THE EFFECTS OF EMBRYONIC DRUG STIMULATION ON LATER BEHAVIOR OF PEKING DUCKS

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THE EFFECTS OF EMBRYONIC DRUG STIMULATION

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PREFACE

The objective of this study is to assess the effects of a central nervous system stimulant administered pre- and postnatally on the later behavior of the domestic Peking duckling. A measure of approach and following is employed to determine the drug's influence on the ducklings' sensitive period for attachment formation.

I wish to express my appreciation to my thesis adviser, Dr. Barbara Weiner, for her invaluable assistance both during the course of this study and throughout my graduate program. I also wish to thank my other committee members, Dr. Arthur Harriman and Dr. Elliott Weiner, for their guidance in the preparation of the experimental design and the final text.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The young of many species of birds possess well-developed sense organs, are physiologically mature enough to be capable of locomotion, and require little parental care upon hatching. Such precocial young, as they are called, which include domestic chicks, goslings, and ducklings, tend to follow the first moving object they see--usually one of their parents. This initial tendency on the part of the newly-hatched creature to approach and follow is a response to stimulation in its early environment. Once the young bird has become attached to a particular object, it will follow only that object. It is this following response which keeps the young bird well within the range of the protective parent. The behavior pattern characterized by this spontaneous act of recognition is called imprinting (literally, "stamping in"). Imprinting has been defined as the very rapid learning of a following response which occurs in certain animals during a sensitive early stage of development. These formations of early attachments to particular moving objects, most likely birds of their own species in their natural habitats, act as the primary basis for the subsequent development of most avian social structures.

Summarizing the evidence available at that time, one leading investigator concluded that:

imprinting is peculiar in the following respects:

> (1) The process is confined to a very definite and very brief period of the individual life, and possibly also to a particular set of environmental circumstances. (2) Once accomplished it is often very stable--in some cases perhaps totally irreversible. (3) It is often completed long before the various specific reactions to which the imprinted pattern will ultimately become linked are established. (4) It is supra-individual learning--a learning of the broad characteristics of the species--for if this were not so and the bird at this stage learnt (as it can easily do later) the individual characteristics of its companion, the biological effect would be frustrated (Thorpe, 1956, p. 129).

The first point is of great significance in the development of imprinting. This brief sensitive period early in the animal's life is called the critical period. A young bird is much more likely to form an attachment to an object presented to it during this period than to an object presented much earlier or later in its life. The limits and duration of the critical period have been the subject of much scientific investigation, and are believed to be a function of species differences, the nature of the stimulation used to elicit the following response, the duration of the actual imprinting session, and other factors such as incubation and rearing conditions. The period during which the domestic chick can be successfully imprinted has been studied extensively by Hess (1959). Hess suggested that the limits of this critical period are determined by the chick's inability to run after the stimulus at very early ages and by the tendency to avoid any novel stimuli later in life. An illustration of the results typically encountered by Hess is presented in Figure 1.

One of the little understood factors which limits the sensitive period of imprinting is the accumulation of enough experience in the young animal's posthatch surroundings to make novel stimuli apparent.

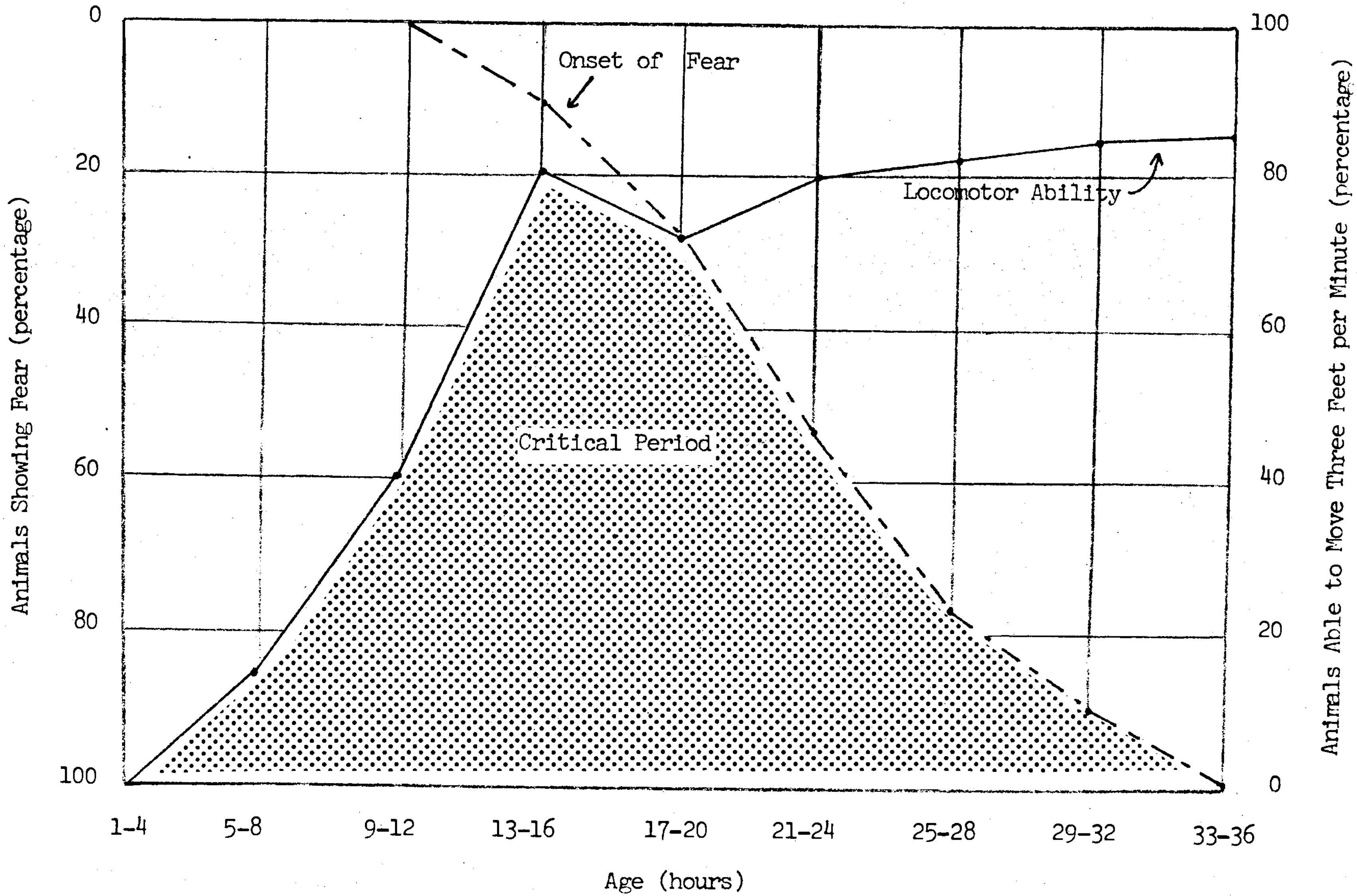

Figure 1. Limitations of the Critical Period for the Domestic Chick. (After Hess, 1959)

It has been speculated that the neonates form an internal representation of the outstanding features around them and subsequently withdraw from stimuli which do not concur with this image. At the end of the critical period, even a subtle alteration of the environment can trigger substantial avoidance reactions.

