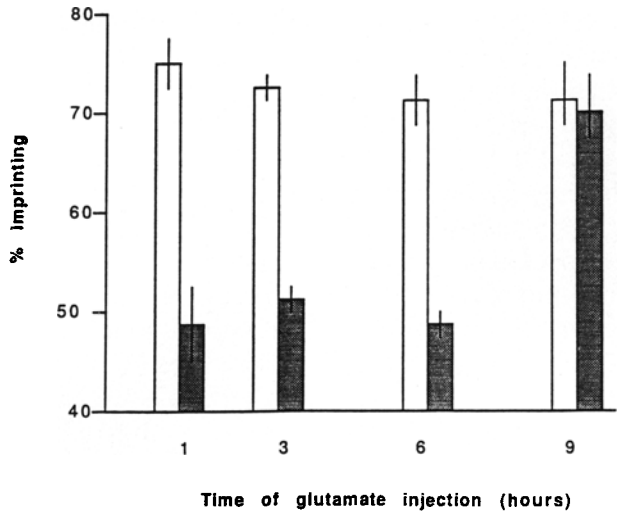

Fig. 3. The effect on recall of administering monosodium glutamate to either the left (white bars) or right (gray bars) hemisphere at various times after imprinting. Recall is expressed as percentage imprinting, which indicates the degree of preference that the chick shows for the imprinting stimulus, measured in terms of approach to the imprinting stimulus in a choice test between the imprinting stimulus and an unfamiliar stimulus *(see text)*. Recall was tested at either 8 h after training (for the first 3 injection times) or 24 h after training (for the 9 h injection time and a repeat of the 6 h injection time; the latter gave the same result as when tested at 8 h after training). Treatment of the left hemisphere had no effect on recall; all of these groups showed over 70% imprinting. By contrast, treatment of the right hemisphere at 1, 3, or 6 h after training blocked recall; these groups showed no preference for the imprinting stimulus over the unfamiliar stimulus (50% imprinting scores). Treatment of the right hemisphere at 9 h after training had no effect on recall, indicating that the cellular events disrupted by the elevated levels of glutamate were completed by this time.

likely to be outside the IMHV region. Furthermore, the action of glutamate on this region in the right hemisphere prevents the chick from accessing the memory from the left IMHV. In other words, the longer-term changes in the left IMHV (increased glutamate receptor number and affinity, and increased synaptic apposition length) cannot be the sole mechanism or site of memory storage. If this were the case, glutamate treatment of the right hemisphere should not prevent recall of the memory stored in the left IMHV. Leakage of glutamate into the left hemisphere cannot be the explanation for the result, because direct glutamate treatment of the left hemisphere is without effect on recall. Apparently, the learning experience leaves biochemical and structural traces in the left IMHV, but other forebrain sites in the right hemisphere become essential for recall.

The postulated analogous functions of the right IMHV and the mammalian hippocampus (Horn, 1990) deserve some further consideration, although it must be noted that the avian brain contains an anatomical structure, known as the hippocampus, that projects to IMHV (Bradley et al., 1985) and as for the mammalian hippocampus, it is larger in species that rely on spatial abilities for survival (Healy and Krebs, 1991; Sherry et al., 1992). Nevertheless, similar neurochemical changes are induced by learning in both the avian IMHV and the mammalian hippocampus. Lynch et al. (1982) have, for example, shown increased ^{3}H-glutamate binding in hippocampal slices of rat brain following afferent stimulation that induces long-term potentiation (LTP), suggesting that membrane receptors may undergo lasting change following behavioral stimulation. Scatchard analysis of the binding data for this

preparation indicated that the increased binding is caused by an increase in glutamate receptor number rather than affinity (Lynch and Baudry, 1984). The increased density and affinity of glutamate receptors in IMHV following imprinting may therefore be linked to induction of LTP or a related electrophysiological change.

The performance of rats on spatial tasks correlates inversely with the number of NMDA binding sites in the hippocampus and frontal neocortex (Wenk et al., 1989). Furthermore, NMDA antagonists impair spatial learning performance (Danysz et al., 1988; Morris, 1989). However, the nature and extent of the behavioral effects of NMDA antagonists is under active study, and these effects may not be confined to memory block *per se* (*see* Keith and Rudy, 1990; Pontecorvo et al., 1991). In the rat, NMDA receptors are rather wide-spread in the neocortex (Cotman et al., 1987), where they may either have a similar role in LTP and memory formation (Artola and Singer, 1987) or they may influence other cognitive functions. Further study of NMDA receptors in the chick brain may well raise similar questions about the specificity of their function and anatomical location.

Bradley and coworkers have measured an LTP-like response, which they term "persistent potentiation" (PPR), in IMHV in brain slices from the left side of the forebrain. Bursts of stimulation pulses have been shown to produce a 20–40% increase in amplitude of response in IMHV that persists for almost 2 h (Bradley et al., 1988, 1991a). Like LTP, PPR occurs only if NMDA receptors are functional and stressed by tetany (Bradley et al., 1991b). Activation of the NMDA receptors is necessary for PPR, but the NMDA-component of the response is not potentiated. Rather, non-NMDA-sensitive receptors in excitatory circuits are potentiated. All of these properties of PPR have much in common with LTP in the mammalian hippocampus. Thus, in the chick, as in the rat, PPR may lead to the increase in glutamate receptor number and affinity that occurs after imprinting.

