Chapter 1

Introduction

During certain periods of ontogeny the behaviour of animals is able to change significantly in response to external factors, whereas during later stages of development these same factors may not be as influential on the animal's behaviour. These periods of development have been termed sensitive periods, critical periods, sensitive/critical phases or privileged periods (Immelmann and Suomi, p. 423, 1981). A specific type of learning that occurs during a sensitive period of development is termed filial imprinting. Filial imprinting is a naturally occurring phenomenon whereby the young of precocial species, such as chicks and ducklings, rapidly form an attachment to the parent bird as the result of merely being exposed to it.

Over the last 50 years imprinting has been extensively studied (Lorenz, 1935; Bateson, 1966; Sluckin, 1972; Horn, 1985; Bolhuis, 1991). However, despite much knowledge on the processes of imprinting itself, we still know very little of the physiological factors which might control the sensitive period for imprinting. Instead, research has focused on characterising the behavioural aspects of imprinting, its sensitive period and the neurochemical aspects associated with the imprinting memory formation. This is surprising since one of Lorenz's (1935) original postulates was that "a quite definite physiological developmental condition in the young bird" (p. 127) was responsible for determining the *critical period* for imprinting.

Our understanding of the physiological mechanisms underlying sensitive periods for certain events in mammals, particularly plasticity of the visual cortex, is now at a stage where it may provide a useful framework within which to study the cellular factors involved in limiting imprinting to a sensitive period of development. For example, the N-methyl-D-aspartate (NMDA) receptor system and the noradrenergic system have both

been implicated in synaptic plasticity in the visual cortex of mammals (Rauschecker, 1991).

The following section reports the results of a pilot experiment showing that chicks treated early in life with a mixture of an NMDA receptor antagonist (ketamine) and an α_2-adrenergic agonist (xylazine) (which acts presynaptically to reduce the release of noradrenaline, Starke *et al.*, 1989) have an unprecedented extension of the sensitive period for imprinting. As the thesis develops, the mechanisms underlying the extended sensitive period caused by treatment with the mixture of ketamine and xylazine (KX) will be elucidated.

1.1 A Pilot Experiment Showing the Effect of Ketamine-Xylazine on the Sensitive Period for Imprinting

Thirty-six chicks, from three separate hatches, were tested in this experiment. On day 18 of incubation the eggs were transferred to a dark incubator, situated inside a dark-room. One day after hatching the chicks were randomly assigned to two groups. One of the groups received an injection of a mixture of ketamine (55 mg/kg), and xylazine (6 mg/kg) made up to a volume of 0.1 ml by the addition of sterile, pyrogen free 0.9% saline. A numbered leg band was placed on each chick before returning it to the dark incubator, where it was left to recover from the effect of KX. The other group of chicks were used as controls. These chicks were also leg-banded and then returned to the incubator. Four hours later all chicks were removed from the incubator and placed in the dark-rearing cages. Chapter 2 (page 43) provides details of the method used to rear the chicks in the dark.

The chicks were imprinted on the 8th day after hatching using a method adapted from the one used by Horn and his colleagues (e.g. Horn, 1985). (See Chapter 2, page 46 for details of the imprinting method and full testing procedures.) In this pilot experiment the chicks were imprinted on a rotating stuffed feral fowl. They were exposed to the

imprinting stimulus for two hours, after which they were returned to the dark room where they had access to food and water. The alternative stimulus used in the simultaneous choice test was a rectangular box (12 cm × 10 cm × 23 cm; width × breadth × height). The narrow sides of the box were black and the wide surfaces were pink. Attached to one of the pink surfaces was a wad of shredded paper, intended to give the box some visual texture. Testing began an hour after the end of training. The chicks were given another preference test on the following day, 24 ± 2 hours after their training session. From these tests percent preference scores were calculated according to the method of McCabe *et al.* (1982) (see methods, page 54). Using this method, a chick that directs all of its activity towards the familiar stimulus will score 100%, a chick that directs all of its activity towards the unfamiliar stimulus will score 0%, while one which exhibits no preference by directing its activity towards both stimuli equally would score 50%.

The mean group scores are presented in Figure 1-1. The data from each test was arcsine transformed (Winer, 1971) and analysed separately using two-tailed t-tests. For each test the percent preference scores of each group were compared to each other, and to the no-preference level of 50% (45% after arcsine transformation). In the test 1 h after training the percent preference scores of both groups were significantly higher than the no-preference level of 50% (untreated $t = 4.90$, $p = 0.0001$; KX-treated $t = 2.43$, $p = 0.029$, both two-tailed t-tests), indicating that both groups showed a significant preference for the imprinting stimulus. However, in the test 24 h after training, after a longer retention interval, it was only the KX-treated group that showed a significant preference for the imprinting stimulus ($t = 4.04$, $p = 0.0012$, two-tailed t-test). Additionally, the percent preference score of the KX-treated group was significantly higher than the percent preference score of the untreated group ($t = -3.21$, $p = 0.003$, two-tailed t-test).

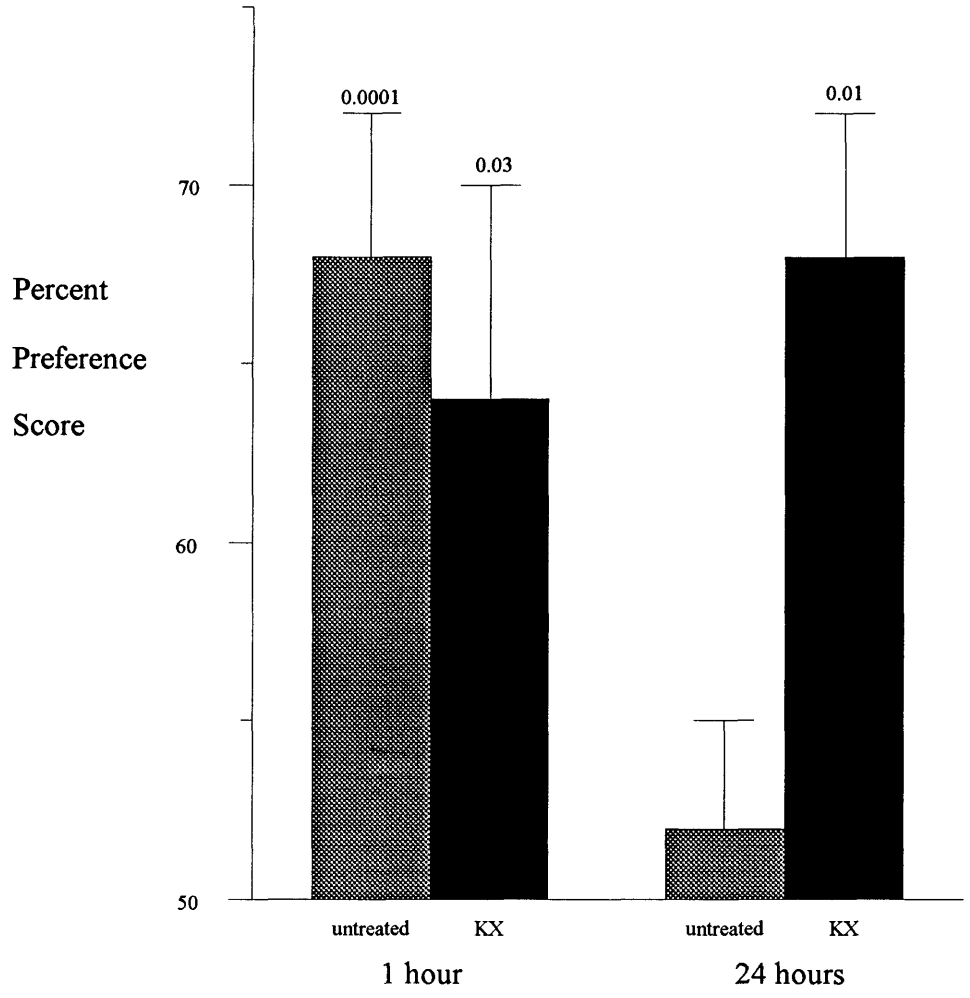

Figure 1-1. Mean ± SEM percent preference scores of untreated and ketamine-xylazine-treated chicks. Results from the tests 1 h and 24 h after training are presented. The grey bars represent the untreated chicks, the black bars represent KX-treated chicks. In the test 1 h after training both groups showed a significant preference for the imprinting stimulus. However, in the test 24 h after training only the KX-treated group showed a significant preference for the imprinting stimulus. The percent preference score of the KX-treated group in the test 24 h after training was also significantly higher than the percent preference score of untreated group in the test 24 h after training. Only the untreated group in the test 24 h after training failed to show a significant preference for the imprinting stimulus. The value above the error bars is the p value from a two-tailed t-test between the percent preference scores of a group and the no-preference level of 50%.

These results indicate that treatment with KX enables a long-term imprinting memory to be formed in chicks that are over a week old, at a time when untreated chicks are clearly unable to form such an imprinting memory. An investigation into the mechanism by which KX acts to extend the sensitive period for imprinting will hopefully provide a

valuable insight into the processes that are responsible for controlling sensitive periods in neural development.