The fact that some types of stimulation attract the young bird and others repel it has been clearly established. Loud, singular cheeps, known as distress calls, coupled with movement away from the stimulus, indicate withdrawal. Rapid twittering, or contentment calls, along with movement toward the source of stimulation signal the attractiveness of the object approached by the newly-hatched bird.

Surprisingly, experiments have shown that the tendency to approach or withdraw from an object is not governed by the animal's primary needs for food or warmth. Indeed, attachments are formed in spite of an obvious lack of any real physical value in the object for the animal. As Sluckin (1965) has stated, imprinting does not depend on the presence of physiological rewards.

The third and fourth characteristics of imprinting cited by Thorpe, although not included in most scientific investigations about imprinting, deserve some comment at this point. The attachments formed to the releasing stimuli enable these stimuli to trigger certain behavior patterns much later in the avian's life, such as sexual behavior. Through the process of imprinting, these stimuli seem to acquire a permanent ability to release various types of social behavior. This concept is somewhat akin to the approach taken by Gardner Murphy (1947) to personality formation. Murphy proposed that each of us has an innate general tendency to respond to classes of stimuli. With the passage of time, this tendency becomes less and less general and focuses on a narrower range of stimuli--the familiar stimuli. Thus we have a tendency to like familiar things more and more. According to Murphy this "canalisation" is a kind of learning altogether different from conditioning; in fact, Murphy (1960) considered canalisation to be quite similar to the early views of imprinting.

The realization that the approach and following behaviors characteristic of imprinting are not directly related to physiological needs, but to some innate drive, has sent psychologists scurrying to their laboratories in search of some way of identifying the main features of this mysterious phenomenon. The following chapter is a historical survey of the significant work on this type of behavior, along with a look at a provocative area of research which has arisen from the initial investigations of imprinting.

CHAPTER II

REVIEW OF THE LITERATURE

The Imprinting Phenomenon

One of the earliest reports of imprinting occurred in 1873, when Spalding noted that incubator hatched chicks tended to follow persistently the first moving object to which they were exposed. While experimenting with pigeons around the turn of the century, Craig (1908) found that in order to cross two different species it was necessary to rear the young of one species under the adults of the other. Mature birds so reared preferred mates of the same species as their foster parents. Heinroth (1910) spurred interest in this phenomenon when he showed that geese would respond to humans rather than adults of their own species if they were exposed to humans just after hatching. Using Heinroth's observations as a basis for his experimentation, Lorenz (1935) analyzed the functional significance of stimuli involved in releasing this type of social behavior in birds. Lorenz's investigations illustrated the fact that, for certain species of birds, a wide variety of animate or inanimate objects could acquire the capacity to evoke certain types of behavior which were normally directed toward members of the same species.

Lorenz considered the conditions under which an object acquired this capacity to be unique; and gave the process a special name--imprinting. In order to distinguish imprinting from other types of learning, Lorenz emphasized three aspects of the imprinting process. The first, and most significant feature of the imprinting process was that it could occur only during a very definite period in the young animal's life. During this short period the organism was assumed to be at a critical stage of physiological development. Thus initial exposure to the object of imprinting must occur within several hours after hatching. The effect of this exposure was shown by the fact that the young animal approached and persistently followed the "surrogate parent" in filial fashion.

The second important feature of imprinting was what Lorenz considered to be its irreversibility. The stimulus to which an animal was exposed during its critical period became the preferred, and often the only, stimulus that animal would follow. Once the preference was established, it remained quite stable throughout the animal's life. Lorenz undoubtedly believed that imprinting modified the organism's behavior in ways which were extremely resistant to change.

A third characteristic of imprinting which distinguished it from other types of learning was the fact that imprinting resulted in attachment not to specific features of an object but to its general characteristics. Thus when a bird became imprinted to a certain stimulus it transferred its approach and following responses to all members of the species to which its "parent" belonged. Lorenz fostered the concept of imprinting as a method of acquiring "consciousness of species." In addition, Lorenz postulated that the first object to elicit a social response also released related responses, such as sexual behavior, later in the animal's life.

The first systematic investigations of imprinting were published in 1951. Working with several species of ducks and a variety of breeds of chickens, Ramsay (1951) discovered that sound and color were important variables in the recognition of the stimulus. However, most of Ramsay's experiments involved the exchange of parents and young, without introducing models or decoys. Fabricius (1951) was able to determine approximately the critical age at which several species of ducklings would imprint most successfully. Both Ramsay and Fabricius apparently considered imprinting to be a process which increased the selectivity of the releasing mechanism for social behavior, insuring that the animal would respond to a relatively limited number of stimuli.

In a more recent publication, Lorenz (1955) placed considerably more emphasis on the innate aspects of the imprinting process, a view which was essentially upheld by Thorpe (1956). Thorpe postulated that many species of birds at the time of hatching possessed a neurosensory mechanism that was responsive to stimuli provided by a variety of moving objects quite unlike the parent, as well as to stimuli provided by the parent. Since the activation of this mechanism released the following response, this lack of selectivity rendered the animal's social behavior susceptible to control by almost any relatively large moving object. Because this condition was not biologically adaptive, the animal had to acquire a preference for a specific class of objects very early in life. The establishment of this preference occurred in the critical period of development, making the releasing mechanism more selective and limiting the number of object configurations which would evoke social behavior.

Lorenz (1957) made use of this concept of an innate neurosensory mechanism in explaining the differences in "imprintability" among different species. In some species of birds, the following response could not be released by any object other than the parent. However, some species (e.g., the graylag goose and mallard duck) could be imprinted to moving objects that vary considerably in size, shape, and color. These differences in imprintability were assumed to be due to species differences in the releasing mechanism at the time of hatching.

