THE EFFECTS OF METOSERPATE HYDROCHLORIDE ON THE NEONATAL APPROACH BEHAVIOR OF DOMESTIC CHICKS

by

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INTRODUCTION

In many species of animals, the survival of the offspring is dependent upon the close proximity of one or both parents. In an altricial species, such as man, where the neonate closely resembles an unborn fetus and is developmentally restricted with regard to mobility and perceptual capabilities, this proximity is due largely to the activity of the parents. In precocial species, however, the young organism comes into the world possessing functional, if not fully developed, sense organs and is capable of locomotion. In these non-altricial species, the goat for example, close proximity of the young to the parent is largely dependent upon the behavior of the young. Typically, the neonate of a precocial species forms an exclusive bond or attachment with the parent soon after (within hours of) birth or hatching and will persist in closely following the adult figure. Lorenz (1935) has used the word "Pragung" or "imprinting" to describe this filial response.

Spalding (1873), in one of the first systematic observations on imprinting, reported that domestic chicks will follow any moving object including humans and, if allowed to follow that human, will, when returned to the hen, leave her and persist in following any person within sight. Although discussed by Spalding as early as 1873 and referred to by James (1890), it was not until the pioneering work of Lorenz (1935, 1937) that imprinting was subjected to intense experimentation as a unique learning phenomenon. And it was Lorenz who first demonstrated that this unique learning process was confined to a relatively short period of time in the beginning of the organism's postnatal life. This short period in the early lives of most precocial neonates has been termed the "sensitive

period" by Fabricius (1951), Hinde, Thorpe, and Vince (1956), and Guiton (1959).

The duration of this period of sensitivity varies greatly from species to species. Fabricius (1951), using young tufted ducks, found a gradual decline in the number of naive birds in which the following response could be elicited after just a few hours posthatch. Similar findings have been reported in domestic chicks by Guiton (1958), Jaynes (1957), and Hess (1959a).

Imprinting and Level of Arousal

Although a number of factors important in the imprintability of a neonate have been examined by numerous authors (Sluckin, 1973; Hess, 1973; Rejecki, 1973), probably the most widely discussed is the level of arousal. For example, Tolman (1963) observed 12 isolated chicks every hour for 24 hours during which he recorded the number of hours the chicks were maximally awake. The data he presented was almost identical to the data presented by Ramsay and Hess (1954) which depicted the percentage of ducklings for each 4-hour age group after hatch which achieved perfect imprinting scores 24 hours after exposure to the imprinting stimulus. From his data Tolman concluded that the critical period for imprinting could be interpreted in terms of internal changes in arousal level.

Hess (1962b) measured emotionality in terms of the defecation rate of 200 Vantress broiler chicks, all within the 14-16 hr. "peak of imprinting susceptability" (Hess, 1973, p. 244). It was found that all the chicks that defecated in the imprinting situation also approached and followed the model. Similarly, Schulman (1969, 1971) measured heart rate and hyperstriatal EEG from young domestic chicks and turkeys in the

presence of an imprinted stimulus. His results showed that imprinted chicks and poults had consistently lower heart rate variability than those control birds which were not imprinted. Also, imprinted birds of both species exhibited similar patterns of sustained EEG arousal when presented with the imprinting stimulus which distinguished them from the non-imprinted control birds.

Effects of Increased Emotionality on the Approach and Following Response

There appears to be at least two aspects of the neonate's environment that could possibly lead to an increase in fear. Sluckin (1960), Sluckin and Salzen (1961), and Salzen (1962) have differentiated between a fear of moving objects or fear of the static environment (ground). Salzen (1962) investigated fear responses of chicks raised in isolation or communally in a static environment or an environment containing both static and moving objects. He reported that, regardless of age, 11 chicks responded to a new ground with some display of fear. Salzen concluded that all chicks, whether raised socially or isolated, had, as a reference, that environment with which they were accustomed, and that fear or increased emotionality resulted from a contrast between the familiar environment and the novel new environment.