Since a single injection of KX was capable of extending the sensitive period for imprinting, the timing of the injection may be crucial. That is, the effect of the KX treatment may be restricted to a sensitive period of development. In the present experiment the chicks were injected soon after hatching. In fact, they were injected during the sensitive period for imprinting. At this time the neural systems involved in imprinting are likely to be at a very susceptible state. It follows that an appropriate neurochemical manipulation of these systems, when they are particularly sensitive, might therefore produce the dramatic effects observed, whereas at later stages of development the effect may not occur.

The effect of the KX mixture may be mediated by either the NMDA receptor, the α_2-adrenergic system, or a combination of the two. Both of these systems have been implicated in neural plasticity (Fox and Daw, 1993; Kasamatsu and Shirokawa, 1985). A study by Rauschecker and Hahn (1987) actually used a KX mixture at an anaesthetic dose to prevent a shift in ocular dominance columns from occurring (see page 24). Rauschecker *et al.* (1990) later showed that this same result could be achieved using ketamine by itself, thus implicating the NMDA receptor. It is possible that the KX mixture extends the sensitive period through directly preventing an imprinting memory from forming or it may act to delay the normal maturational processes which end the sensitive period, the effect being to keep the imprinting system in a plastic state. The mechanisms through which this occurs may be similar to those that are involved in maintaining plasticity in the kitten visual cortex (e.g. Rauschecker, 1991, and see page 24).

The rest of the introduction to this thesis will review the literature associated with three major areas, in order to place the above finding and associated postulates into a broader perspective.

Firstly, the sensitive period for imprinting and the factors which control it shall be reviewed. Secondly, plasticity in the visual cortex of mammals shall be examined with an

emphasis on the neurochemical and physiological factors that are known to be involved in this plasticity. Finally, the neuronal events associated with the formation of an imprinting memory will be briefly reviewed. Through these three main areas possible mechanisms by which KX can extend the sensitive period for imprinting will be suggested.

1.2 Imprinting and its Sensitive Period

During the sensitive period for imprinting, precocial species will approach and follow the first visually conspicuous stimulus to which they are exposed, rapidly learning its characteristics and forming a long-lasting bond to it (Lorenz 1935). As a result, the young bird will demonstrate filial behaviour; that is, it will approach and remain close to the imprinting stimulus, showing signs of contentment when the stimulus is near. If the young bird becomes separated from the imprinting stimulus, it may become distressed and will attempt to move towards the stimulus. Imprinting may thus be measured by a range of different behaviours, all of which indicate the attachment to a "parent object". In the laboratory setting the most common measure of imprinting is the *following response* directed to the object to which the chick has been previously exposed. Using an imprinting wheel, such as the one shown on page 49, it is possible to quantify the approach of a chick towards a familiar stimulus relative to its approach towards a novel stimulus.

The phenomenon of imprinting has provided us with an excellent model for the study of memory (Horn, 1985; 1990; Andrew, 1991). The fact that imprinting is restricted to a sensitive period of development suggests that it may also help us to understand the mechanisms that control the temporal limits of other neural events that are restricted to sensitive periods of development.

The first formal reports of imprinting behaviour were those of Spalding (1873) and Heinroth (1910). However, Lorenz (1935) is generally considered to be the person responsible for providing the first theoretical account of imprinting.

Lorenz regarded imprinting as a non-typical form of learning because it occurred only "during a narrowly defined period in an animal's life" (p. 127) and unlike all other forms of learning it could not be forgotten. He reported that in some species the learning process involved in imprinting was very rapid. Thus, the investigators that followed Lorenz based their studies upon a rapidly established memory that was only able to be formed during a very early stage in the life of a young precocial bird, and which was also thought to be a very stable form of memory.

The term *critical period* was replaced with the term *sensitive period* because of the growing body of evidence showing that imprinting could occur outside of the narrowly confined time defined and inferred by the term critical period (Bateson, 1966).

The notion of the sensitive period for imprinting is, on the surface, very straightforward. A period is said to occur during which chicks will imprint on practically any stimulus. Obviously, in order to determine the length of the sensitive period, imprinting must be measured. Paradoxically, the method used to measure imprinting itself contributes to the length of the sensitive period (Bateson, 1966, 1979a). If the method used to imprint an animal incorporates factors that are known to enhance the ability of an animal to imprint (e.g. Moltz and Stettner, 1961), the measured sensitive period will be of a greater duration than that obtained if one were to use a different method, incorporating factors less favourable for the formation of an imprinting memory. These factors are not limited to those occurring at, or even around, the time of imprinting or testing. From pre-hatching through to the final imprinting test, potentially every experience that an animal has can influence its subsequent ability to imprint. These factors will now be discussed.

1.2.1 Factors influencing the sensitive period for imprinting

Before hatching the development of a chick may be influenced by external factors. Perhaps the most important factors are auditory and visual influences, but a number of less obvious ones have also been identified. For example, atmospheric pressure (Bateson, 1974), odours, vibrations or tactile stimulation including the heartbeat of the

young or its incubating parent (Fischer, 1972; Salk, 1962) may all contribute to the development of the embryo and may influence its subsequent imprinting preferences. Shapiro (1981) noted that artificially incubated eggs are deprived of a potentially major source of pre-hatching influence that would otherwise be provided by the incubating parent. While this may be true, in the laboratory it is necessary to carefully control the incubating conditions so that the experimenter knows exactly what stimulation the embryo has received, thus allowing the replication of these conditions.

Pre-hatching auditory influences on the subsequent behaviour of precocial birds have been well documented. Grier *et al.* (1967) demonstrated that chicks could discriminate between a 200 Hz tone that had been played during incubation and a novel tone. Gottlieb (1965) found that the last day of incubation was the most critical of the pre-hatching days for auditory learning, even though chicks are able to hear and indeed begin to vocalise at least two days prior to hatching (Gottlieb and Vandenbergh, 1968). Thus, during the latter stages of embryonic development the chick is influenced by its auditory environment.

Peking ducklings show an unlearnt preference for the maternal call of their species (Gottlieb, 1979). The preference has been shown to be based on the repetition rate of the call (approximately 4 notes/sec.) (Miller and Gottlieb, 1978). Although the responsiveness has been shown to develop prior to experience with their own, sibling or maternal vocalisations, auditory stimulation at the rate of 4 notes/sec during the later stages of incubation is required to maintain this specificity (Gottlieb, 1979). If ducklings are devocalised and isolated from auditory stimulation, the range of vocalisations that they are responsive to broadens such that they will respond to a slowed (2.3 notes/sec.) recording of the maternal call of their own species, or to the maternal call of the chicken, which also repeats at 2.3 notes/sec. (Gottlieb, 1978). Thus, although the preference has been shown to be unlearnt, experience during the latter embryonic stages serves to maintain the preferences for the specific characteristics of the call.

Bobwhite quail (*Colinus virginianus*) chicks also show a preference for the maternal call of their own species without prior experience of it (Heaton *et al.*, 1978).

However, Lickliter and Virkar (1989) showed that chicks displayed this unlearnt preference for their species-specific maternal call only during the first 48 h post-hatching. At 72 or 96 h post-hatching, an unlearnt preference was able to be shown only for a stuffed bobwhite quail hen emitting the bobwhite maternal call (a species specific maternal model). The bobwhite chicks were able to discriminate between their species-specific maternal model and a stuffed hen of another quail species (scaled quail, *Callipella squamata*) emitting the bobwhite maternal call. Thus, the unlearnt preferences of bobwhite quail chicks are highly specific for visual and auditory characteristics that are possessed by members of their own species.

Younger bobwhite quail chicks display a species-specific preference based on an auditory preference alone, while older chicks only show this species-specific preference if the auditory stimulation is combined with an appropriate visual stimulus. Lickliter and Virkar (1989) point out that the order of these developmental phases (for example, auditory preferences before visual preferences) is consistent with the order in which the underlying systems develop. The inference is that the attractiveness of a stimulus is dependent on the maturation of the neural systems that process the sensory information. The more developmentally advanced that the systems are, the greater is their capacity to control the behaviour of the animal. In a related study (Lickliter, 1990) the shell and inner shell membrane overlying the air space of the egg was removed for the last 36 hours of incubation. During this time the embryo was exposed to a 15-W light pulsed at three cycles per second. Chicks treated in this way and reared with other hatchlings no longer showed the unlearnt preference for their species-specific call presented alone in the first two days post-hatching. Instead, they showed a preference for a combined audio-visual, species-specific stimulus. The patterned visual stimulation prior to hatching appeared to have accelerated the development of the visual system, resulting in the inability of the species-specific maternal call to elicit filial behaviour at a time when it normally would have done so. Thus, in the bobwhite quail chick there is good evidence for an interaction between the visual system and the auditory system in the control of filial behaviour.

Recent work by Lickliter and his co-workers have shown the importance of social experience with conspecific siblings (Banker and Lickliter, 1993; McBride and Lickliter, 1993). These studies have shown that the predisposition to approach the maternal bobwhite model 72 h after hatching was dependent on social rearing with conspecific siblings. If a chick's eyes are covered from hatching, but otherwise the chick is reared normally, it will not exhibit the preference for a maternal model. Similarly, chicks reared in visual isolation from other chicks, but able to hear their vocalisations and see other aspects of the rearing environment do not exhibit the predisposition. In another condition, chicks reared in separate compartments but able to see other chicks through a glass partition also did not exhibit a predisposition. Most interesting was the experiment in which it was shown that single bobwhite quail chicks that were reared with a group of scaled quail chicks (which are of a similar size and have the same incubation period as bobwhite quail) did not exhibit the unlearnt preference for the maternal model. Thus, the stimulation that was needed to induce the species specific preference was highly species specific. Some combination of, as yet undetermined, characteristics unique to bobwhite quail chicks must be responsible for the species-specific preference. These studies demonstrate that a complex interaction between the sensory systems contributes to the behavioural development of the animal.