The view of imprinting as a process which increased the specificity of a hypothetical releasing mechanism necessitated the responsibility of specifying the behavioral events involved in imprinting. A great deal of empirical examination has been focused on the behavioral events to which the imprinting process is functionally related. Smith and Hoyes (1961) utilized an array of visual stimuli for eliciting the approach response in domestic chicks. Salzen (1962) investigated the relation between imprinting and the onset of the fear response in several types of domestic fowl. Smith and Bird (1963) studied the effects of distant intermittent stimuli on the approach response of the chicken. Klopfer and Hailman (1964) examined a wide spectrum of perceptual preferences for imprinting in domestic chicks. An excellent review of the significant work on the characteristics and contexts of imprinting up to 1966 was published by Bateson (1966). More recent work on this subject includes that of Pedersen (1971) concerning the duration of exposure to the stimulus object. Hoffman _et al._ (1972) found that some stationary stimuli could acquire the capacity for control of the approach response. Stratton (1971) attempted to define the response contingencies in the following behavior of ducklings and to determine the nature of reinforcement provided by the imprinted stimulus. This shift of emphasis from the view of imprinting as essentially innate to the view of it as a learning process proved largely unsuccessful, however. Stratton was unable to demonstrate any consistent effect upon the following response by the environmental factors present over long periods of time.

Much of the pioneer experimental work in imprinting was done by Eckhard Hess. In 1959, Hess published a summary of his major findings after working with almost every species of fowl from pheasants to Peking ducks to Vantress broiler chicks. One outstanding contribution of Hess's work was his evidence for the differences between imprinting and visual discrimination learning. First of all, visual discrimination learning was faster and more resistant to extinction when trials were separated by periods of rest. On the other hand, imprinting was most effective when massed practice was used. Secondly, visual discrimination learning was most effective if the experience was most recent; in imprinting, primacy of experience was most effective. Another important difference lay in the fact that the administration of painful stimulation increased the effectiveness of imprinting, while aversive stimulation caused avoidance reactions to a visual discrimination stimulus.

A major portion of Hess's article was devoted to a characteristic of imprinting which is still a locus of uncertainty in experimental findings--the critical period.

The Critical Period

Imprinting is known to occur during a specifiable sensitive period very early in life. This period has also been called the critical period because it is believed that filial attachments cannot be formed at any other time. Fabricius (1951) reported maximal following at 12 hours after hatching in three species of ducks. Although Lorenz (1935) thought that the sensitive period of ducklings occurred only within the first few hours of life, Fabricius and Boyd (1954) reported the highest proportion of followers in mallard ducklings at ages ranging from 25 to 50 hours. Using domestic chicks, Jaynes (1957) assessed approach and following responses to a moving object at various ages and found the youngest chicks to be most responsive, with following markedly decreased after the first week of life. Ramsay and Hess (1954) found the critical period for imprinting in mallard ducklings to range from five to 24 hours after hatching, reaching an optimal level at thirteen to sixteen hours of age. This finding was later substantiated by Hess (1959), who also found that the mallard ducklings who consistently scored the highest in a test of following were in the thirteen-to-sixteen-hour-old group.

In his classic study, Gottlieb (1961) investigated the critical period for imprinting in the domestic Peking duckling, and found that it extended from eight to approximately 27 hours after hatching. Although he could see no clear optimum time for exposure to the imprinting stimulus in terms of posthatch age, Gottlieb reported a marked peak in his percentage-following curves at 27 days when he converted the ducklings' ages to developmental age; i.e. age calculated from onset of incubation. (The normal length of time for the gestation period of Peking ducklings is 24 to 26 days.)

According to Jaynes (1957), the most likely explanation for the variety of results obtained in the different experiments on critical periods for imprinting was that the limits and duration of the critical period were an extremely delicate function of several important variables:

(1) Species differences. Considerable species differences in ease of imprinting exist among geese, Bantams, mallards, Muscovy ducks, and Peking ducks, as well as others.

(2) Nature of the stimulus. There appeared to be innate preferences for stimulus objects, including the color, form, size, movement, and sound of an imprintable object. Jaynes thought it possible that a less specific stimulus could extend the critical period for some neonates.

(3) Duration of imprinting session. When the lengths of the imprinting sessions used in previous experiments were compared, it was discovered that the longer the session, the greater proportion of birds were imprinted.

and other important factors such as methods of incubation (forced vs. still-air incubators) and rearing (individual vs. communal housing).

Gottlieb (1961) placed the blame for such a disparate conglomeration of critical period findings on the use of posthatch age as a baseline for behavioral development. In Gottlieb's opinion, posthatch age was only a rough approximation and one that obscured such delicately timed processes as the neurophysiological and neuromuscular developments of the embryo. According to Gottlieb, posthatch age disregarded the varied lengths of individual incubation periods. Also, Gottlieb's findings suggested that these important organismic developments relevant to imprinting took place independently of hatching, and they were more stable than the factors governing time of hatching. Hatching, as a mechanical event, could be initiated by a less well-developed neuromuscular system than that which is later involved in imprinting. Contrary to most leading experimenters in this field, Gottlieb conceived of imprinting as dependent upon a more mature state of the organism than was required for hatching. While successful hatching depended upon the heat and humidity of the air around the shell, readiness for imprinting depended upon metabolic factors which influenced the whole embryo. Gottlieb added that "alteration of the metabolism of the developing embryo would probably show a greater effect on the critical period for imprinting than on the time or success of hatching" (p. 285).

Drug Effects

When compared with the vast amount of work on the imprinting phenomenon itself, relatively little has been done with the effects of drugs on imprinting. Even so, all but one of the experiments published to date have dealt with drugs administered after the animal has hatched.

In an attempt to reduce emotionality and slow metabolism, thereby lengthening the critical period, Hess (1957) administered meprobamate to ducklings at twelve hours of age and tested for imprinting at 14, 16, 24, and 26 hours of age. Almost as an afterthought, Chlorpromazine and Nembutal were also used as test conditions. Chlorpromazine allowed good imprinting at all ages, but emotionality did hinder imprinting in the Nembutal group. The meprobamate group showed little evidence of emotionality, but this drug's muscle relaxant effects nullified its effectiveness and made imprinting almost impossible.

In a personal communication with Smith and Bird (1963), James indicated that injections of testosterone within 24 hours of hatching did not effect the chick's response to visual flicker (small sources of flickering light); but when the chick was three days old, testosterone did depress that response.