Moltz and Rosenblum (1958b) investigated the effects of prior experience with the environment before confronting the bird with the novel imprinting stimulus. Their procedure was to allow each Peking duckling one hour exposure in the runway before exposing it to a moving box in the same runway. Their control subjects were allowed exposure to a different runway before being exposed to the test runway. Their results show that the control group exhibited more following than the experimental group

that was habituated to the test runway. They concluded that the control birds had a greater degree of emotionality due to the newness of the environment and were thus driven more strongly toward the imprinting model.

Along this same line, Sluckin (1960) and Sluckin and Salzen (1961) have shown the numerous forms of environmental changes such as loud noises can cause an imprinted chick to run toward and follow more closely, a familiar moving object. Guiton (1961) also has shown that a chick can be made to follow the imprinting stimulus more closely if the experimenter mildly frightens the bird. Pitz and Ross (1961) have also demonstrated that a sudden intense noise sounded whenever a chick came within six inches of the imprinting stimulus greatly enhanced the filial response.

Kovach (1963), using 60 Vantress broiler chicks (18, 32, and 48 hours of age), demonstrated the effects of electric shock on imprinted behavior varies with age. His shocked groups received eleven 3.0 ma. shocks, each approximately 0.5 sec. in duration, while in the imprinting situation. Specifically, the animal was allowed to follow a blue ball around a 10-ft. circular runway. The electric shock was administered through wing electrodes once on the first turn, twice on the second turn, three times on the third turn, and four times on the fourth turn. His results showed that the mean number of feet the chicks followed the stimulus was double for the shocked group at 18 hrs. At 32 hrs. of age, the non-shocked birds followed more than the shocked animals. For the 48-hr. group (well past the traditionally accepted critical period), the shock had almost a complete inhibiting effect upon following behavior.

In an expansion of this previous study, Kovach and Hess (1963) used 120 subjects of the same species (Vantress broiler chicks) using three

age groups of 14, 18, and 32 hrs. posthatch and two different shock intensities coordinated with two different numbers of exposures to the shock. In this study, while continuing to use 3 ma. for 11 exposures for one group, they additionally employed a group which received 1 ma. for 27 exposures. The procedure for this study was the same as the previous study.

Their results replicated the findings of Kovach (1963) so far as the subjects receiving 11 exposures of the 3 ma. intensity are concerned. However, when the two different shock intensities (1 ma. and 3 ma.) with the same number of exposures (27) were compared, the results indicated marked differences. For the 14-hr. age groups, the 1 ma. shock facilitated the following response similar to the eleven 3 ma. shock treatment group. However, when the number of exposures was increased to 27 using the 3 ma. intensity, the treatment had an inhibitory effect. When the 32-hr. age groups were compared it was found that the inhibitory effect of the 27 exposures of 1 ma. shock was more than doubled for the 27 exposures of 3 ma. group. From these data Hess (1970) concludes that "administration of rather strong and frequent electric shock (27) in the course of the laboratory imprinting exposure during the critical period interferes only slightly with the animal's tendency to follow; . . . whereas, animals shocked at lesser intensities or with lesser frequencies, on the other hand, follow more than unshocked controls if--and only if--they were at the critical period."

Moltz, rosenblum and Halikas (1959) demonstrated that an increase in emotionality associated with the imprinting environment produces an increase in the approach and following response of young ducklings. In

their study, they exposed young ducklings to an imprinting stimulus for 25 min. a day for 6 days. Two groups were then shocked either in or out of the runway on Days 7, 8, 9, and 10 in the absence of the imprinted stimulus. Their results show that subjects that received shock in the runway subsequently approached faster than those subjects shocked outside the runway. The authors concluded that this increase in approach speeds was due to increased emotionality associated with the apparatus.