Arguably one of the most important factors influencing the development of the chick embryo is the amount of light exposure that it has received. Light stimulation during incubation is thought to accelerate the general development of the embryo, possibly through non-visual mechanisms. Shutze *et al.* (1962) have shown that eggs incubated in complete darkness have significantly longer incubation periods than eggs incubated in the light. Furthermore, light stimulation during the first 42 hours of incubation has been found to significantly accelerate the rate of embryonic growth (Siegel *et al.*, 1969). A non-visual mechanism may be responsible for mediating the effect because prior to day 15 the pupillary response is absent (Heaton, 1976) and before day 17 of incubation the optic tectum and the eye are not responsive to visual stimulation (Peters *et al.*, 1958).

One of the best examples of light stimulation affecting the development of the nervous system comes from the light-induced development of visual pathways in the chick. During later stages of incubation the head of the chick is orientated such that the right eye can be stimulated by light passing through the egg shell during incubation, while the left eye is positioned against the body and is not stimulated. In the chick there is an almost complete decussation of the optic nerves at the optic chiasma (Cowan *et al.*, 1961). The primary visual projections from the right eye go almost exclusively to the left side of the brain, and primary visual projections from the left eye go almost exclusively to the right side of the brain. Two regions of the brain receive the bulk of the primary visual information, the optic tectum and the thalamus. In the chick there are more visual projections from the left side of the thalamus to the hyperstriatum than from the right side of the thalamus to the hyperstriatum (Rogers, 1990). This is thought to occur because the right eye, and therefore the left side of the brain, receives more visual stimulation. If during the later stages of incubation the left eye is exposed to light and a patch is applied to the right eye, the asymmetry of the visual projections may be reversed (Rogers and Sink, 1988).

Light stimulation can accelerate neural growth, and as Lickliter and Virkar (1989) have shown, can significantly alter the behaviour of the chick, presumably by accelerating the development of the underlying neuronal systems. Thus, an immature visual system could be a limiting factor in the formation of an imprinting memory. Dimond (1968) has postulated that visually stimulated chicks may learn better than chicks without visual stimulation. Visual deprivation may retard the development of the visual system and impede visual learning until the visual system has developed sufficiently.

Exposing dark-reared chicks to light for a half hour period prior to training has been shown to facilitate their approach to an imprinting stimulus (Bateson *et al.*, 1972; Bateson and Seaburne-May, 1973). This period of light exposure is referred to as *priming* and it is thought to activate the visual system, enabling light-exposed chicks to form an imprinting memory more rapidly than chicks that did not receive the light exposure (Bateson and Wainwright, 1972). Similarly, Cherfas (1977) reported that the

ability of chicks to discriminate between beads in a passive avoidance test was enhanced if they were given a period of patterned light experience prior to the tests. Again, this indicates that previous visual experience has a positive influence on learning visual tasks.

It is important that during a priming period, before the controlled imprinting session, the chicks are not given the opportunity of forming an imprinting memory of a stimulus in their environment. A number of studies have reported that socially reared chicks or ducklings will not imprint as well as isolated controls, showing little interest in the imprinting stimulus. (e.g. Guiton, 1959; Sluckin and Salzen, 1961; Polt and Hess, 1964; Gottlieb and Klopfer, 1962). In these studies it is likely that the socially reared animals would have imprinted on their cage-mates, thereby initiating an ending to the sensitive period for imprinting, while the isolated animals would not have had the opportunity to do so. However, isolated chicks will form preferences for their home-cage environment, as Bateson (1964b) demonstrated that chicks preferred boxes covered with a similar pattern to the walls of their home-cage to boxes covered with an unfamiliar pattern. Thus it is now common practice to dark-rear animals prior to imprinting in order to control their visual experience (Bateson, 1991, p. 118).

Irrespective of the stage of development of the visual system, the physical state of the chick also plays an important role in its ability to imprint. Immediately after hatching chicks are fatigued and pay very little attention to their environment. In hen hatched broods, chicks spend most of their first day sleeping, or are nestled up to the hen and do not have the opportunity of learning her visual features (Bateson, 1987). The arousal level of chicks increases up to 12 hours after hatching (Tolman, 1963) and a sharp increase in locomotor ability occurring between 5-8 hours and 13-16 hours post-hatching has also been reported (Hess, 1959b). It appears then that 12 hours after hatching is the minimum age at which a chick is normally able to demonstrate imprinting behaviour.

It is beyond doubt that there exists a period during which chicks are more likely to form an imprinting memory than at other times in their life. However, the evidence that it is confined to a sharply defined period, 13-16 hours after hatching (Ramsay and Hess, 1954) is not conclusive, and the value of empirically defining the sensitive period for

imprinting to a pre-determined period of development is questionable because the length of the sensitive period is governed by its onset and offset; parameters which are themselves influenced by many factors. While much effort has been devoted to determining the factors that can affect the ability to imprint, the interpretation of these results has been confounded, largely because of the non-uniformity of the various imprinting methods that have been employed. For instance, some studies measured imprinting by the *following response* of the chick during an exposure period (e.g. Jaynes, 1957; Guiton, 1958, 1959) while others have used a discrimination test in which a chick must choose between the familiar stimulus and a novel stimulus (e.g. Ramsay and Hess, 1954).

Ramsay and Hess (1954) used mallard ducklings and Cochin bantam chicks to determine the sensitive period for imprinting. This study revealed a sensitive period for imprinting in ducklings and chicks of between 9 and 20 hours post-hatching, with a peak occurring 13-16 hours post-hatching. The results were much more convincing for the ducklings than for the chicks, both in terms of the imprinting performance and the number of chicks. A total of 92 ducklings were tested, while only 26 chicks were used. These were spread over seven age-groups. In the chick study, four groups comprised only three chicks each, two groups contained five chicks while one group contained four chicks. However, the results did indicate a similarity between the sensitive period for chicks and ducklings.

1.2.2 Extended sensitive periods

Many studies have sought to experimentally extend the sensitive period for imprinting in chicks. For example Moltz and Stettner (1961, see page 20) manipulated the amount and type of visual stimulation that the chicks received prior to exposure to the imprinting stimulus, and Hess (1957) treated chicks with meprobamate in order to alleviate their fear (fear is thought to impede the formation of an imprinting memory, see page 21). Other studies have shown extended sensitive periods by enhancing the imprinting

qualities of a stimulus, for instance by pairing it with an auditory stimulus (Case and Graves, 1978; Smith and Nott, 1970 and see page 15).

Sluckin (1962) reported preliminary results showing that dark-reared, isolated chicks showed a preference for a familiar stimulus over an unfamiliar stimulus on day 15 post-hatching. These chicks (n = 6) were first exposed to an imprinting stimulus on day 8 post-hatching for "one to four hours" (Sluckin was not more specific than this). The same procedure was repeated the following day. On the fifteenth day after hatching the chicks were again exposed to the imprinting stimulus for one to two hours and at the end of this period they were given a three minute discrimination test using a red feather duster as the alternative stimulus. The chicks showed a preference for the familiar stimulus. Additionally, four chicks were first trained on day 15 post-hatching, and again on day 22. At this time there was no indication that the chicks were able to discriminate between the stimuli.

Thus, the sensitive period for imprinting was reported to have been extended to day 8 post-hatching by dark-rearing. These results must be interpreted with caution as no detailed methodology was reported and only a small number of chicks were used. Unfortunately, the experiment was presented only in a preliminary form (Sluckin, 1962), but no follow-up work has been forthcoming (in the second edition of his book (p. 80 1972) Sluckin reported only the results from the 1962 paper). Nevertheless, the experiment of Sluckin (1962) does indicate that the sensitive period for imprinting can be extended for a significant length of time if the visual experience of the chick is manipulated.

1.2.2.1 Manipulating properties of the stimulus

One method of enhancing the ability to imprint involves manipulating the properties of the stimulus itself. A live hen model has been shown to elicit imprinting more effectively and more permanently than another visually conspicuous moving object (a toy windmill) (Boakes and Panter, 1985). In fact, ten Cate (1989) has demonstrated in Japanese quail chicks that a living hen is a more effective stimulus than either a stuffed,

non-moving or moving hen. Strongest imprinting attachment was achieved with the live hen, while the moving hen was a superior imprinting stimulus compared to the stationary hen, which elicited no response. The strength of attachment to the live hen was correlated with the amount of positive responses shown by the hen towards the chicks. ten Cate's study clearly shows that the stimulus plays an important role in the elicitation of the filial response, and this includes stimulus chick interactions. It is also probable that the hen's vocalisations played an important role in the elicitation of the filial responses as another method known to facilitate the filial response is to pair the imprinting stimulus with an auditory stimulus.