After participating in an experiment which indicated the possible involvement of autonomic arousal of neural activity during imprinting, Kovach (1964) decided to study the effects of various autonomic drugs upon the following behavior of young chicks. Kovach administered adrenergic stimulants (amphetamine, ephedrine sulphate, and epinephrine), a cholinergic stimulant (neostigmine), a cholinergic blocking agent (atropine), and three additional pharmacological agents (hexamethonium, ergotamine, and ergonovine)--each at 8, 14, 18, 24, and 32 hours of posthatch age. The behavior produced by the adrenergic stimulants was remarkably similar to that observed after the administration of painful stimulation (Kovach & Hess, 1963). The chicks' performance under the influence of the cholinergic stimulant neostigmine was below that of the controls, but not significantly different. The cholinergic blocking agent atropine, which is also a strong central stimulant, significantly facilitated following at eight hours, while interfering with following at ages beyond eight hours. Kovach suggested that this paradoxical effect may have been due to the fact that the intensity of central excitation produced by atropine was above the optimal level for imprinting at maturational states beyond eight hours. Contrary to Kovach's expectations, all the remaining experimental drugs facilitated imprinting at all ages.

Kovach (1964) summarized the most interesting conclusion of his study in the following statements:

> It appears that any agent which will produce general activation of the CNS will facilitate the following behavior at the earliest ages. It is likely that the involvement of sympathetic stimulation in the elicitation and establishment of behavior patterns during the early critical periods. . .does not go beyond the general activating role of the adrenergic neurohumoral mechanisms associated with the reticular activating system (p. 187).

All drugs used in Kovach's experiment facilitated following behavior at an age which had been previously identified as prior to the critical period for imprinting in chicks; i.e. an age at which following behavior was not yet present under normal conditions. Kovach's findings showed that this view of the critical period was incorrect. Lack of following at very early ages was not due to incomplete muscular development, nor was the critical period a strick function of posthatch age. According to Kovach, the age of a subject was an important variable only to the extent that it correlated with the maturational state of excitability of the CNS. When Kovach pharmacologically increased that level of excitation, he observed following at an age when the excitability of the CNS was ordinarily too low to produce following behavior under normal circumstances. In other words, Kovach succeeded in manipulating, by means of drugs, what was previously considered to be a fairly "fixed" pattern of behavior. It is also noteworthy that the effects of the drugs used by Kovach on the initial imprinting performance were retained at an age well beyond the critical period (52-60 hours), in the absence of any further administration of drugs.

Although not directly concerned with the imprinting phenomenon, the findings of Schrold and Squires (1971) helped to further illustrate the nature of the effects of one type of stimulant on the newly hatched avian. The purpose of their experiment was to observe the effects of the stimulant d-amphetamine on five day old chicks under conditions of normal activity. The chicks were observed in groups of four and scored every 15 minutes for one or two hours after drug administration. The predominant behavioral signs exhibited by these birds were wing droop, down on metatarsus, trunk against floor, head up, rump up, and significantly increased twittering. However, when the chicks were pretreated with a drug which protected them from these effects, injections of d-amphetamine produced a marked increase in the level of general excitation, with a considerable amount of aggressive behavior (esp. pecking).

In a later paper, Schrold (1972) conducted an investigation of the effects of d-amphetamine in combination with various antidepressants and some psychotropic drugs on the behavior of three to five day old chicks. The antidepressant imipramine and the relatively unknown drug pipradrole showed the most apparent effects, causing greatly increased locomotion about one hour after injection. Unfortunately, very little is known about the neurochemical mechanisms underlying the behavioral effects of these drugs.

At present, the only published work on the effects of prenatally administered drugs in the avian embryo was undertaken by Green and Meeker (1972). Their investigation was launched in order to determine the effects of a CNS stimulant, d-amphetamine, and a CNS depressant, Nembutal, on hatch viability, weight gain, postnatal activity, and emotional development. Using very small dosages, Green and Meeker injected each egg with its assigned drug once daily from Day 14 to Day 18 of incubation. Contrary to their expectations, the researchers found that the Nembutal group showed significantly higher levels of activity than either the Amphetamine or Control (saline) groups on an open-field test, as well as other posthatch behavioral measures.

Although research concerning the effects of drugs on imprinting has proven quite fruitful, one major aspect of the entire imprinting process has received considerably more attention in the past few years.

The Stimulus Situation

As mentioned earlier, one of the most plausible explanations for the variety of findings in experiments concerned with the imprinting process was the nature of the stimulus used to educe the approach response. Through the process of trial and error, investigators of this subject slowly discovered a set of feasible and effective objects which appeared successful in evoking the initial approach movements.

Working with young coots, gulls, and ducklings, Nice (1953) found that simple hand movements like that used in sketching were not sufficient to initiate imprinting. However, Weidmann (1958) observed that ducklings would approach a human being if the person were walking slowly or moving from side to side while seated.

In an attempt to more explicitly define the nature of the "sufficient" stimulus situation for the approach response, Smith (1960) used a rectangular run approximately ten feet long. The sides of the run were covered with opaque brown paper and the top was covered with fine muslin. Smith's first stimulus was a 12-inch disc of white Bristol Board, on which he had painted a black 45° sector, placed at one end of the run. The disc rotated clockwise at one revolution per 1.5 seconds, driven by a small, silent electric motor. Chicks were placed in the center of the run, at a right angle to the stimulus object, and their behavior was observed for five minutes. Despite the unmistakably favorable response to this stimulus, Smith designed another experiment in which he compared the response to this stimulus with the response to an identical disc which was moved up and down in a vertical position at one end of the run. From a comparison of two groups of newly hatched chicks, the white disc with the black sector which rotated slowly in one plane was significantly more effective in producing approach responses than the similar object moving away from the chick, but not rotating.

Smith and Hoyes (1961) discovered that a number of intermittent visual stimuli presented silently at a distance of three to five feet from the chick would not only induce approach responses and "contentment" chirps, but also be chosen in discrimination and choice situations. James (1959) found that flickering sources of light were sufficient stimuli for imprinting chicks. Subsequent work by James (1960) resulted in the definition of an approximate critical period for these responses to flickering light which was almost identical to that reported for objects moving away from the subject. Smith and Bird (1963) decided to further document the effectiveness of the rotating white 12-inch disc with a black 45° sector by comparing it with a flickering patch of light. The proportions of chicks approaching each stimulus were not significantly different on the initial presentation of the stimuli. However, when the chicks' performance on subsequent days was compared, the experimenters found that the chicks exposed to the flashing light ceased to approach that stimulus. The overall findings of their study show that, as a group, chicks exposed to the rotating disc continued to respond better. The final conclusion made by Smith and Bird was that this experiment provided evidence that the rotating sector/disc was an "intrinsically more attractive stimulus."