In brain slices taken from chicks of various ages, Bradley et al. (1991c) have found that the glutamate receptor antagonist, D,L-2-amino-5-phosphovaleric acid (APV) has a greater effect in preventing PPR in slices taken from dark-hatched chicks aged between 3 and 5 d, compared to both younger and older chicks. In light-hatched chicks the probability of producing PPR is high on the first day posthatching (Bradley et al., 1991d). Thus, the probability of producing PPR may reflect an in vivo sensitive period during which glutamate receptor changes can occur in response to imprinting, or it may reflect a sensitive period for another type of learning (Bradley et al., 1991d).

The studies of imprinting discussed so far have concentrated on visual imprinting and the role of the IMHV region, but other regions of the forebrain may also be involved in imprinting, particularly to auditory stimuli. Reexposure of 7-d-old guinea fowl chicks to their auditory imprinting stimulus (a tone) causes increased metabolic activity, as indicated by uptake of 2-deoxyglucose, in three rostral brain regions, including areas of the hyperstriatum, an auditory area called MNH encompassing part of the rostromedial neostriatum and hyperstriatum ventrale, and LNH in the rostrolateral neostriatum and hyperstriatum ventrale (Maier and Scheich, 1987). A similar result was found for domestic chicks reexposed to an auditory imprinting stimulus, and there was a 47% loss of dendritic spines on large type I neurons in MNH of the imprinted vs control chicks (Wallhäusser and Scheich, 1987). MNH receives auditory input via the dorso-caudal neostriatum and it has reciprocal projections back to this latter region. It is those neurons that project from MNH to the dorsocaudal neostriatum that lose spines when imprinting occurs (Dörsam et al., 1991).

It is possible that MNH plays a role in auditory but not visual imprinting, but it should be noted that MNH is adjacent to IMHV. In fact, it seems likely that regions of the forebrain other than IMHV are involved in visual imprinting. Salzen et al. (1975) found that lesions of the lateral neostriatum impaired imprinting to objects suspended in the chick's home-cage, whereas lesions that included IMHV had no effect. The discrepancy between this study and those of Horn's group apparently depends on the

nature of the visual imprinting stimulus, since Salzen (1991) has now found that IMHV lesions do impair imprinting to a moving patterned stimulus suspended in the home-cage. Thus, task and stimulus type may influence the brain regions involved in memory formation. Further research needs to address the possibility of regional differences in the neurochemical changes involved in memory formation, although these seem somewhat less likely to occur. In fact, Braun et al. (1992) report that administration of the NMDA receptor-antagonist APV into MNH blocks auditory imprinting.

Neural Plasticity and the Sensitive Period for Imprinting

Once imprinting has occurred, the sensitive period for it closes and the chick will not imprint on another stimulus, or only with much greater difficulty (Bolhuis and Bateson, 1990). Having formed an attachment to a particular individual or object, the young chick avoids other individuals or novel objects. If, however, the chick is reared in darkness or, at least, prevented from seeing patterned-light, the imprinting period can be extended until the end of the first week of life, but not longer (*see* Sluckin, 1972). Apparently, during the first week of life the sensitive period for imprinting remains open until exposure to the appropriate stimulus occurs, and then it is closed. In other words, the neurochemical events that occur with imprinting learning must not only encode the memory, but also actively close the sensitive period.

Little consideration has been given to the neurochemical events that may close the sensitive period. Some researchers argue that the sensitive period is closed by behavioral mechanisms, because the imprinted chick avoids strange objects and so it is no longer in a position to imprint even though it may still be neurobiologically capable of doing so. On the other hand, it is highly probable that there are neurochemical changes that prevent the imprinted brain from reimprinting. They may be the same subcellular processes that encode the memory, or concomitant, but different, processes. The increase in glutamate receptor number and affinity that follows imprinting may, for example, close the sensitive period. However, it is not known for how long the glutamate receptor number and affinity is increased following imprinting. The maximum delay period between imprinting and assaying that has so far been tested is 8 h. It is possible that these increases are transient (*see* McCabe and Horn, 1991), and are even followed by a decrease in glutamate receptor activity, the latter marking the end of the sensitive period for imprinting.

In cats, active glutamate-sensitive NMDA receptors have been associated with the sensitive period for neural plasticity, but not with its termination. The activation of NMDA receptors has, for example, been shown to be essential for modifications in the cat visual cortex in response to experience. During a sensitive period in early postnatal development, neural connections in the visual cortex are modified in response to the visual input received. If the lid of one eye of the kitten is sutured, the cells in the visual cortex become dominated by the open eye (Hubel and Wiesel, 1970). This sensitive period has recently been shown to coincide with elevated levels of ^{3}H-glutamate binding sites in the primary visual cortex (Bode-Greuel and Singer, 1989). The binding sites were found to increase dramatically between the second and fourth week of life, and they remained elevated throughout the sensitive period. Toward the end of the sensitive period, they declined to adult levels, suggesting that the sensitive period is closed by a fall in the number of active receptors for glutamate.