In ducklings and chicks pairing auditory and visual stimuli can enhance imprinting (Gottlieb, 1971; Case and Graves, 1978; Storey and Shapiro, 1979). There is some evidence in ducklings that maternal calls are more effective than visual stimuli in eliciting the filial response. This was true even when using a silenced, live hen model (Gottlieb, 1971; Storey and Shapiro, 1979). Case and Graves (1978) deprived chicks of visual experience to day 4 post-hatching and showed an attachment to a combined audio-visual stimulus on day 7 post-hatching. Isolated chicks, which otherwise received normal visual stimulation, did not show a preference at this age indicating that the visual deprivation was a significant factor in this result (Case and Graves, 1978). Smith and Nott (1970) also used a combined audio-visual presentation and were able to show *following behaviour* in chicks that were visually isolated and tested on day 10. However, the *following response* of the day 10 chicks was not as great as the response of the chicks which started the trial on days 1, 2 or 3. Nevertheless, *following* was observed, and with repeated exposure to the stimulus the chicks developed a strong preference for it, indicating that an audio-visual model is a particularly strong imprinting stimulus, capable of eliciting filial behaviour much later than normal.

The approach used by MacDonald (1968) was to prevent chicks from imprinting by repeatedly injecting them with a barbiturate. Chicks were injected with sodium pentobarbitone (either 10 mg/kg or 15 mg/kg) three times per day until day 4 when they were imprinted. Treated chicks showed significantly higher imprinting scores than the

control groups that received equivalent volumes of sterile water. Apparently, by preventing the chicks from imprinting on features of their environment, it was possible to keep the sensitive period open until the test stimulus was presented.

1.2.2.2 Experiments in which an unusually long period of exposure has been used

With prolonged periods of exposure it is possible to demonstrate that imprinting can occur outside of the period during which it normally occurs, even after a chick has imprinted. Salzen and Meyer (1968) demonstrated that imprinting preferences could be reversed if chicks were subsequently exposed to a second stimulus. In their experiment they showed that chicks initially exposed to a ball of one colour would show a discriminating preference for that stimulus over a novel coloured ball of a similar size. If chicks were subsequently exposed to the second ball for a period of three days they would then demonstrate a preference for that stimulus over the first stimulus. This not only suggests that imprinting is non-permanent, it also suggests that imprinting can occur at a much later age than was previously thought. However, Cherfas and Scott (1981) have demonstrated that the primary imprinting object is more permanent than the second stimulus on which a chick imprints. They, like Salzen and Meyer (1968), showed a reversal in imprinting preferences in chicks after exposing them to a second imprinting stimulus for three days. However, after three days without exposure to any imprinting stimulus the preferences of chicks reverted to the first stimulus that the chicks were exposed to. This indicates that, while a preference for a second stimulus may be formed, the first stimulus imprinted upon results in a more permanent preference.

Many studies have thus demonstrated that imprinting can occur beyond the sensitive period first proposed by Lorenz (1935) and which was experimentally shown to occur by workers such as Ramsay and Hess (1954) and Hess and Schaefer (1959). In contemporary imprinting studies chicks are usually aged between 15-30 hours post-hatching. For example in our laboratory Johnston *et al.* (1993) used chicks 24 h post-hatching, and the laboratory of Horn typically uses chicks aged 15-30 hours post-

hatching (e.g. McCabe and Horn, 1988, 1991; Ambalavar *et al.*, 1993). Other studies from Horn's laboratory have shown that chicks dark-reared to 45 hours post-hatching (Bolhuis *et al.*, 1989) or 60 hours post-hatching (Davies *et al.*, 1985) display a preference for the stimulus on which they were trained. It is apparent that filial imprinting can, and indeed, does occur outside of the sensitive period originally proposed by workers such as Hess (1959a). In all of these experiments care has been taken to avoid exposing chicks to stimuli on which they may imprint. Normally this is achieved by rearing chicks in the dark. However, the complex nature of filial imprinting is exemplified by the fact that chicks may develop a preference for a particular class of stimuli that they have not seen before. The following section addresses this phenomenon.

1.2.3 Predispositions

Many studies have shown that the young of some precocial species will preferentially respond in a filial manner to stimuli that possess certain characteristics. The nature of the characteristics that the young respond to varies between species. I have previously cited examples of ducklings that will preferentially respond to tones that are repeated approximately 4 times/sec. (Gottlieb, 1971), and bobwhite quail chicks which show an unlearnt preference for the species specific maternal call in the first two days post-hatching, and from 2-4 days post-hatching show a predisposition for a species-specific maternal model comprising a stuffed bobwhite hen emitting the bobwhite maternal call (Lickliter and Virkar, 1989 and see page 8). Domestic chicks also show a predisposition to approach certain objects in preference to others. Schaefer and Hess (1959) and Gray (1961) demonstrated that some colours were more effective in eliciting filial behaviours than others. More recently, in domestic chicks, it has been demonstrated that naive chicks will demonstrate a preference for objects that possess features that resemble the head and neck region of a fowl (Johnson and Horn, 1988). This predisposition has been examined in some detail.

From the results of studies investigating lesions of the intermediate medial portion of the hyperstriatum ventrale (IMHV) it was noted that chicks with bilateral lesions of the IMHV were severely impaired in their ability to acquire a preference for a red box, or if the IMHV was lesioned after training the expression of a preference for the box was prevented (Horn and McCabe, 1984, and see page 29). In contrast, chicks trained on a fowl showed no such deficits. It was proposed that different mechanisms were responsible for these preferences; the preference for the box was proposed to be dependent upon an intact IMHV, while the preference for the fowl was independent of the integrity of the IMHV. Further evidence that two systems underlie the expression of the preferences was provided by Davies *et al.* (1985) who reported that the noradrenergic neurotoxin, DSP4,† impaired the acquisition of a preference for the box but not the hen.

Subsequent studies have revealed that the predisposition develops only after the chick has had some, as yet, unknown stimulation. While the exact nature of the stimulation needed to induce the predisposition is unknown, it has been shown that a two-hour period spent inside the imprinting wheels (similar in design and purpose to the imprinting wheels used in the pilot experiment and which will be used in subsequent experiments) is sufficient to elicit the predisposition. During this period the chick does not have to be exposed to any stimulus at all, as it was shown that chicks placed in the imprinting wheels with only a dim diffuse overhead light developed the predisposition while chicks that had been left in a dark-incubator did not develop the predisposition (Johnson *et al.*, 1985). Johnson *et al.* (1989) showed that the development of a predisposition occurred only during a sensitive period of development. Chicks were placed in the imprinting wheels for a two hour period at either 12, 24, 36, 42 or 48 hours after hatching in an attempt to induce the formation of a predisposition. Only those chicks that had been placed in the imprinting wheels at 24 or 36 hours after hatching developed the predisposition, as evidenced by their demonstrating an unlearnt preference for a fowl when tested 24 h later. Davies *et al.* (1992) later published an extension of this

† N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride

experiment showing that DSP4 treatment extended the period during which the chicks could display the predisposition. Chicks treated with DSP4 showed a preference for the fowl when placed in the wheels at 42 and 48 hours after hatching, but not at 12, 24 or 36 hours after hatching. Thus, DSP4 treatment prevents the normal expression of the predisposition, but allows the predisposition to be expressed at a later stage when it normally does not occur. It would be interesting to determine if this effect could be maintained in older chicks by repeatedly administering DSP4.

The study of Davies *et al.* (1992) measured dopamine and noradrenaline levels in the IMHV of chicks. There was no significant correlation between the level of noradrenaline in the IMHV and the preference for the fowl in DSP4-treated or control chicks. However, in control chicks there was a negative correlation between percent preference scores and dopamine levels in the IMHV, which led these authors to the conclusion that the dopaminergic system was involved in the developing predisposition. Caution must be exercised in this interpretation as the IMHV, from where the dopamine was measured, is not thought to be associated with the developing predisposition. The assumption must be that the levels of dopamine and noradrenaline in the systems underlying the formation of the predisposition parallel that which occurs in the IMHV. The authors suggest that the paleostriatal complex, which shows a developmental rise in dopaminergic receptors (MacDougall *et al.*, 1989) may be involved in the developing predisposition.

Whatever the neurochemical systems that may be involved in the developing predisposition, the revelation that at least two systems underlie the expression of filial preferences is certainly important for elucidating the mechanisms for the ending of the sensitive period for imprinting.

1.2.4 The ending of the sensitive period for imprinting

There exists some disagreement as to how the sensitive period for filial imprinting ends. Basically, there are two proposed mechanisms. The original view of Lorenz was that imprinting could only occur during a discrete phase of development, probably

associated with a specific state of physiological development (Lorenz, 1935). It is now thought that the ending of the sensitive period for imprinting is an experience-dependent event. Once an animal has imprinted on a stimulus, it is difficult to reimprint it and the sensitive period is said to have ended (Bateson, 1979a).

Hess (1973) pointed out that there are a number of events which roughly coincide with the ending of the sensitive period. Increasing fear is one of the most important of these. Generally a fearful animal will avoid novel objects and hence will not have the opportunity to imprint on them.

There are two schools of thought as to the development of fear behaviour. One ascribes it to an endogenous process, developing independently of imprinting (e.g. Spalding, 1873; Ramsay and Hess, 1954; Schaller and Emlen, 1962) but which signifies the end of the sensitive period. The other, regards fear behaviour as a product of imprinting or learning the characteristics of an environment (e.g. Bateson, 1964c).

Regardless of the position one takes on this issue, it is agreed that fear behaviour impairs the formation of a new attachment. If an animal is given sufficient time to overcome its fearfulness of an object it may eventually respond to it in a filial manner (Bateson, 1964c). In fact, Bateson showed a strong relationship between the point in time when avoidance behaviour ceased and following behaviour commenced, suggesting that the same system regulates both behaviours.