In the most recent experiment conducted by Smith et al. (1970), a somewhat improved stimulus situation produced even better approach results. The apparatus was a "run" made of sound absorbent panels painted matt white. The imprinting stimulus was a 30.5 cm diameter disc, with a 45° red sector, attached to a small electric motor and rotated at a speed of 30 ± 10 revolutions per minute. The floor of the run was graduated, enabling the experimenters to record the chicks' progress. After exposure to the imprinting stimulus, all chicks were required to discriminate between it and another stimulus, a rotating white disc on which thin black stripes had been painted. When both stimuli were presented in the choice situation, responses to the rotating sector/disc were significantly better. Smith _et al_. interpreted this finding as further confirmation of their hypothesis that the rotating sector/disc was an intrinsically more appealing stimulus for eliciting approach responses in domestic fowl.

A few of the intriguing issues which have arisen from the wealth of information about the imprinting process, as well as a proposal for investigation of a previously ignored question, will be discussed in the next chapter.

CHAPTER III

STATEMENT OF THE PROBLEM

The idea that there are periods in the development of an individual during which certain experiences acquire a lasting influence on his or her later behavior is not new. Freud suggested that certain patterns of behavior in the various stages of psycho-sexual development could become fixed and later mold the personality. As mentioned earlier, Murphy postulated that, at critical periods in a person's life, general needs evolve into specific preferences and can determine one's lifelong tastes. In his analysis of the critical period for socialization, Scott (1962) considered this sensitive period to be the most crucial learning phase of all. Although it is extremely difficult to ascertain these periods in human beings, observations of lasting attachments in young precocial birds have produced a great deal of documented research.

In addition to the environmental factors influencing the young animal, the process of physical maturation plays an invaluable role in setting the limitations for the time during which the animal can be imprinted successfully (Schneirla, 1956; Slucking, 1965). Gottlieb (1961) added a new dimension to this view of the critical period by proposing that the animal's state of readiness for imprinting was a function of its metabolic development, and not its age in terms of hatching time. In the same study, Gottlieb also postulated that altering the avian embryo's metabolism would affect its critical period more than its hatching time.

At the present time, relatively little work has been published on the effects of drugs on the imprinting process. It is possible that this is due, at least in part, to the lack of established dosage levels for drug use with newborn avians. Only one investigation (Green & Meeker, 1972) dealt with the prenatal injection of pharmacologic agents, but it did not attempt to document the drugs' effects on any measure of imprinting.

The avian embryo has been shown to be a particularly convenient subject for the study of prenatal development because embryonic growth is largely independent of the parent, which allows a clear experimental separation of fetal and maternal effects (Gold, 1972). The present study used one species of domestic fowl to observe the effects of pre- and postnatal pharmacologic manipulations on the critical period for imprinting. If a drug which has been found to facilitate following behavior when administered postnatally were injected into the developing embryo, it is possible that the critical period during which the organism is at its maximal level of imprintability would be observed at a significantly earlier posthatch age than that reported by previous investigators. This would represent evidence that (a) there may be some observable connections between embryonic stimulation and posthatch measures of imprinting, and (b) it may be possible to manipulate the readiness for imprinting by increasing the speed with which metabolic events occur in the developing central nervous system of the embryo.

Specifically, it was hypothesized that the final measure of imprinting would yield the following results:

1) _Ss_ receiving ephedrine sulphate prenatally imprint significantly earlier than _Ss_ receiving saline prenatally.

2) *S*s receiving ephedrine sulphate postnatally imprint significantly earlier than *S*s receiving saline postnatally.

3) *S*s receiving ephedrine sulphate both pre- and postnatally imprint significantly earlier than *S*s receiving ephedrine sulphate only prenatally, only postnatally, or *S*s receiving saline in both conditions.

CHAPTER IV

METHOD

Subjects

_S_s were 48 fertilized eggs of Peking ducks, a highly domesticated breed of _Anas platyrhynchos_, obtained from a local commercial hatchery.

The technique to assure similar embryonic stage prior to artificial incubation was essentially the same as that used by Gottlieb (1961). Before being placed in the incubator, the eggs were refrigerated at 40-50° F. for at least 24 hours. After refrigeration, the eggs were held at 75-80° F. for 6 hours before placement in the incubator. This procedure assured that each duckling began incubation in a similar embryonic state, which was necessary in order to calculate the developmental age of each duckling later in the experiment. According to Gottlieb's study (1961), the chilling procedure will kill any embryo which may have developed beyond the initial single layer stage while not destroying any embryo in which "no cell division has taken place beyond that which is normally present at the time the egg is laid (p. 424)."

The eggs were placed in a still-air incubator (Brower Mfg. Co., Quincy, Illinois) at a mean temperature of 102° F., after being washed in a mild disinfectant solution (Green & Meeker, 1972). During incubation, eggs were turned twice daily from Day 5 to Day 26, and cooled for 10-15 minutes daily from Day 7 to Day 21. Eggs were sprayed with distilled water at room temperature daily from Day 5 to Day 24. Each egg was candled to check fertility and embryo viability on Days 7, 14, 21, and prior to drug injection. All damaged and infertile eggs were removed from the incubator and discarded.

Prehatch Procedure

The remaining eggs were randomly divided into two treatment groups: ephedrine sulphate and saline. Earlier studies (Myers & Stettner, 1968; Green & Meeker, 1972) have shown that younger organisms have a much lower tolerance for drugs than do adults of the same species. Therefore, all prehatch dosage levels were set at .03 cc/dose. On the day of injection (Day 24), a small puncture was made in the equator of each shell. All injections were administered under sterile conditions. To prevent dehydration, clear nail polish was used to seal the entry site following each injection (Green & Meeker, 1972).

Ephedrine sulphate injection is a sympathomimetic agent. The mechanism of action of this agent is fairly well known (Goodman & Gilman, 1970). It has pronounced stimulating effects on the central nervous system, raises blood pressure, dilates the pupils, and stimulates the respiratory center; and these effects are slower and more prolonged than after adrenaline. Although isolated instances of toxicity have been reported when ephedrine was given in excessive doses within a short period of time, the drug has shown no consistent cumulative effects and has been administered to humans in dosages as large as 400 mg with no apparent harmful side effects.