In an attempt to more closely define the perameters of the effects of electric shock on the imprinted response, Barrett, Hoffman, Stratton and Newby (1971), after exposing ducklings on Days 1, 2, and 3 to an imprinting stimulus, subjected these imprinted birds to either response-contingent, response-noncontingent, or no shock on Day 5. Their results indicate that while contingent (punishing) shock interfered with the approach and following response, noncontingent shock enhanced that filial response.

Thus, it would appear that a general increase in emotionality enhances the filial response, whereas, if this increase in emotionality is response contingent it may suppress that response. An exception to this generalization may be found in an experiment reported by Roehling and Schulman (1973) who found a traditional conditioned suppression effect may be obtained during the imprinting situation if an auditory CS previously associated with electric shock outside the imprinting situation is delivered when chicks are running toward an imprinted stimulus in a straight runway. Therefore, it would appear the relationship between imprinting and shock-produced emotionality is not yet completely understood.

Effects of Drugs on Imprinting

While a number of investigators have examined the effects of external stimulation, such as loud noises, new environments, and electric shock, on the imprinting response, an additional research strategy has more recently come into prominence: the chemical manipulation of autonomic activity. Kovach (1964) investigated the hypothesis that the neural activity associated with imprinting is primarily autonomic activity. In addition, he hypothesized that this autonomic activity is on an "uninterrupted continuum of the maturation of autonomic excitability and would implicate the latter not only in the termination of the critical period but also in its onset and extent" (Kovach, 1964, p. 183). In his investigation, Kovach used 450 Vantress broiler chicks as subjects divided into five age groups of 8, 14, 18, 24, and 32 hrs. old. He then further divided each age group into eight experimental groups and one control group giving ten subjects in each group. Each experimental group was to receive subcutaneous injections of one of eight autonomic drugs while control subjects received injections of 0.03 cc water containing 0.9% benzylalcohol. The subjects were hatched in the dark and isolated upon hatching.

His apparatus consisted of the traditional circular imprinting runway with a blue ball 6.5 in. in diameter serving as an imprinting stimulus. After all his subjects were exposed to the imprinting object at the appropriate age they were placed back in isolation until they were tested at 52-60 hrs. No injections were given before the final test.

Kovach's results indicate that the administration of nearly all of these drugs has a similar effect to the administration of painful

stimulation used by Kovach and Hess (1963); that is, a facilitative effect at an early age and an interference effect at later ages. This effect was clearly demonstrated in the administration of the sympathetic stimulants.

With the exception of the cholinergic stimulant neostigmine, all drugs used facilitated following behavior at 8 hours. Kovach interpreted these results as indicating that instead of the onset of the critical period being due to the development of the motor capabilities, rather it is likely that it is due to the maturational state of the excitability of the CNS. "Artificial increase in excitation produces following at an age when the excitability of the nervous system is still too low for the elicitation of the following response by the normal stimulation of the imprinting experiment" (Kovach, 1964, p. 185).

Along a similar line, Bradford and McDonald (1969) investigated the effects of sodium pentobarbitol (a CNS depressant) on the approach response. In the first experiment of their investigation it was hypothesized that once an association was formed in a non-drug (ND) state, low doses of the depressant should not interfere with the strength of the approach response unless the response is drug state dependent (Overton, 1964). This latter case was tested by reversing drug conditions between the acquisition and test trials. In this experiment, 144 chicks were administered either 5-, 7-, or 10-mg/kg sodium pentobarbitol or sterile water 7-15 min. prior to exposure to an imprinting stimulus for three days of training. The subjects were then randomly assigned to one of two reverse or no reverse groups (drug training-drug test, drug training-no drug test, no drug training-no drug test, or no drug training-drug test) for

each dosage level and tested on the fourth day under the new conditions.