In contrast, Moltz and Stettner (1961) showed that the two behaviours did not necessarily share the same relationship. Ducklings fitted with translucent goggles, and thus reared without patterned light, demonstrated less avoidance than ducklings that received normal visual stimulation. The ducklings that received no patterned visual stimulation were able to imprint 48 hours after hatching, while isolated ducklings that were allowed normal visual stimulation were able to imprint no later than 24 h after hatching. At 72 hours post-hatching there was no difference in the following scores of the two groups. Neither of the groups showed significant imprinting despite the fact that the visually deprived chicks showed significantly less avoidance. Thus, in this instance, fear and avoidance behaviour develop independently of imprinting. This experiment also

demonstrates that depriving ducklings of patterned visual stimulation extends the period during which they may imprint. (This could be attributed to slowing the development of the visual system or to denying the animals the opportunity to imprint.)

In an experiment designed to determine the influence of imprinting on fear and avoidance behaviours, Schaller and Emlen (1962) reared chicks in complete darkness in order to prevent them from imprinting. In this way, it was thought that the development of fear or avoidance behaviour could be measured independently of imprinting. Birds raised in this manner developed their avoidance behaviour at a rate comparable to chicks reared in the light, indicating that a developmental process, largely independent of sensory experience, was involved in the development of fear behaviour. This conclusion has attracted criticism from Bateson (1964c) who disagreed on the grounds that the dark environment was itself a sensory experience and that the birds would be able to detect a difference between the familiar dark environment and the test stimulus. As evidence, Bateson (1964b) has shown that chicks will form a preference for their rearing environment, preferring a patterned box resembling their home-cage to a box with a novel pattern. Since Bateson (1964c) hypothesised that the development of fear behaviour is dependent upon an animal imprinting, the implication was that in the absence of a suitable imprinting stimulus, an animal must imprint on a sub-optimal stimulus. It follows then, that chicks reared in the absence of light may eventually become accustomed to this environment. However, whether they would ever become imprinted on the dark, and so become photophobic, is another question that has yet to be tested.

Taking a somewhat more direct approach to determine the relationship between fear and the ability to imprint, Hess (1957) tried to modulate fear behaviour in the hope that this would allow chicks to imprint. Hess used the minor tranquilliser, meprobamate to suppress the fear responses of Mallard ducklings. He noted that the emergence of emotional responses such as fear begin to appear in ducklings at 20 hours post-hatching, coinciding with a sharp decrease in percent correct imprinting responses, which peaked at 16 hours. Hess reasoned that by reducing the fearfulness of the ducklings with

meprobamate it should be possible to extend the period in which they were able to imprint. In fact, meprobamate (25 mg/kg) did extend the period in which imprinting was able to occur but not by the mechanism which Hess had proposed. Three control groups were used, one group was given water, another given chlorpromazine (15 mg/kg) which was intended to control for the metabolic effects of meprobamate, and a fourth group was given nembutal (5 mg/kg) to tranquillise the animals without metabolic effects.

When treated 12 hours post-hatching and trained 14-16 hours post-hatching, imprinting was adversely affected in the groups treated with meprobamate and nembutal, while the chicks treated with water or chlorpromazine were able to imprint. When treated 12 hours post-hatching and imprinted 24 h post-hatching the water and nembutal treated groups failed to imprint, while the meprobamate and chlorpromazine groups did imprint. It was concluded that the period for imprinting was "stretched" not because of a reduction in fear but because metabolism had been reduced or, in support of his (Hess, 1959a) Law of Effort theory, meprobamate as a muscle relaxant "cut into the muscular tension or other afferent consequences and thus nullified the effectiveness of the imprinting experience" (p. 731). With present day knowledge, it is difficult to agree with Hess's (1957) conclusion, or indeed his Law of Effort theory because the amount of activity during training does not necessarily correspond to the strength of imprinting. For example, Bateson and Jaeckel (1974) have shown that there is only a weak correlation between training activity and strength of imprinting. It is more likely that the treatments either temporarily prevented an imprinting memory from forming or slowed neural development.

Clearly, many efforts have been made to manipulate the sensitive period for imprinting. The methods used have varied. These include manipulating the developing animal's sensory environment (e.g. Moltz and Stettner, 1961; Sluckin, 1962), which either prevents the formation of an imprinting memory, or slows down the development of the nervous system. Drug treatments, such as sodium pentobarbitone, have also been utilised to prevent an imprinting memory from forming for a period of time (MacDonald,

1968). In the pilot experiment of this thesis KX treatment and the dark-rearing conditions could have acted on similar mechanisms.

In the following section another form of experience-dependent plasticity and the factors involved in the plasticity it will be examined. This will then be compared to the underlying neurochemical and synaptic properties of the region of the chick brain known to be involved in imprinting.

1.3 Plasticity in the Visual Cortex

1.3.1 The effects of dark-rearing on sensitive periods in the visual cortex

Other than imprinting, one of the best examples of sensory experiences modulating the nervous system is experience-dependent plasticity that occurs in the visual cortex of the kitten. Without normal visual stimulation during a sensitive period of development, neural connections within the visual cortex will not develop properly. In kittens that have received normal visual stimulation, cells of the striate cortex reach a mature state by the end of the sixth week of life (Pettigrew, 1974; Derrington and Fuchs, 1981). Fregnac and Imbert (1978) reared kittens in the dark and examined the visual responsiveness of cells in the visual cortex. They found no difference between dark-reared and light-reared kittens before the age of 17 days. After this age there was an increase in orientation specific cells in light-reared kittens, while in dark-reared kittens there was an increase in cells that had no orientation specificity.

Other studies have looked at the effect of dark-rearing on the formation of ocular dominance columns. Normally the segregation of ocular dominance columns occurs by the age of six weeks (LeVay *et al.*, 1978). Swindale (1981) reported that dark-rearing to the age of six weeks prevents ocular dominance columns from forming. However, if kittens were given normal visual experience after six weeks of dark-rearing, the normal pattern of ocular dominance columns was able to be established. In kittens dark-reared

to seven months, it was not possible to establish the normal pattern of ocular dominance columns.

1.3.2 Pharmacological manipulation of plasticity in the visual cortex

For synaptic modifications to occur in the visual cortex, postsynaptic cells require activation above a critical threshold. Plasticity may be prevented by reducing the activity of the cells such that they remain below the critical threshold for plasticity. As has been mentioned, one method of doing this is through dark-rearing. Another method is to pharmacologically reduce the activity of the visual cortex.

The NMDA receptor system (on which ketamine acts) and the noradrenergic system (on which xylazine acts) have both been implicated in plasticity of the visual cortex. Recently, much emphasis has been placed on the role of the NMDA receptor in visual plasticity. The most convincing line of evidence for a role of the NMDA receptor in visual cortical plasticity comes from studies in which amino phosphonovalarate (APV), a competitive NMDA receptor antagonist was infused into the visual cortex of kittens that had been monocularly deprived (Bear *et al.*, 1990; Gu *et al.*, 1989; Kleinschmidt *et al.*, 1987; Miller *et al.*, 1989). These studies used osmotic minipumps to infuse APV into one side of the visual cortex, leaving the other side as a control. The hemisphere that was infused with APV showed no shift in ocular dominance, while the control hemisphere showed the expected shift.

Rauschecker and co-workers (Rauschecker and Hahn, 1987; Rauschecker *et al.*, 1990) have shown that the non-competitive NMDA receptor antagonist, ketamine, prevents ocular dominance shifts in kittens. In these experiments, dark-reared kittens were given daily periods of monocular exposure. Immediately after the exposure they received an injection of ketamine. This procedure was repeated over three days. Control kittens that instead received repeated injections of saline showed a shift in ocular dominance columns towards the undeprived eye, while ketamine treatment prevented the changes from occurring after the light exposure. Studies such as these have implicated the NMDA receptor in plasticity of the visual cortex.

If the NMDA receptor has a critical function in visual cortical plasticity, its number and function may vary with age and visual experience. Bode-Greuel and Singer (1989) reported an increase in NMDA-sensitive glutamate receptor binding in visual cortex slices peaking at 4-6 weeks of age. Fox *et al.*, (1992) reported a delay in the loss of functional NMDA receptors in dark-reared kittens, aged 6-7 weeks. Compared to normal, light-reared kittens, a greater proportion of cells in the visual cortex of dark-reared kittens were inhibited by APV. However, Reynolds and Bear (1991) reported no such decline in NMDA receptor number as a function of age in normally reared kittens. In their study, [^{3}H]-MK-801 binding showed a sharp increase between days 7 and 35. After this age, although the ability for plastic changes normally decreases, there was no significant difference in [^{3}H]-MK-801 binding. Thus, it would appear that the number of NMDA receptors does not correlate well with the sensitive period for plasticity in the visual cortex. Strengthening this conclusion was the fact that dark-rearing kittens to 40 days actually decreased the density of [^{3}H]-MK-801 binding by up to 30% in the visual cortex. Dark-rearing then, has the effect of decreasing the number of NMDA receptors. Reynolds and Bear (1991) point out that normal synaptic plasticity can occur in animals that have been dark-reared after subsequent light exposure (Swindale, 1981) and that it would be interesting to determine if, after a 40 day period of dark-rearing, light exposure has the capacity to increase the levels of NMDA receptors.