Of the 48 viable eggs which began incubation on Day 1, 14 embryos died during the first three weeks of incubation. On Day 24, half of the remaining 34 eggs were injected with ephedrine sulphate, and the other half received injections of saline. Twenty-five ducklings finally hatched at the end of the gestation period, including 13 of those prenatally injected with saline and 12 ducklings who were prenatally injected with ephedrine sulphate. Hatching time was recorded with an accuracy of $\pm$ 1 hour. Newly hatched ducklings were allowed to remain in the incubator for 2-3 hours, at which time they were removed and placed in small individual wire living cages with aluminum sides. This method of housing insured visual but not auditory isolation. Ss then remained in the light of an infrared heat lamp at a temperature of 85-90° F. for 24 hours a day. Neither food nor water was available until the completion of the experiment (Kovach & Hess, 1963; Kovach, 1964).

Apparatus

The imprinting apparatus consisted of a runway 1.82 m long X 30.7 cm wide X 30.7 cm high, constructed of plywood painted matte gray (Smith et al., 1970). The top of the apparatus was covered at all times by a fine mesh wire screen. The approach stimulus was a 25.5 cm diameter disc of white poster board, on which a 45° sector was painted red. This disc was attached to the drive shaft of a small electric motor located outside the apparatus, which rotated clockwise at a speed of 30 $\pm$ 5 revolutions per minute. The floor of the runway was graduated from zero at the center to ten at a position 7.5 cm from either end, the graduations being 7.5 cm apart (Smith et al., 1970).

For the testing sessions, the apparatus was modified to form a truncated "V". During these sessions, the original approach stimulus was paired with the discriminative stimulus, which consisted of a 25.5 cm diameter disc of white poster board on which were painted 1.28 cm wide black stripes, 1.28 cm apart. The discriminative stimulus was mounted on the drive shaft of another electric motor rotating at the same speed (Smith et al., 1970).

Posthatch Procedure

Upon hatching, each S was randomly assigned to one of two posthatch experimental conditions: 1) 3 mg ephedrine sulphate injection per kg of body weight or 2) 3 mg saline solution per kg of body weight (Moore, 1974). According to this procedure, half of the group which received ephedrine sulphate and half of the group which received saline in the embryonic state were injected with the drug 1 hour before the training session. The remaining halves of each prehatch group received an injection of saline 1 hour before the training session. Before being transferred to individual housing, each duckling was randomly assigned to one of the following posthatch training times: 6, 10, or 14 hours after hatching. The assignment of Ss to levels of each of the three independent variables (prehatch condition, posthatch condition, and imprinting time) is illustrated in Figure 2.

One hour before the designated imprinting time, each S was weighed and injected intraperitoneally with the calculated dose of the assigned drug, then returned to its home cage for the remainder of the hour (Kovach, 1964). At the appropriate time, each S was placed in the center of the apparatus, facing the wall of the runway, and timing was begun.

The first time recorded was the animal's latency to move (LTM), i.e. the time, in seconds, between first being placed in the runway and making its first move (Smith et al., 1970). Each training session lasted 10 minutes; and the Ss' performance was recorded every 15 seconds

Prenatal Injection	Postnatal Injection	Imprinting Time (hours) 6	10	14
a_1 Ephedrine Sulphate	b_1 Ephedrine Sulphate	GROUP I		
		N = 2	N = 2	N = 2
	b_2 Saline Solution	GROUP II		
		N = 2	N = s	N = 2
a_2 Saline Solution	b_1 Ephedrine Sulphate	GROUP III		
		N = 2	N = 2	N = 2
	b_2 Saline Solution	GROUP IV		
		N = 2	N = 2	N = 2

Figure 2. Experimental Design.

by noting the grade the duckling had reached. Thus, a duckling having a very short LTM which reached a position just in front of the stimulus before the end of 15 seconds, and stayed there for the remainder of the session, would receive 40 entries of 10 grades--a total score of +400, the maximum attraction score. Movement away from the stimulus was scored negatively on the same scale, resulting in a possible maximum aversion score of -400. These scores were referred to as "following scores." In both training and testing conditions, position preference was controlled for by presenting the disc(s) at alternate ends of the runway in random order for each S.

Each animal was tested for strength of imprinting in the modified apparatus at 40 hours of age (Kovach, 1964). No drugs were administered at the time of testing. Testing sessions were essentially the same as training sessions, with the addition of the discriminative stimulus, which was placed at the opposite end of the truncated runway from the approach stimulus. Each testing session was 5 minutes in length, with the Ss' performance being scored every 15 seconds. Scoring for the testing sessions was exactly the same as the system used for the training sessions, so that the maximum attraction score was +200 and the maximum aversion score was -200. Thus the two dependent variables were the LTM and following scores recorded in the testing session.

CHAPTER V

RESULTS

Of the 48 eggs which began incubation, 51% hatched. Two percent of the eggs hatched on Day 26, 35% hatched on Day 27, 8% hatched on Day 28, and 6% hatched on Day 29. Because only 50% of these hatched Ss exposed to the approach stimulus in the training session survived to participate in the testing session, it was impossible to obtain even one measure of the following for each experimental group at each post-hatch age level. Therefore, the absolute distance travelled in the apparatus by each S during the testing session was found, and the means for each cell calculated, in an attempt to uncover any possible remaining sources of response variance. The absolute distance scores, along with the latency and following scores, of the surviving Ss are presented in Table 1.

Due to the high mortality rate, there were not enough Ss at each treatment level to provide a sufficient N for statistical analysis of the testing data. However, the results reported in Table 1 show that a surprising number of Ss in Groups I and II, who received ephedrine sulphate in the prenatal injection, died before they could be tested for imprinting in the discrimination situation. It is interesting to note that the following scores and absolute distance scores of the two Ss who survived in these groups were considerably lower than those of Ss in either of the groups receiving saline in the embryonic injection (Groups III & IV).

TABLE I

MEAN SCORES FOR SURVIVING SS ON ALL MEASURES

Group	Posthatch Age (in hours)	N	LTM (in seconds)	Following Scores	Absolute Distance Scores
I	6		266	+2	1
(Drug/	10	1			
Drug)	14				
II	6				
(Drug/	10				
Saline)	14	1	38	-10	6
III	6	2	36.5	+30	16
(Saline/	10	2	112.5	+34	13.5
Drug)	14	1	59	-29	9
IV	6	1	53	+100	32
(Saline/	10	2	215	-28	12.5
Saline)	14	2	205.5	+30	13.5

A test of the proportion of the number of surviving members in each group revealed that Groups III and IV had a significantly higher proportion of living *S*s than Groups I and II ($z = 3.26$, $p < .001$). Thus a significantly greater number of ducklings receiving an injection of ephedrine sulphate in the empbryonic state died before the time of testing (48 hours of posthatch age). Figure 3 shows the number of ducklings either surviving for or dying prior to testing in terms of which prenatal treatment they received.