Blockage of NMDA receptors prevents the experience-dependent modifications that occur in the kitten cortex. Kleinschmidt et al. (1987) applied the NMDA receptor antagonist APV to the striate cortex of kittens when they had one eyelid sutured. The drug was administered slowly over 1 wk by placing an osmotic minipump into area 17. Electrophysiological recordings made at the end of 1 wk revealed that the APV exposed cortex had resisted the effects

of monocular deprivation. In other words, blocking the NMDA receptors had prevented the neural plastic changes that normally occur in response to monocularity during this sensitive period.

The effects of monocular deprivation in the kitten can be reversed if, still within the sensitive period, the originally sutured eyelid is opened and the other eyelid is sutured (Blakemore and Van Sluyters, 1974). The cortical neurons shift to being responsive to the newly opened eye and unresponsive to the newly closed eye. This reversal of the effects of monocularity is also NMDA receptor-dependent, since it can be prevented by APV (Gu et al., 1989).

Similar to the effect of APV, the anesthetic ketamine–xylazine blocks the cortical cell modifications that occur in the kitten in response to monocularity. Rauschecker and Hahn (1987) gave kittens daily brief (20 min) monocular experience followed by ketamine–xylazine anesthesia that lasted for 1 h. The treatment, which continued until the kittens had received 30 h of monocular exposure, had a retrograde effect on the cortical plasticity, preventing the shift in ocular dominance. A control group received the anesthesia after a delay period of 1 h following each monocular exposure, and these kittens showed the expected ocular dominance shift. Subsequent experiments tied the blockage of neural plasticity to the action of ketamine, which blocks NMDA receptors, and not to xylazine, an α-adrenoceptor agonist (Kossel et al., 1987; Rauschecker et al., 1990).

Recent experiments by C. H. Parsons in my laboratory have shown that treatment of chicks on d 1 after hatching with the ketamine–xylazine mixture can extend the sensitive period for imprinting into the second week of life (Parsons and Rogers, 1992). In these experiments the chicks were incubated, hatched, and reared in complete darkness. Even under these conditions control chicks will not imprint in the second week of life, d 8 in these experiments. By contrast, chicks treated with ketamine–xylazine shortly after hatching were found to imprint well on d 8. In the retest on d 9, they showed about a 70% preference for the imprinting stimulus, compared to 50% in controls. The effect is, however, not specific to ketamine and, therefore, the NMDA receptor alone, as is the case for neural plasticity in the kitten; neither ketamine nor xylazine administered alone extends the sensitive period. Apparently, both NMDA and α-adrenergic receptors are involved, a result that may not be too surprising since noradrenergic mechanisms have been implicated in neural plasticity in the kitten (Kasamatsu et al., 1979; Bear and Singer, 1986) and, in particular, in imprinting in the chicken (Davies et al., 1985). A more recent report by Shirokawa et al. (1989) has tied the plasticity of ocular dominance columns in the kitten to β_1-adrenoceptors. Specificity of receptor subtype has not yet been studied in the chick. Davies et al. (1985) administered the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP4) to chicks prior to imprinting training with either the rotating box or stuffed hen. The treated chicks exposed to the box showed significantly reduced imprinting, but those exposed to the hen were unaltered. This implicates noradrenergic receptors in imprinting to some stimuli and not others. Apparently, noradrenergic receptors facilitate imprinting to artificial stimuli, or less complex stimuli. Consistent with the result obtained using DSP4, Davies et al. (1983) have shown that imprinting on the box causes a significant rise in noradrenalin levels in the chick forebrain, measured immediately after training and up to 50 h after training. It should be noted, however, that the experiments by Parsons and Rogers (1992), showing extension of the sensitive period for imprinting into the second week of life, used both the box and the stuffed hen as imprinting stimuli, and no difference was found for these two stimuli. This result is, however, consistent with that of Johnson et al. (1989), who found that the predisposition to approach a stuffed fowl in preference to a box was confined to a sensitive period in the first 2 d of life and thereafter learning about the box does not differ from learning about the fowl.

Collectively, these studies implicate an interactive role for noradrenergic and glutamatergic receptors during the plastic phase of neural development in the chick, when imprint-

ing occurs. Since the duration of the sensitive period is extended by modulating the activity of these receptors, one might predict that the NMDA receptor number would be elevated in the second week of life in chicks that have received prior treatment with ketamine–xylazine.

If a change in NMDA receptor number, presumably a decline, does indeed mark the end of the sensitive period for neural plasticity and imprinting in chicks, it might also be the neurochemical basis for the shift from early forms of learning ability to later, more adult, forms of learning ability. For example, chicks in their first week of life show poor learning performance on the task requiring them to discriminate grain from a background of pebbles (Rogers, 1986). In the second week of life, their ability to perform this task is markedly improved. Of course, the improvement in this ability could depend on the maturation of an entirely different set of neurochemical processes, or it could be linked to the decline in neural plasticity for imprinting. Nevertheless, the end of the sensitive period for imprinting phases into the beginning of a period in which plastic changes must subserve other forms of learning. The latter may differ either quantitatively or qualitatively from early learning, such as imprinting, that occurs during the sensitive period of maximal neural plasticity. Possibly, this shift from early to later forms of learning ability is delayed or prevented in the chicks treated with glutamate injected into the left hemisphere on d 2 *(see earlier)*, and this may be why they are slow at learning the pebble floor task. There are many exciting openings for further research aimed at understanding the neurobiology of sensitive periods in development and learning ability.