Carmignoto and Vicini (1992) showed that NMDA receptor mediated excitatory postsynaptic currents (EPSC) are longer in the visual cortex of young rats but decrease as a function of age, concomitant with reduced plasticity. Dark-rearing delayed the change in the NMDA receptor-mediated EPSC. Similarly, daily treatment with the sodium pump inhibitor, tetrodotoxin, also prevented the NMDA receptor mediated changes in the visual cortex. Thus, they infer that the developmental changes are greatly influenced by neural activity. These authors further suggest that the normal, activity-dependent change may be related to a change in the subunit composition of the NMDA receptor, as has been shown to occur for acetylcholine receptors in the development of the neuromuscular junction (Mishina *et al.*, 1986). If this is correct, then the properties of the NMDA

receptor change as a function of activity, and in doing so they alter the rate of Ca^{2+} influx. A high rate of Ca^{2+} influx, represented by a longer duration of the EPSC, is favourable to synaptic plasticity. Dependent upon activity, the duration of the EPSC is reduced, the effect of which is to alter the rate of influx of Ca^{2+} and thus decrease the opportunity for synaptic changes.

Taken together, these studies support the proposal that NMDA receptors are at least involved in the synaptic changes associated with the sensitive period for plasticity of the visual cortex.

Other studies, however, have cast doubt on the role of the NMDA receptor in visual plasticity. Perhaps the chief concern is that their action in the visual cortex may not be as specific to synaptic plasticity as it has been shown to be in the hippocampus. For example, NMDA receptor antagonists applied to the visual cortex cause a reduction in visual responsiveness (Miller *et al.*, 1989; Rauschecker *et al.*, 1990), and they are known to be involved in the transmission of patterned visual information (Fox *et al.*, 1989). This is cause for serious concern as it is known that procedures which reduce the activity of the visual cortex, such as dark-rearing or infusion with tetrodotoxin (Reiter *et al.*, 1986) prevent synaptic changes from occurring.

Other transmitter systems also have the capacity to modulate visual cortical plasticity. Functionally, (gamma amino-butyric acid) GABA has the potential to antagonise the NMDA receptor by preventing the voltage-dependent Mg^{2+} block of the Ca^{2+} channel, associated with the NMDA receptor, from being removed (Artola and Singer, 1987). In the kitten visual cortex there is an increase in binding of the selective $GABA_A$ receptor agonist, [^{3}H]-muscimol, which peaks at around the fourteenth week of life (Shaw *et al.*, 1984). Given the antagonistic properties of GABA towards the NMDA receptor, and assuming that the NMDA receptor is essential for neural plasticity, a plausible mechanism is provided for the ending of the critical period for plasticity in the visual cortex.

1.3.3 The role of noradrenaline in plasticity of the visual cortex

There is conflicting evidence concerning the role of noradrenaline (NA) in visual plasticity. Kasamatsu and Pettigrew (1976, 1979) reported that treatment with 6-hydroxydopamine (6-OHDA), a noradrenergic toxin, prevented a shift in ocular dominance columns in monocularly deprived kittens. However, a number of studies by other workers failed to replicate these results (Daw *et al.*, 1983, 1984, 1985a, 1985b). Only when 6-OHDA was directly infused into the visual cortex was plasticity prevented. It was suggested that the prevention of plasticity was due to a side effect of the 6-OHDA treatment and not entirely due to depletion of noradrenaline. The alternative explanation was that the direct infusion of 6-OHDA also reduced the acetylcholine levels, and that it was this combination that was responsible for the loss of plasticity (Bear and Singer, 1986). In this particular study though, NMDA was used as an excitotoxin directed against the cholinergic system. Given the present state of knowledge, it is possible that the effect is due to the removal of glutamate containing axons, which are now the focus of most of the attention in the study of plasticity in the visual cortex.

Further work by Kasamatsu and his co-workers (Kasamatsu and Shirokawa, 1985; Shirokawa and Kasamatsu, 1986) showed that an infusion of β-adrenergic antagonists into the visual cortex of kittens prevented a shift in ocular dominance columns in monocularly deprived kittens. A similar study used the α_2-adrenergic agonist, clonidine to reduce the amount of noradrenaline released. This treatment also prevented a shift in ocular dominance columns although the effect was not permanent and plasticity was again present after the antagonist treatment had worn off (Nelson *et al.*, 1985). The recovery from the effects of the β-adrenergic antagonists could be accelerated by infusion of noradrenaline into the cortex (Shirokawa and Kasamatsu, 1987).

Some of the most remarkable studies implicating the noradrenergic system in visual plasticity are those in which plasticity was induced by the infusion of noradrenaline into the visual cortex of monocular adult cats. Heggelund *et al.* (1987) demonstrated a reduction in binocularity of cats that had noradrenaline infused into their visual cortex.

Kasamatsu *et al.* (1979, 1981) reported the induction of visual plasticity in adult cats, well past the sensitive period for visual plasticity. In these studies the concentration of noradrenaline was increased, either by a direct infusion of noradrenaline into the visual cortex, or by stimulating the locus coerulus. The resulting shift due to monocular lid suture was not as strong as that which occurs in kittens at the peak of their sensitive period, but more impressive results were reported for dark-reared cats (Shirokawa *et al.*, 1989), probably due to the extension of the sensitive period that results from dark-rearing.

In summary, many different neurochemical systems have been shown to be involved in the plasticity of the visual cortex. Rauschecker (1991) suggests that the function of the noradrenergic system is to accelerate the consolidation of synaptic changes. There is likely to be some interaction between most of the neurochemical systems involved, and it is doubtful as to whether one system can be singled out as the system involved in the plasticity of the visual cortex. Indeed, NMDA receptors are known to be located on some noradrenergic neurones, and their stimulation promotes the release of norepinephrine, which can be prevented by EAA (excitatory amino acid) antagonists (Blandina *et al.*, 1992).

Theoretically, KX treatment and the resulting extension of the sensitive period for imprinting could be due to an action on the NMDA receptor system, the noradrenergic system or a combination of both. The next section seeks to provide an insight into where the drugs may be acting, by reviewing information on the cellular basis of the formation of an imprinting memory.

1.4 The Intermediate Medial Portion of the Hyperstriatum Ventrale - an Area Known to be Involved in Imprinting

The first studies examining the neural basis of imprinting were concerned with localising the area of the brain involved in laying down the memory. Initially, the incorporation of tritiated lysine into acid insoluble protein was found to be greater in the

roof of the forebrain than the base of the brain or the mid-brain in imprinted chicks (Bateson *et al.*, 1969). A similar result was obtained for the incorporation of tritiated uracil into ribonucleic acid (Bateson *et al.*, 1972). Subsequent studies indicated that the amount of uracil incorporation was positively correlated with the preference for the imprinting stimulus (Bateson *et al.*, 1975). A more precise localisation of the area involved was obtained using an autoradiographic technique in which the IMHV was identified as being an area with a high incorporation of tritiated uracil (Horn *et al.*, 1979). Using the 2-deoxyglucose autoradiograpic technique another group showed a similar area to be metabolically active following imprinting (Kohsaka *et al.*, 1979).

1.4.1 Lesioning studies

Further evidence that the IMHV area is important in the formation of an imprinting memory was obtained through lesioning studies. Bilateral lesions of the IMHV before training prevent the formation of an imprinting memory (McCabe *et al.*, 1981). Bilateral lesions of the IMHV three hours after training prevent the expression of an imprinting preference. Lesions that were placed in the visual Wulst or the lateral region of the forebrain did not impair imprinting, indicating that the effect had some degree of specificity.

However, another group has identified the lateral neostriatum of the forebrain as being important in establishing and maintaining imprinting preferences (Salzen *et al.*, 1975; Salzen *et al.*, 1978). In the studies of Salzen's laboratory, lesions of the anterior forebrain, encompassing the IMHV, did not affect imprinting preferences. The discrepancy between the results of Salzen *et al.* (1975, 1978) and the results reported by McCabe *et al.*, (1981) may be explained by the different methods of imprinting employed by these two laboratories. Salzen's group reared chicks with an imprinting stimulus for at least two days, while in the experiments of McCabe *et al.* (1981) the chicks were exposed to the imprinting stimulus for only three hours.

Further studies by Horn and co-workers have revealed an asymmetrical functioning of the left and right IMHV (Horn *et al.*, 1983). In order to determine the role of the left

and right IMHV only one IMHV was lesioned at a time. Chicks were trained with both IMHV intact. Three hours after training they were anaesthetised and either their left or right IMHV was lesioned. A subsequent choice test, 15-20 hours later revealed that all chicks displayed a preference for the imprinting stimulus. The remaining IMHV was lesioned and again, 15-20 hours later the chicks were tested for an imprinting preference. In this test the chicks that had been lesioned first in the left IMHV showed a preference for the imprinting stimulus, indicating that in the absence of the left IMHV the subsequent expression of the imprinting memory is dependent on areas of the brain other than the IMHV. In contrast, if the right IMHV was lesioned first the left IMHV became crucial for the expression of the imprinting memory. Thus, it is implied that there are two memory systems involved in imprinting in the chick. One of these systems involves the left IMHV, the other involves another, as yet unknown region of the brain. It has been postulated that the right IMHV acts as a temporary store, that slowly transfers the imprinting memory to other areas of the brain (Horn, 1990).