		State of *S*s at Testing: Alive	Dead
Prenatal Injection	Ephedrine Sulphate (Groups I & II)	2	10
	Saline Solution (Groups III & IV)	10	2

Figure 3. Survival Rate as a Function of Prenatal Injection

A comparison of the proportion of surviving *S*s in Groups I and III versus Groups II and IV was not significant ($z = 0$). This indicates that the nature of the posthatch injection had no effect upon the animal's chances for survival.

CHAPTER VI

DISCUSSION

The proposal that embryonic drug stimulation would facilitate following behavior at an age prior to the normal critical period for imprinting was not supported. Furthermore, it was found that this particular drug (ephedrine sulphate), when injected into the embryo on the 24th day of incubation, had a lethal effect at some point between 24 and 48 hours of posthatch age. Even the 2 of 12 ducks receiving the drug prenatally who lived showed lower movement scores (following and absolute distance) than the other Ss in Groups III and IV.

Ephedrine sulphate injection was used successfully in doses of 20 mg/kg by Kovach (1964) in his work with Vantress broiler chicks 8 to 32 hours old. However, as is the case with an overwhelming majority of the published studies concerning imprinting, Kovach failed to report either the percentage of eggs which hatched of the original number placed in incubation or the percentage of Ss who completed the retention test of those who were initially exposed to the stimulus in the training session. The data resulting from the present experiment point to the critical importance of reporting such facts so other experimenters might have some basis for comparison. As it is, the amount of time and expense involved in establishing even a rough idea of the mortality rate associated with drug stimulation (especially embryonic) would be prohibitive for one individual. Future investigators of this phenomenon would do well to include such vital statistics in their published papers.

Taken by itself, the 51% total hatch rate found in this study could be an indication of the need for a change in incubation conditions (the present experiment used a still-air incubator while a majority of the recent work on imprinting employed forced-air incubators). This finding could also be explained in terms of the embryonic administration of substances into the egg, since a similar number of eggs injected with either agent failed to hatch. The former explanation is a little more plausible, however, in light of the fact that fourteen of the eggs which began incubation died by the end of the second week in the gestation period. A third possibility exists--that the abnormally low hatch rate was due to the interaction of the incubation conditions and the violation of the egg by foreign substances before hatching. The only published work which represented an attempt to assess the effects of prenatally administered drugs on postnatal behavior (Green & Meeker, 1972) reported a 36% mortality rate for the first two weeks of gestation alone. Unfortunately, Green and Meeker failed to report either the final hatch rate, which would have given some indication of the effects of penetration of the shell, or the number of Ss surviving to completion of the posthatch measures of behavior.

It appears that avian embryos can be chemically challenged in the shell and still produce viable young, but the nature of the chemical substance is of crucial importance. While saline solution has no obvious harmful effects, ephedrine sulphate--even in such small doses--is definitely not a suitable experimental drug when administered prenatally. Green and Meeker (1972) pointed out the problem areas of prenatal drug administration in the following statement: "Since it is unknown how much of the drug is able to penetrate the blood brain barrier, or what concentrations must be administered for optimal effect, much initial work has still to be done." Beyond that, any conclusions drawn from the results of the present experiment, besides the need for further study of the experimental variables, would be sheer speculation.

BIBLIOGRAPHY

Arbib, M. A. Organizational principles for embryological and neurophysiological processes. International Journal of Neuroscience, 1971, 2, 207-217.

Balaban, M., & Hill, J. Perihatching behavior patterns of chick embryos (Gallus domesticus). Animal Behavior, 1969, 17, 430-439.

Barnes, C. D., & Etherington, L. G. Drug dosage in laboratory animals: A handbook. Berkeley: University of California Press, 1965.

Bateson, P. P. G. The characteristics and context of imprinting. Biological Reviews, 1966, 41, 177-220.

Cole, S. O. Comments on generalizations related to the experimental effects of amphetamine. Journal of General Psychiatry, 1972, 87 (1), 99-103.

Csanyi, V., Gervai, J., & Adam, G. Two time-dependent responses of chicks to imprinting stimuli. Journal of Comparative and Physiological Psychology, 1973, 83, 13-18.

Dews, P. B., & Skinner, B. F. Techniques for the study of behavioral effects of drugs. Annals of the New York Academy of Science, 1956, 65 (4), 247-356.

Dimand, S. J. Approach behavior and embryonic visual experience in chicks: Studies on the effect of rate of visual flicker. Animal Behavior, 1972, 20, 413-420.

Fabricius, E., & Boyd, H. Experiments on the following reaction of ducklings. Wildfowl Trust Annual Reports, 1952/53, 6, 84-89.

Fisher, A. E. Chemical stimulation of the brain. Scientific American, 1964, 210 (6), 60-68.

Gilbraith, J. C. Personal communication with the former professor of Poultry Science, Oklahoma State University, May, 1974.

Glick, S. D. Facilitation or impairment of learning by d-amphetamine as a function of stimuli. Psychopharmacologia, 1971, 21 (4), 356-360.

Gold, P. S. The effects of sensory stimulation during embryogeny on growth, hatching, and imprinting in the domestic chick. Dissertation Abstracts International, 1972, 32 (10-B), No. 6074.

Goodman, L. S., & Gilman, A. (Eds.) The pharmacological basis of therapeutics. 4th ed. New York: Macmillan, 1970.

Gottlieb, G. Developmental age as a baseline for determination of the critical period in imprinting. Journal of Comparative and Physiological Psychology, 1961, 54 (4), 422-427.

Gottlieb, G. Following-response initiation in ducklings: Age and sensory stimulation. Science, 1963, 140, 399-400.

Gottlieb, G. Prenatal behavior of birds. Quarterly Review of Biology, 1968, 43, 148-174.

Gottlieb, G. Neglected developmental variables in the study of species identification in birds. Psychological Bulletin, 1973, 79 (6), 362-372.

Gottlieb, G., & Klopfer, P. H. The relation of developmental age to auditory and visual imprinting. Journal of Comparative and Physiological Psychology, 1962, 55 (5), 821-826.