Passive Avoidance Learning

The IMHV and LPO regions of the chick forebrain are also involved in learning and memory of a passive avoidance task involving pecking at a bead coated with methylanthranilate (Rose, 1989). This task is used in a number of laboratories studying the neurobiology of memory (Andrew, 1991; Gibbs and Ng, 1977; Rose, 1991a,b). As mentioned earlier, the chick is trained by pecking at a bitter-tasting bead (usually a red bead) coated with methylanthranilate. This evokes a disgust response from the chick and it will avoid pecking a similar (red) bead presented at retest. The chick will, however, peck at a bead of another color. Following passive avoidance learning, a cascade of cellular processes occurs spanning time-courses ranging from minutes to hours (Rose, 1991b). Coincident with the training and immediately afterward there is enhanced uptake of glucose in the IMHV and LPO. In fact, as for imprinting, the left and right IMHV and LPO regions are differentially involved. Performance of the task results in different patterns of neural activity, detected by uptake of [^{14}C] 2-deoxyglucose (2-DG) in the left and right hemispheres (Rose and Csillag, 1985). The chicks were given a pulse of 2-DG lasting for 30 min just after training with the methylanthranilate-coated bead. Compared to controls, which pecked at a bead coated with water, the trained chicks showed increased accumulation of 2-DG in the IMHV and the LPO, the greatest changes occurring in the IMHV and the LPO of the left hemisphere.

The neurochemical changes that follow this event include first phosphorylation of the presynaptic membrane protein kinase C substrate, B50, (Burchuladze et al., 1990) along with translocation of protein kinase C and genomic activation of the protein oncogenes c-*fos* and c-*jun* (Anokhin et al., 1991). Somewhat longer-term changes (1–6 h after training) include the synthesis of the protein tubulin (Anokhin and Rose, 1991; Scholey et al., 1991), synthesis of pre- and postsynaptic glycoproteins (Bullock et al., 1990; McCabe and Rose, 1985) and bursting of neuronal activity (Mason and Rose, 1987). Neuronal "bursting" activity, recorded as extracellular multiunit activity from the IMHV, lasts for up to 12 h after training (contrast this to the decline in bursting activity at 6 h after imprinting; Davey and Horn, 1991, and *see earlier*). Finally, at 12–24 h after training, morphological changes can be detected in synaptic structures. These include lengthening of the synaptic apposition zones and increased density of dendritic spines (Patel and Stewart, 1988;

Stewart et al., 1984; and *see later*). It is possible that these changes are part of a linked sequence or cascade, as Rose (1989, 1991b) suggests, but to prove this it is first necessary to determine whether all of the changes occur in the same brain locations.

The neurochemical changes and changes in subcellular structure that occur soon after passive avoidance learning are largely localized in the left, and not the right, IMHV, although some of the studies do not give information on hemispheric differences since they have used the entire forebrain. There is an increase in the concentration of synaptic membrane-bound protein kinase C, compared to soluble protein kinase C, that occurs in the forebrain. This enzyme phosphorylates the B-50 protein, which becomes located at the synapse within 1–6 h after training (Ali et al., 1988). Consistent with this, amnesia is produced by injecting melittin or H7, inhibitors of this enzyme, into the left hemisphere (Burchuladze et al., 1990). Incidentally, protein kinase C has been shown to modulate NMDA receptor activity in rat hippocampal slices (Aniksztejn et al., 1992).

Glutamate receptor binding is increased in the left IMHV, and not in the right IMHV. Using quantitative receptor autoradiography, Stewart et al. (1992) have shown that 30 min after passive avoidance training there is a significant (39%) increase in NMDA-sensitive ^{3}H-glutamate receptor binding in the left IMHV. There is also an increase in binding to MK801-sensitive glutamate receptors in the left IMHV. This parallel increase in both glutamate receptor subtypes is consistent with expectations since MK801 is a noncompetitive antagonist of the NMDA receptor that acts by binding to the NMDA ion channel complex. In the same study, no changes were found in ^{3}H-AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid) binding 30 min after training. Because AMPA binds to the quisqualate receptor subtype of glutamate receptors, it can be concluded that the changes are specific for the NMDA receptor subtype of glutamate receptor. It should be noted that the increase in NMDA receptor binding following passive avoidance learning occurs much earlier than following imprinting (6–8 h after imprinting). This may suggest differential involvement of NMDA receptors in shorter and longer term processes of memory formation of the two different tasks. Therefore, whether NMDA receptor activation occurs at several steps in memory formation, rather than being specific for acquisition or longer-term retention, needs to be determined. Possibly its more delayed occurrence following imprinting relates the earlier discussed hypothesis of Horn and Johnson (1989) of increasing "depth" of processing as contextual information is added to the imprinting memory, but the hypothesis relates to the right hemisphere rather than the left.