A further examination of the roles of the left and right IMHV was undertaken by Horn *et al.* (1983). In this experiment the left or right IMHVs were lesioned before exposing chicks to an imprinting stimulus. Subsequent tests revealed that it was still possible to form an imprinting memory with only one IMHV intact. It did not matter if this was the right or the left IMHV. However, when the remaining IMHV was lesioned after training, the chicks did not show a preference for the imprinting stimulus. As the lesioning occurred some 21 hours after training, sufficient time had elapsed for it to have been transferred to another site (Cipolla-Neto *et al.* 1982). There was no evidence that this occurred; if it had occurred, its subsequent recall was prevented. Thus, an imprinting memory can be formed with only one IMHV but the subsequent expression of this memory is dependent on the integrity of that IMHV. If it is destroyed, the memory can not be expressed. Under these circumstances the right IMHV functions like the left IMHV and is crucial for the expression of the memory.

Functionally, the left and right sides of the brain have been shown to have different roles in the expression of social behaviour. Male chicks showed marked differences in

their ability to discriminate between objects on which they have been imprinted depending on the eye that is used (Vallortigara and Andrew, 1991). Chicks using the left eye are superior in discriminating between a cage-mate and an unfamiliar chick. Further experiments using table-tennis balls as imprinting stimuli revealed that the left eye system was more capable of discriminating between individual features of stimuli, while those chicks using the right eye system could distinguish between those features that placed a stimulus in the category of the social companion. Although this shows that the left and right sides of the brain have different functions in recognising a familiar stimulus, caution must be exercised before ascribing these properties to the left and right IMHV because many different neuronal systems may be involved in these complex recognition tasks.

1.4.2 Biochemical correlates of imprinting

Several studies have measured glutamate receptor binding in the IMHV of the chick after imprinting. McCabe and Horn (1988) reported an increase in NMDA-sensitive glutamate receptor binding in the left IMHV of chicks exposed to an imprinting stimulus. It was inferred that the binding was associated with the learning process of imprinting because the increased binding occurred only in the left IMHV and in Experiment 3 of McCabe and Horn's (1988) study there was a positive correlation between binding levels in the left IMHV and percent preference scores. Further studies by McCabe and Horn (1991) have shown that the increased binding does not occur until 6 to 8½ hours after training and thus may be correlated with the expression of the imprinting memory and not its acquisition. Johnston *et al.* (1993) have shown that the increase in [^{3}H]-glutamate receptor binding in the left IMHV is due to an increase in both the number and affinity of glutamate receptors.

The NMDA receptor also appears to play a role in the acquisition of an imprinting preference (McCabe *et al.*, 1992). APV, a competitive antagonist of the NMDA subtype of the glutamate receptor has been shown to impair imprinting. In this study APV was infused into the left IMHV of chicks which had their right IMHV lesioned. This

preparation was used in order to make the left IMHV by itself essential for the formation of an imprinting memory (Horn *et al.*, 1983). APV infusion resulted in an impairment of the imprinting memory. One explanation that was proposed was that APV interfered with a long term potentiation (LTP)-like phenomenon (McCabe *et al.*, 1992). LTP is a form of synaptic plasticity whereby the ease of transmission across a synapse is increased (Collingridge, 1992 and see page 36). LTP is thought to underlie the long term changes associated with many forms of synaptic plasticity including learning and memory (Morris *et al.*, 1990; Bliss and Collingridge, 1993). Antagonists of the NMDA receptor, such as APV, prevent LTP from occurring and have also been shown to interfere with memory function in other animal models (e.g. Morris *et al.*, 1990; Bolhuis and Reid, 1992 and see page 38).

1.4.3 Synaptic morphology of the IMHV

Further evidence for a specific role of the left IMHV in the formation of an imprinting memory came from an electron microscopic study of the synaptic morphology of the IMHV. This study revealed specific changes in the left, but not the right, IMHV after imprinting (Horn *et al.*, 1985). In this study chicks were exposed to an imprinting stimulus for either 20 or 140 minutes. It had previously been shown that a training time of 20 minutes was insufficient to establish an imprinting preference while chicks trained for 80 minutes showed a preference for an imprinting stimulus (Bateson, 1979b). Thus, the chicks that were trained for 140 minutes should have formed an imprinting memory while those that were trained for 20 minutes would not have formed the preference. The left and right IMHV regions were examined by electron microscopy. The only difference in the synaptic morphology between the two groups was found in the left IMHV. In the left IMHV the mean length of the post-synaptic density was larger in the chicks trained for 140 minutes compared to chicks trained for 20 minutes.

1.4.4 Neuronal activity in the IMHV

Studies investigating the neuronal activity in the IMHV have provided more evidence for a role of the IMHV in imprinting learning. Bradford and McCabe (1992) trained chicks by exposing them to either a red or blue box. Twenty hours later the chicks were tested; on the basis of their preferences they were grouped into weak, medium or strong 'imprinters'. Under anaesthesia, recordings of spontaneous multiple unit activity in the left IMHV were made. There was a positive correlation between mean firing rate and imprinting preference. In contrast, a previous study had shown a negative correlation between approach during training and spontaneous firing rate in the left IMHV of anaesthetised chicks (Payne and Horn, 1982). The reason for the discrepancy may be attributed to either the time at which the recordings were made or the experience of the chicks immediately prior to the recording period. Payne and Horn recorded one hour after training, without testing their chicks, instead inferring that a preference was present on the basis of their activity during training, which is known to be weakly correlated with the strength of imprinting (Bateson and Jaeckel, 1974). Bradford and McCabe (1992) recorded at least 20 hours after training, after they had tested the chicks for an imprinting preference in a test during which the chicks were sequentially exposed to the imprinting stimulus and an unfamiliar stimulus. The testing procedure may have stimulated neurones of the IMHV involved in the recognition part of the memory formation, which may have increased the spontaneous firing. Alternatively, the increase in spontaneous firing could be related to changes in NMDA receptor density, which occurs 6-8½ hours after training and which is thought to represent consolidation of the imprinting memory (McCabe and Horn, 1991).

1.4.5 Synaptic transmission in the IMHV

A role for NMDA mediated events in synaptic transmission in the IMHV was determined through the work of Bradley and his co-workers. They conducted a series of

experiments that investigated the neurochemical and synaptic properties of the IMHV *in vitro*. They used sections containing the IMHV from the left forebrain and showed that a short latency diphasic field response could be elicited by low frequency stimulation (Bradley *et al.*, 1988). The initial phase of the response was negative and of a short duration, while the second phase was positive and of a longer duration. Removal of Ca^{2+} from the bathing medium enlarged the initial, negative phase, and nearly abolished the positive phase. Similarly, the addition of Mg^{2+} to the bathing medium also abolished the positive phase of the response, but had no effect on the initial negative response. The positive phase is thought to represent the post-synaptic neural response and is probably mediated by the NMDA receptor. The addition of the competitive NMDA receptor antagonist (AP5) to the bathing medium eliminates most of the post-synaptic response, while the non-specific excitatory amino acid antagonist kyurenic acid completely eliminates the response (Bradley *et al.*, 1990).

One of the most interesting aspects of this study was the revelation of a strong GABA-ergic inhibition. In the presence of the $GABA_A$ antagonist bicuculline methiodide, the duration of the post-synaptic response was increased some five-fold. Aside from the increased response in the magnitude and duration of the post-synaptic response, all other characteristics were the same. The post-synaptic response was reduced with the addition of APV and was completely abolished when kyurenic acid was added to the medium. Bradley *et al.* (1990) proposed that two types of glutamate receptors are present on those neurones that are responsible for the post-synaptic response; the NMDA receptor, which is responsible for the longer duration response, and the kainate/quisqualate receptor, responsible for the shorter duration response that was left after the NMDA receptor was blocked by APV. The authors do not think that the NMDA receptor could be solely responsible for the long duration post-synaptic response. Instead they have proposed a 'reverberating circuit' to account for the length of the post-synaptic response. Under this scheme a stimulated neuron would excite a positive feedback loop containing more glutamatergic neurones that feed back to the original cell.

The self-exciting local circuits are able to sustain a form of LTP, termed post tetanic potentiation (PTP). Using a preparation similar to the one just described, Bradley *et al.*, (1991a) gave a train of 300 stimuli at 5 Hz, followed by 10 minutes of test stimuli at 0.1 Hz, and then a further 300 stimuli at 5 Hz. Half of the attempts at producing the potentiation were successful. The persistent potentiation of the response (PPR) typically lasted for two hours and was more likely to occur in sections of the medial hyperstriatum ventrale that included the IMHV, than in the surrounding sections.