Gottlieb, G., & Kuo, A. Y. Development of behavior in the duck embryo. Journal of Comparative and Physiological Psychology, 1965, 59, 183-188.

Green, P. C., & Meeker, R. Effects of prenatally-administered drugs on peri- and postnatal behavior in the Peking duck. Proceedings of the 80th Annual Convention of the American Psychological Association, 1972, 7, 87-88.

Hafez, E. S. (Ed.) The behavior of domestic animals. 2nd ed. Baltimore: Williams & Wilkins, 1969.

Henton, M. B. Stimulus coding in the species-specific perception of Peking ducklings: An analysis of critical elements of their prenatal emergence. Dissertation Abstracts International, 1971, 32 (3-B), No. 1876.

Hess, E. H. Effects of meprobamate on imprinting in waterfowl. Annals of the New York Academy of Sciences, 1957, 67, 724-732.

Hess, E. H. Imprinting. Science, 1959a, 130, 133-141.

Hess, E. H. Two conditions limiting critical age for imprinting. Journal of Comparative and Physiological Psychology, 1959b, 52, 515-518.

Hoffman, H. S., Eiserer, L. A., & Singer, D. Acquisition of behavioral control by a stationary imprinting stimulus. Psychonomic Science, 1972, 26 (3), 146-148.

Jacobson, M. The development of specific neuronal connections. Science, 1969, 163, 543-547.

Jaynes, J. Imprinting: The interaction of learned and innate behavior. II. The critical period. Journal of Comparative and Physiological Psychology, 1957, 50, 6-10.

Klopfer, P. H., & Gottlieb, G. Imprinting and behavioral polymorphism: Auditory and visual imprinting in domestic ducks (Anas platyrhynchos) and the involvement of the critical period. Journal of Comparative and Physiological Psychology, 1962, 55 (1), 126-130.

Klopfer, P. H., & Hailman, J. P. Perceptual preferences and imprinting in chicks. Science, 1964, 145, 1333-1334.

Kovach, J. K. Effects of autonomic drugs on imprinting. Journal of Comparative and Physiological Psychology, 1964, 57 (2), 183-187.

Kovach, J. K. Development and mechanisms of behavior in the chick embryo during the last five days of incubation. Journal of Comparative and Physiological Psychology, 1970, 73, 392-406.

Kovach, J. K., & Hess, E. H. Imprinting: Effects of painful stimulation upon the following response. Journal of Comparative and Physiological Psychology, 1963, 56 (2), 461-464.

Milliken, G. C. The development of filial behavior in ducklings. Behaviour, 1972, 43, 13-47.

Moltz, H. Imprinting: An epigenetic approach. Psychological Review, 1963, 70, 123-138.

Moore, J. Personal communication with the Chairman, Department of Pharmacology, University of Oklahoma Health Sciences Center, July, 1974.

Oppenheim, R. W. Some aspects of embryonic behavior in the duck, Anas platyrhynchos. Animal Behaviour, 1970, 18, 335-352.

Oppenheim, R. W. Prehatching and hatching behaviour in birds: A comparative study of altricial and precocial species. Animal Behaviour, 1972, 20, 644-655.

Oppenheim, R. W. An experimental investigation of the possible role of tactile and proprioceptive stimulation in certain aspects of embryonic behavior in the chick. Developmental Psychobiology, 1972, 5 (1), 71-91.

Oppenheim, R. W. An investigation of various egg-opening techniques for use in avian behavioral embryology. Development Psychobiology, 1973, 6, 53-68.

Paulson, G. W. Maturation of evoked responses in the duckling. Experimental Neurology, 1965, 11, 324-333.

Pedersen, E. E. Duration, intermittency of pretest exposure, and imprinting in the domestic chick. Dissertation Abstracts International, 1971, 31 (12-B), No. 7641.

Ramsay, A. O. Familial recognition in domestic birds. Auk, 1951, 68, 1-16.

Russell, R. W. Drugs as tools in behavioral research. In L. Uhr and J. G. Miller (eds.), Drugs and behavior. New York: Wiley & Sons, 1960.

Salzen, E. A. Imprinting and fear. Symposium of the Zoological Society of London, 1962, 8, 197-217.

Salzen, E. A. Imprinting and the immobility reactions of domestic fowl. Animal Behaviour, 1963, 11, 66-71.

Salzen, E. A., & Sluckin, W. The incidence of the following response and the duration of responsiveness in domestic fowl. Animal Behaviour, 1959, 7, 172-179.

Schrold, J. Behavioural effects of d-amphetamine alone and in combination with antidepressants, antihistamine or other psychotropic drugs in young chicks. Psychopharmacologia, 1972, 23 (2), 115-124.

Schrold, J., & Squires, R. F. Behavioral effects of d-amphetamine in young chicks treated with p-Cl-phenylalanine. Psychopharmacologia, 1971, 20, 85-90.

Sidman, M. Drug behavior interaction. Annals of the New York Academy of Sciences, 1956, 65, 282-302.

Smith, F. V. Towards definition of the stimulus situation for the approach response in the domestic chick. Animal Behaviour, 1960, 8 (3-4), 197-200.

Smith, F. V., & Bird, M. W. Varying effectiveness of distant intermittent stimuli for the approach response in the domestic chick. Animal Behaviour, 1963, 11, 57-61.

Smith, F. V., & Hoyes, P. A. Properties of visual stimuli for the approach response in domestic chicks. Animal Behaviour, 1961, 9, 159-166.

Smith, F. V., Nott, K. H., & Yarwood, A. Brain protein synthesis and the approach response of chicks to visual stimulation. Brain Research, 1970, 21, 79-90.

Spooner, C. E. Observations on the use of the chicken in the pharmacological investigation of the central nervous system. (Doctoral dissertation, University of California at Los Angeles) Ann Arbor, Mich.: University Mikrofilms, 1964.

Stettner, L. J., & Matyniak, K. A. The brain of birds. Scientific American, 1968, 218, 64-76.

Stratton, J. W. Response contingencies in the following behavior of imprinted ducklings. Dissertation Abstracts International, 1972, 32 (11-B), No. 6701.

Uhr, L., & Miller, J. G. (Eds.) Drugs and behavior. New York: Wiley & Sons, 1960.

Williams, J. T. Developmental age and the critical period for imprinting. Psychonomic Science, 1972, 27 (3), 167-168.

Yuiveler, A., Gresnough, W., & Geller, E. Biochemical and behavioral effects of magnesium pemoline. Psychopharmacologia, 1968, 13, 174-180.

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