Confirmation of the role of NMDA receptors, rather than the non-NMDA kainate/quisqualate subtype, in passive avoidance learning has been provided by investigation of the effects of glutamate, NMDA, MK801, AMPA, and quisqualate on the accumulation of inositol phosphates from breakdown of phosphoinositides in vitro in tissue from trained and nontrained chicks (Bullock et al., 1992). At 30 min after training there is a significant decrease in glutamate-stimulated accumulation of intracellular inositol phosphates in prisms of tissue taken from the left IMHV, compared to both the right IMHV and to samples taken from control chicks. No such effects were seen in samples taken at either 5 min or 3 h after training. Administration of MK801 just prior to training abolished the effect of training. Thus, there appears to be transient activation of NMDA receptor channels, and not quisqualate/AMPA receptors, in the left IMHV during the early phases of memory formation.

Furthermore, intracranial administration of MK801 at 1 h before or 5 min after training produces amnesia in chicks tested at 3 or 24 h later (Burchuladze and Rose, 1992). There was, however, no amnesia when these MK801-treated chicks were tested 30 min after training, the time at which the NMDA and MK801 binding is increased after training. Nevertheless, administration of MK801 at 1 or 6 h after training does not cause amnesia at 24 h, which suggests that NMDA receptor activity at or close to the time of training is necessary to enable the formation of

long-term memory. In the same study, intracranial administration of non-NMDA glutamate antagonists had no affect on memory of the passive avoidance task, adding support for the role of NMDA-sensitive glutamate receptors rather than kainate or quisqualate receptors.

Although the biochemical studies have located changes in the left IMHV only, there is neurochemical evidence for a role of the right IMHV in the memory formation. Barber and Rose (1991) report that injection of 2-deoxy-D-galactose, which inhibits brain glycoprotein fucosylation, into the right hemisphere causes amnesia of the passive avoidance task, whereas injection of this drug into the left hemisphere has no effect on memory recall. This particular result is consistent with an earlier finding of a lateralized increased incorporation of fucose into glycoproteins in the right forebrain base (containing LPO) of trained chicks (McCabe and Rose, 1985).

There is considerable evidence that long-term memory formation is associated with enhanced incorporation of precursor sugars into glycoproteins, these being destined for the synaptic membrane where they alter neurotransmission (Rose and Jork, 1987). At 12–24 h after training the mean number of neurotransmitter containing vesicles per synapse in the left IMHV is increased, the density and size of dendritic spines on large multipolar projection neurons is increased (Stewart et al., 1984; Stewart, 1991), and the spine head diameter is increased (Patel and Stewart, 1988). The IMHV regions of trained chicks are strikingly asymmetrical; for example, the number of vesicles per synapse in the left IMHV exceeds that of the right by more than 50% (Stewart et al., 1984).

In addition to IMHV, the LPO and possibly the paleostriatum augmentatum (PA) and lateral neostriatum regions are involved in memory formation of the passive avoidance task. At 30 min after passive avoidance training Stewart et al. (1992) found a significant increase in NMDA-sensitive ^{3}H-glutamate receptors in the left LPO. This increase was of the same order of magnitude as that occurring in the left IMHV. MK801 binding also increased in the left LPO, whereas ^{3}H-AMPA binding showed no change, again indicating a specific effect for NMDA vs non-NMDA receptor type. MK801 binding increased in the right LPO. At 24 h after training, the mean postsynaptic thickening length in the LPO region is greater on the right side in controls and on the left side in chicks trained in the passive avoidance task (Stewart et al., 1987). Both sides of the LPO show a significant increase in the density of synapses. A more recent study by Hunter and Stewart (1991) found a significant increase in synaptic density (of around 30%) in the left LPO at 24 h after training, and lesser (10%) increase at 48 h after training. At 48 h after training the right LPO had approx an 18% increase in synaptic density. These changes in synaptic density in the LPO regions are matched by increases in the number of dendritic branches and increased dendritic length (Lowndes et al., 1991).

Stewart et al. (1987) also examined the subcellular structure of the PA region of the forebrain, and found no asymmetry or changes with training in the postsynaptic density thickening in this region of the forebrain. They did, however, find that the density of synapses and the number of vesicles per synaptic bouton to be higher in the right PA of controls, and that this asymmetry was removed by training. Further evidence for the involvement of PA in the memory formation comes from a study that demonstrated changes in neurotransmitter levels in PA as a consequence of imprinting (Bullock et al., 1987). The acetyl choline content of PA was reduced by up to 48% in trained chicks, the change being greater in the left PA. The study also included measuring the titers of antibodies raised to synaptic vesicle proteins. Training reduced the titer in left PA of the antibodies to synaptic vesicles. Incidentally, related changes also occurred in IMHV and LPO.

There is some evidence that, as for auditory imprinting, the lateral neostriatum in the right hemisphere may also be involved in passive avoidance learning, since injection of glutamate into the right, but not the left, neostriatum causes amnesia (Patterson et al., 1986). By contrast, injection of glutamate into the left, but not the right, IMHV causes amnesia. It should be noted that the spread of glutamate was not traced in this

study; that is, although the IMHV and lateral neostriatum were targeted, other areas of the forebrain could have been affected. Nevertheless, different regions of each hemisphere appear to be used to store memory of the task, and possibly different forms of memory are laid down accordingly. Yet, other evidence suggests a role of the left lateral neostriatum, as well as the left IMHV and LPO, in passive avoidance memory formation: At 30 min after training a significant 44% decrease in NMDA-sensitive ^{3}H-glutamate binding occurs in left lateral neostriatum, as opposed to an increase in left IMHV and LPO (Stewart et al., 1992).