In a related experiment, the visual experience and age of the chick was shown to influence the probability of producing a potentiated response (Bradley *et al.*, 1991b). In this experiment two groups of chicks were used, a dark-hatched group and a light hatched group. Both groups were derived from eggs that were dark-incubated. Chicks that received visual experience were transferred to a lighted incubator as embryos, two days before hatching. Dark-hatched chicks were removed from the dark-incubator 18 hours after hatching. Thus, light-hatched chicks received nearly three more days of light exposure than dark-hatched chicks. Compared to dark-hatched chicks, light-hatched chicks had an increased probability of producing a potentiated response at 8-24 h post-hatching. However, at 2-3 days post-hatching there was a marked decrease in the probability of producing the potentiation in light-hatched chicks. This contrasts sharply with the increased probability of producing a PPR from 2-5 days after hatching in dark-hatched chicks. After this period, the probability of producing the potentiation is the same for both dark-hatched and light-hatched chicks. If one were to ignore the hatching age and instead 'zero' the age of the chicks from the onset of light exposure, the probability of inducing potentiation in dark-reared chicks is similar to that shown by the light-reared chicks. Thus, the development of the potentiated response in the IMHV, which is an NMDA mediated event, is influenced by the visual experience of a chick. We may speculate that a component of the formation of an imprinting memory involves a change in synaptic efficacy, represented by the potentiated response. The probability of achieving the potentiated response varies with the visual experience of the chick, just as does the probability of forming an imprinting memory. As the potentiated response is

NMDA-receptor dependent it follows that the ketamine component of the KX mixture has the potential to disrupt LTP-like processes in the IMHV. One possible mechanism for the extended sensitive period achieved by KX treatment is that the mixture, like dark-rearing, delays the ability to produce a potentiated response.

1.4.6 Long term potentiation

The enhanced synaptic transmission of the IMHV is thought to be similar to, although not identical to LTP of the mammalian hippocampus, being less stable, of lower magnitude and produced with less predictability (Bradley *et al.*, 1991a). LTP is an activity-dependent form of synaptic modification that has attracted much attention due to its possible role in learning and memory (Bliss and Collingridge, 1993). It has also been suggested that LTP may be involved in many different forms of synaptic modifications such as experience dependent plasticity of the visual cortex (Rauschecker, 1991). In the Schaffer collateral-commissural pathway of the hippocampus LTP is typically induced by high frequency stimulation of the post-synaptic cell for a brief period of time (Larson and Lynch, 1986). LTP is said to have occurred if subsequent low frequency stimulation elicits a persistent increase in the amplitude of the excitatory response. Potentiation is classified as LTP if it persists for over an hour. Potentiation lasting for less than an hour is classified as short term potentiation. Post-tetanic potentiation lasts for a minute or so after the conditioning phase and is independent of the NMDA receptor. Both short-term potentiation and LTP are both prevented by NMDA receptor antagonists (Collingridge, 1992).

Excitatory amino acid receptors play a critical role in LTP. NMDA receptors are thought to mediate the induction of LTP, while non-NMDA receptors, such as quisqualate, are responsible for the mediation of low frequency transmission before and after generation of LTP. In the hippocampus, APV does not suppress synaptic transmission evoked by low frequency stimulation of the Schaffer collateral-commissural pathway, while a general EAA antagonist such as g-D-glutamylglycine does suppress this transmission. The presence of APV reversibly blocks the induction of LTP, while the

removal of APV allows the induction of LTP with the application of another stimulus (100 Hz for 1 sec) (Collingridge *et al.*, 1983). Drugs such as ketamine or phencyclidine, which block the ion channel associated with the NMDA receptor also block the induction of LTP (Wong *et al.*, 1988). NMDA receptors are concentrated in the superficial layers of the mammalian neocortex (Cotman *et al.*, 1987) and it is possible that an LTP-like process is present in the neocortex, which could provide a physical basis for the activity-dependent changes of the visual cortex.

However, early attempts at inducing neocortical LTP produced limited results (Bliss *et al.*, 1968). Hippocampal induction proved much easier and so efforts were concentrated in this area (Bliss and Lomo, 1973). It was not until relatively recently that neocortical LTP was successfully induced, when Artola and Singer (1987) demonstrated a form of synaptic enhancement that was blocked by APV. The induction of neocortical LTP is reported to be easier in immature slices (Komatsu *et al.*, 1988), which provides support for the idea that LTP has a functional role in synaptic plasticity since it is easier to induce synaptic changes in younger brains.

Conflicting results have been reported for the role of the NMDA receptor in neocortical LTP. APV reduces the probability of establishing LTP (Artola and Singer, 1987, Sah and Nicoll, 1991; Bear *et al.*, 1992) and has also been shown to induce long term depression (Artola *et al.*, 1990; Hirsch and Crepel, 1991; 1992). In apparent contrast to the hippocampus, a form of LTP may be established in the neocortex in the presence of AP5 (Komatsu *et al.*, 1991. Arondiadou and Teyler (1992) report that LTP of this sort appears to be stronger in the presence of AP5.

It is thus clear that aspects of mammalian neocortical LTP differ from that of hippocampal LTP. This may be due in part to the lack of a large afferent input such as the Schaefer collateral-commissure of the hippocampus (Collingridge, 1992). It may also be due to the fact that in the cat visual cortex the NMDA receptor may contribute to the normal synaptic transmission to a greater extent than in the hippocampus (Miller *et al.*, 1988). In the hippocampus, AP5 application does not prevent normal synaptic transmission, which it does in the visual cortex.

As knowledge of the NMDA receptor complex increases, it is becoming apparent that mechanisms that prevent NMDA receptor activation, such as blocking the strychnine-insensitive glycine site associated with the NMDA receptor, also prevent the induction of LTP (Oliver *et al.*, 1990). Suffice it to say here that LTP is a process that has been shown to be mediated by the NMDA receptor (Collingridge, 1992; McNaughten, 1993).

1.4.7 NMDA receptors and learning and memory

There exists some controversy over the relationship between LTP and learning and memory. LTP is thought to model memory formation (Bliss and Collingridge, 1993), but caution must be exercised before extrapolating this connection to an actual mechanism for the storage of memory. One of the best lines of evidence connecting LTP with learning and memory is the integral role of the NMDA receptor in both processes.

An example of the role of the NMDA receptor in memory formation specifically relating to imprinting has already been given (McCabe *et al.*, 1992 and see p. 31). The NMDA receptor has also been shown to have an important role in other memory systems. The selective NMDA receptor antagonist, AP5 has been shown to disrupt spatial memory in rats but not memory of visual discrimination tasks (Morris *et al.*, 1986a; Morris *et al.*, 1989). Additionally, these effects are remarkably similar to those that have been achieved by lesioning areas of the hippocampus (Morris *et al.*, 1986b; Robinson *et al.*, 1989), indicating that APV disrupts hippocampal LTP. Other NMDA antagonists have been used to impair spatial performance, including ketamine, which has been shown to disrupt performance in a water maze (Wesierska *et al.* (1990). The learning disruption is, however, not restricted to spatial learning tasks. For example, operant discrimination tasks have been disrupted by CPP and MK-801† (Clissold *et al.*, 1991) and radial arm maze performance is disrupted by MK-801 treatment (Shapiro and Caramanos, 1990). These tasks by no means represent all of the studies into the effect of NMDA receptors in

† CPP 4-(3(-phosphonoprop-1-yl)piperazine-2-carboxylic acid
MK-801 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine

learning and memory. In most of the studies reported above, and seemingly in general, the effect of blocking NMDA receptors is evident only in the learning or acquisition stage of the task, with little or no effect on the consolidation or long-term memory formation phases.

The chick, however, seems to provide an exception to this. The work of McCabe and Horn (1988, 1991) in which an increase in NMDA-sensitive glutamate binding in the left IMHV was reported to occur between six and 8½ hours after imprinting. In contrast, in the rat, an inverse correlation between NMDA receptor binding and spatial learning was reported, although this was measured 10 days after acquisition of the task (Wenk *et al.*, 1989). This is consistent with the notion that the NMDA receptor is involved in the acquisition phase of memory formation and not in the long-term memory formation stages. It should also be remembered that in the chick, antagonism of the NMDA receptor prevents the acquisition of the imprinting memory (McCabe *et al.*, 1992).

1.5 An Overview of the Present Study

The current study is concerned with elucidating the mechanism or mechanisms by which KX mediates the extension of the sensitive period for imprinting. Clearly, many factors normally contribute to the ending of the sensitive period for imprinting. The common link between these factors is that they may all be associated with the increasing age and consequently the experience of the chick. Since the experience of the chick probably serves to alter the rate of development of the underlying neural systems that may control the sensitive period for imprinting, KX must reduce the effectiveness of these factors.

At the outset of the investigation three important avenues of inquiry were undertaken. Neurochemical aspects of the extended sensitive period were examined in Chapter 4 by measuring the density of NMDA receptors in the IMHV of day 8 chicks treated with KX or saline. In Chapter 5 the optimal time to administer the KX treatment was determined. Evidence for an important role of the NMDA receptor in the extended

sensitive period was obtained in Chapter 6. From these three chapters it was concluded that antagonism of the NMDA receptor within the first 40 h after hatching allows chicks to imprint on day 8 post-hatching. At this age, like in day 2 chicks, the NMDA receptor plays a role in the extended sensitive period.

In Chapters 5 and 6 it was found that, when tested 24 hours after training, day 8 chicks only showed a preference if they had been imprinted on the hen. Thus, Chapter 7 was concerned with determining whether there was a predisposition for day 8 chicks to approach a hen in preference to a box. It was found that, without prior exposure to the hen, the chicks did not show a preference for the hen.

Chapter 8 was concerned with determining whether dark-rearing plays a significant role in the extended sensitive period shown in chapters that preceded it. This was investigated by imprinting light-reared KX-treated and saline-treated chicks. The effect of KX was such that even light-reared chicks could imprint on day 8. Finally, Chapter 9 investigated the effect that treatment with KX had on behaviours other than imprinting.

The overall conclusions drawn from these experiments are presented in Chapter 10.