More recently the passive avoidance learning task has been useful for showing another cellular aspect of memory formation; the expression of "immediate-early" genes, such as the c-*fos* gene, which codes for a nuclear phosphoprotein implicated in the regulation of transcription of "late genes," that may possibility hold long-term memory (Curran and Morgan, 1987). That is, the early genes may act as nuclear signals for long-term memory formation to be ultimately expressed in terms of synaptic modifications. Expression of the c-*fos* gene is induced after LTP in the hippocampus (Dragunow et al., 1989), and its expression is induced by the activation of NMDA receptors that are linked to calcium channels. A study by Anokhin et al. (1991) has shown that the c-*fos* gene is expressed 30 min after passive avoidance learning. This expression occurs in both the left and right IMHV and LPO, but in control chicks the level of c-*fos* gene expression was found to be higher in the left IMHV. Although the expression of c-*fos* may be triggered by stress or arousal rather than the learning *per se*, this was to some extent controlled for by measuring c-*fos* expression in monocularly trained chicks. The c-*fos* was expressed only on the side of the brain contralateral to the open eye, suggesting that stress is not likely to be a factor controlling its expression.

In this context, it is worth mentioning that training chicks to discriminate food grains from a background of pebbles (a task discussed earlier) also leads to the expression of immediate early genes, c-*fos* and c-*jun*, 30 min after training (Anokhin et al., 1991). The genes were expressed in the forebrain, but the method did not delineate the specific regions involved. The expression of these genes occurred only in chicks that were learning the discrimination, and not in chicks that had been previously trained and were simply being retested on the task. Thus, the immediate early genes appear to be involved in memory formation of more than one task. Other neurochemical correlates of learning the "pebble floor task" have not yet been examined.

Lesioning studies have demonstrated the differential role of the right and left IMHV regions in the laying down of memory of the passive avoidance learning task (Patterson et al., 1990). Chicks that have had the left IMHV lesioned on d 1 posthatching and are trained on d 2 are amnesic when tested for recall on d 3. A similar lesion of the right IMHV does not impair the memory formation.

Although lesioning the right IMHV does not result in amnesia in binocularly trained and tested chicks, it is capable of taking over from the left IMHV in chicks trained and tested monocularly. Sandi et al. (1992) have found that in chicks wearing an eyepatch on the right eye (using the left eye), lesions of the right IMHV disrupt learning and memory acquisition, but that lesions of the left IMHV are ineffective. These rather anomalous results, in comparison to the binocular situation at least, may occur as a consequence of the monocular experience causing shifts in the areas that are used for the memory. In addition, the same researchers have demonstrated that interocular transfer of the passive avoidance task occurs more readily from the right eye to the left (i.e., when training is with the right eye and testing with the left) than vice versa. That is, the left eye has better access to information acquired by the right eye than the other way around.

Pretraining lesions of the left (but not the right) IMHV cause amnesia, but pretraining lesions of the LPO have no effect on recall (Patterson et al., 1990). However, bilateral lesions of IMHV made as little as 1 h after training have no effect on recall, suggesting that the memory must move on to another forebrain site, such as the LPO (Patterson et al., 1990). In fact, bilateral lesions of

the LPO made after training do produce amnesia (Gilbert et al., 1991). This suggests that, in the absence of LPO during training and soon after, the memory is retained in the IMHV. In fact, if the LPO is lesioned bilaterally before training, lesioning of the right IMHV after training results in amnesia. By piecing together these results, Rose (1991b) has suggested a model in which the memory "flows" from left IMHV to right IMHV and then on to the left and right LPO regions. The memory must "flow" on to LPO for long-term storage.

If the memory flows from left to right IMHV and then on to LPO, one might question the functional significance of the structural changes in the synapses that occur in the left IMHV. Their occurrence suggests that, although the main accessible aspects of the memory may flow out of the left IMHV, at least some facet of the memory must remain there. The latter may possibly be retrieved along with the "main" memory, adding to the complexity of the stored and retrieved memory. Patterson and Rose (cited in Rose, 1991c) have presented some evidence that IMHV may store memory of the color cue of the methylanthranilate bead, but not memory of other cues: IMHV lesions prevent color discrimination of beads, but LPO lesions do not. Presumably, LPO may store memory of other, as yet unknown, cues of the beads. Thus, memory of the task may be stored at more than one brain site and in each place in a different representational form.

There are no known direct connections between the left and right IMHV regions, or between IMHV and LPO (Bradley et al., 1985) via which the memory might "flow." Nevertheless, the IMHV regions do connect with the PA and dorsal archistriatum and these, in turn, connect to part of LPO (Benowitz, 1980). As already mentioned, some evidence implicates PA in formation of memory of the passive avoidance task, and the archistriatum has been shown to have a role in imprinting memory (Salzen et al., 1975). If these pathways are the route via which the memory might flow to LPO, lesions of PA or the archistriatum would be expected to interrupt the memory flow. This has not yet been investigated.

Most interestingly, the projections from IMHV to the archistriatum have asymmetrical synapses with dendritic spines and, as a recent study using immunostaining has shown (Csillag, 1991), some of these asymmetrical synapses are immunopositive for glutamate. Also, the projections from the archistriatum to LPO have a wealth of glutamate immunopositive axon terminals at asymmetrical synapses. This suggests that changes in glutamate receptor number or affinity in LPO might follow passive avoidance learning, as they do imprinting (*see earlier*), but other neurotransmitter systems in LPO (especially local circuits that use GABA) are equally likely to be involved in memory formation.

Conclusions

It is becoming increasingly apparent that the memory processes of imprinting and passive avoidance learning of the methylanthranilate bead task have much in common, in terms of the forebrain regions involved, with the lateralization of the memory storage and the neurochemical events that occur. Although there may be differences in the timing of memory shifts from one region of the forebrain to another, and further study may reveal differences in the details of memory formation in each case, imprinting and passive avoidance learning rely on essentially the same neurobiological processes and these, in turn, closely parallel the processes that have been shown to underlie changes in neural connectivity that occur in the cortex of kittens during early development (*see* Rauschecker, 1991). The important role of NMDA-sensitive glutamate receptors in both forms of early learning in the chick and in neural plasticity in kittens is perhaps the strongest link between the two processes.

As discussed for imprinting in this review, and by Rauschecker (1991) for neural plasticity in kittens, although other neurotransmitter systems are also involved in these experience dependent changes, the role of the NMDA receptor is paramount. Stimulation of NMDA receptors opens ion channels for calcium ions to enter the cell and act intracellularly as a second messenger, activat-

ing enzymes that may play a key role in LTP and synaptic plasticity (reviewed by Rauschecker, 1991).

If we are to draw further parallels between early learning and neural plasticity in the chick and neural plasticity of visual cortex in the kitten, the timing and exact nature of the events mediated by NMDA receptors needs to be considered. It is known that ketamine prevents the neural plastic changes that normally occur in response to monocularity in the kitten but, apart from our study of ketamine/xylazine's ability to extend the period of imprinting into the second week of life *(see earlier)*, there has been no study of the effect of ketamine on memory formation *per se* in the chick. We know that NMDA-sensitive glutamate receptors increase in number and affinity following both imprinting and passive avoidance learning, but we do not know whether they change similarly when kittens are, for example, made monocular during the sensitive period. We know that intracranial administration of glutamate into the forebrain hemispheres prevents recall of imprinting (when the right hemisphere is treated) and passive avoidance learning (*see* Ng et al., 1992), but we do not know whether glutamate treatment has any effect on the shift in ocular dominance in kittens.

In the chick, treatment of the left hemisphere with glutamate after imprinting has no effect on later recall (Johnston and Rogers, *see earlier)*, although it is in the left hemisphere (IMHV and LPO regions) where the changes in glutamate receptor number and affinity occur several hours later (Johnston et al., 1988). Thus, it would be interesting to know whether the glutamate treatment of the left hemisphere affects the delayed changes in the glutamate receptors that follow imprinting. Seemingly, it may have no effect, unless regions in the right hemisphere take over to store the memory. Also, when ketamine and xylazine treatment extends the sensitive period for imprinting, does it do so by acting in the left and/or right hemisphere, and what is the effect on glutamate receptor changes that might occur in response to imprinting in the second week of life? All these questions will need to be answered if we are to go beyond merely noting the important roles played by glutamate receptors in early learning in the chick and in the neural plastic changes in the kitten.

Both imprinting and the passive avoidance bead task are forms of learning that occur in chicks soon after hatching. It is possible, therefore, that they have more in common with neural plasticity during development than do other forms of learning that occur later in life. Learning in adult rats has, however, been shown to involve NMDA receptors (Melan et al., 1991). It would now be of interest to study the neurobiological events that correlate with other forms of learning in older chicks so that direct comparison can be made to the early learning paradigms. Learning to discriminate grain from pebbles is a task well suited to this study, since chicks do not perform this task well until the second week of life. It is clearly a form of learning that occurs more readily after the imprinting/early learning period has passed.

It is already known that performance of the pebble-floor task leads to expression of immediate early genes (Anokhin et al., 1991), as it does for passive avoidance learning, but no other neurochemical changes that follow pebble-floor learning have yet been investigated. Nevertheless, from the studies showing that glutamate treatment of the forebrain leads to slower learning of the pebble-floor task, it is likely that glutamate receptors play a role in this form of learning. Furthermore, it would be most important to know whether the same regions of the forebrain (in particular, the IMHV and LPO) are involved in memory storage of the pebble-floor task, and whether the memory shows the same "flowing" from region to region in the brain as it appears to do following passive avoidance learning. These are all questions that could be answered with the available techniques.

The chick brain has a number of asymmetries present in terms of the structure of at least one of the visual pathways (Rogers and Sink, 1988; Adret and Rogers, 1989), in the density of synapses in the hyperstriatum accessorium, HA (Stewart et al., 1992), in the length of the postsynaptic densities in IMHV (Bradley et al., 1981), and

in functional control of behavior (Rogers, 1986, 1991; Mench and Andrew, 1986; Andrew 1988; Gaston, 1984). From the study of glutamate binding discussed in this paper (*see* Fig. 2) there is an indication that in control chicks glutamate binding (receptor number and affinity) is higher in the right HV compared to the left. With a larger sample size this difference may be significant.

Some of the asymmetries in the chick brain are dependent on asymmetrical stimulation of the eyes by light prior to hatching, since the embryo is oriented in the egg so that it occludes its left but not its right eye (Rogers, 1986). This asymmetrical stimulation by light enhances development of the thalamofugal visual projections fed by the right eye (Rogers and Bolden, 1991) and determines the direction of a number of functional asymmetries controlled by the forebrain (Rogers, 1982b, 1990; Zappia and Rogers, 1983). Other asymmetries seem to be unaffected by light stimulation (in particular, the asymmetry of synaptic density and glutamate binding in IMHV) because the chicks used in these studies were incubated and hatched in darkness. Of course, light stimulation before hatching may well generate asymmetries in neurotransmission, including glutamate receptor mechanisms, in other forebrain regions, most likely in those regions receiving visual input. (e.g., it may cause the greater density of synapses in right HA, since the chicks used in the study by Stewart et al., 1992, received light before hatching).

Whereas light stimulation prior to hatching generates some of the asymmetries, learning that occurs immediately following hatching (imprinting or passive avoidance) imposes other forms of asymmetry on the forebrain. The asymmetry in glutamate binding in IMHV is enhanced or reversed (Fig. 2), as also is that of synaptic density in IMHV. This highlights the need to control the light stimulation during incubation of chicks to be used in the study of memory formation, and to report the incubation conditions used in all studies. Although it is known that the chicks used in the imprinting studies were incubated in darkness, those used in passive avoidance training have usually been exposed to light, a factor that may possibly explain some of the apparent, although minor, differences between the memory processes in each case.

There are two most important aspects of understanding that have emerged from the studies using chicks. First, memory is not held in a fixed location in the brain but either "flows" from one region to another leaving neurochemical traces in each site as it moves on or multiple sites are involved. Second, differential use of the left hemisphere is a dominant aspect of all of the memory processes so far investigated. It is unlikely that the flow of memory and its asymmetry is unique to the chicken brain. Hence, it certainly deserves consideration in studies of memory formation and neural plasticity in mammals.

Although this review has focused on the role of glutamate receptors in early learning and development, it is well recognized that other receptor types may also be important in these processes. Some mention has been made of a role for α-adrenergic receptors in imprinting (Davies et al., 1983, 1985). Following passive avoidance training by chicks there are also alterations of the binding levels in the forebrain of QNB to muscarinic receptors at 30 min and 3 h, α-bungarotoxin to nicotinic receptors at 30 min but not 3 h, serotonin to sertonergic receptors at 3 h, and muscimol to $GABA_A$ receptors at 24 h (Rose et al., 1980, Aleksidze et al., 1981; Bourne and Stewart, 1985). The increase in NMDA binding following passive avoidance learning appears to be transient and to coincide with the changes in cholinergic and serotinergic receptors, and to precede a much longer-term increase in GABAergic receptors. Now more precise delineation of the time courses and of potential interrelationships between the changes in the various receptor types is required. Thus, a change in one receptor type may enable a delayed change in another type. Alternatively, the changes in different receptor types may occur in different regions of the forebrain, or the time courses of change may vary in different brain regions. So far, this has not been investigated. The time courses may also be task-specific. As mentioned, the increase in NMDA-sensitive glutamate binding is more delayed following imprinting compared to passive avoidance

learning. Nevertheless, the transient earlier change following passive avoidance learning appears to enable longer-term biochemical changes that encode the memory (Burchuladze and Rose, 1992).

A final point should be made about the need for detailed study of the behavior of the chicks in the tasks used for the study of the neurobiology of learning and memory, particularly with respect to the lateralization. Hemispheric lateralization of the memory trace may either result from lateralized use of the monocular visual fields (most information from each eye is processed by the opposite hemisphere) or it may direct behavior so that the chick shows differential use of the left and right eyes to view the stimulus before it makes a decision to respond. Andrew and Dharmaretnam (1991) have, for example, shown that chicks in their first week of life preferentially use the right eye to view a hen that they have not seen before. This result suggests that imprinted chicks may show biases in eye use at retest, and that this might even be influenced by the nature of the stimulus used and, as Andrew and Dharmaretnam (1991) found, by the age of the chicks. It would be worth investigating whether any form of behavioral bias might correlate with the neurochemical differences following imprinting on the box vs the hen. If, as Horn (1990) suggests, shunting the memory out of the right IMHV to other regions of the hemisphere is associated with adding contextual depth to the imprinting memory, left eye viewing should allow better recognition of the imprinting stimulus in a variety of contexts. Indeed, Vallortigara (1992) has shown that chicks using the left eye recognize individual conspecifics from strangers, whereas those using the right eye do not.

It is also possible to make predictions about eye use in the passive avoidance task based on the memory flow hypothesis of Rose (1991b). At training-test intervals of <30 min the memory is said to be located in the left IMHV, and so the chick might avoid a bead viewed by the right monocular field but not one viewed by the left monocular field. At training test intervals of 30–60 min this situation might be reversed, and for longer delays no eye bias should occur. Thus, the neurochemical findings lead us back to more detailed analysis of the behavior as a way of testing their validity.

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