The results of this experiment showed that during acquisition for all dosage levels except 10 mg/kg there was a marked practice effect over trials. The 5 mg/kg group showed no significant differences from the no drug control group. The 7 mg/kg group, however, showed significantly less improvement than the no drug controls. In addition, there was almost a complete lack of effect of the reverse from the drug to no drug state except for the 10 mg/kg dosage. Bradford and McDonald (1969) interpreted the results of this experiment as, "(a) The drug treatment differentially inhibits the formation of associations during training and (b) The presence of the drug on the retention test does not seriously affect the retrieval of information stored as an association."

In a second experiment in the same investigation Bradford and McDonald (1969) tested the effects of post-trial injection of sodium pentobarbitol in order to determine if the drug impairment found in the first experiment could be caused by a retrograde amnesia-like effect similar to those obtained by Leukel (1957), Pearlman, Sharpless and Jarvik (1961), McGaugh (1966), and Fischer, Campbell and Davis (1965). In this experiment the subjects were 40 Cornish X White Rock chicks treated in the same manner as in Experiment 1 except the injections were administered intraperitoneally (IP) or intracardially (IC) <u>after</u> each trial with an equal dosage of 10 mg/kg.

The results showed that the birds that received an IC injection that resulted in an unconscious state (presumably required in the study of retrograde amnesia) showed impairment in the development of the approach response (see Leukel, 1957 or Pearlman, et al., 1961). This, according

to the authors, "suggests that the associations involved in imprinting develop incrementally over trials and require some post-trial integration or consolidation. Once formed, however, the associations seem to be particularly resistant to external interference" (Bradford & McDonald, 1969).

In an earlier study Hess (1957) investigated the effects of a tranquilizer drug (meprobamate) on the imprinting response. In this investigation Hess administered 25 mg/kg of the tranquilizer in four conditions: (1) drug at 12 hrs., imprint at 24 hrs., and test when the drug had worn off; (2) drug at 12 hrs., imprint at 14-16 hrs., and test when the drug had worn off; (3) imprint at 16 hrs. and test under drug; and (4) drug at 24 hrs., imprint at 26 hrs., and test when the drug had worn off. The control animals were given .3 cc of distilled water. The results of this study show that while meprobamate is effective in reducing fear or emotional behavior, it also reduced imprintability. Briefly, he concluded that: (1) Mallard ducklings aged 12-17 hrs. will not imprint under the influence of meprobamate; (2) ducklings imprinted in the absence of the drug will respond under a standard dosage in the test situation; (3) birds given the drug at 24 hrs. will not be imprinted at 26 hrs.; and (4) ducklings given meprobamate at 12 hrs. can be imprinted at 24-26 hrs.

While the preceding study has shown imprinting to be interfered with by the use of a tranquilizer, Rejecki and Saegert (1971) have demonstrated that with the use of a stimulant (methamphetamine hydrochloride) the imprinting response can be facilitated. In this investigation 24 DeKalb Hybrid (White Leghorn variety) were subcutaneously injected with methamphetamine hydrochloride 40 min. prior to exposure to the imprinting situation. Control birds were given a comparable amount of sterile saline.

Their results are comparable to other studies (e.g., Kovach, 1964) in which amphetamines have been demonstrated to facilitate imprinting.

Statement of Problem

In the extensive imprinting literature one finds little agreement as to the length of the period of sensitivity. Traditionally, the critical period has been thought to extend up to 36 hrs. (Lorenz, 1935, 1937; Fabricius, 1951). Hess (1971) speaks of a period of "maximal susceptibility" of 12-16 hrs. posthatch. Kovach (1964) and Kovach and Hess (1963) manipulated a number of variables believed to be involved in the imprinting approach and following response in birds from 8-32 hrs. after hatch. Hinde (1962a), however, has pointed out that instead of the period of sensitivity being a sharply defined critical period per se, it is more in line with an optimal period for learning. Similarly, more recent investigations (Christopher, 1969; Schulman, 1969, 1971; Roehling & Schulman, 1973; and Schulman & Roehling, 1973) have indicated that instead of a matter of 32-36 hrs., the period of sensitivity may extend as far as 96-120 hrs., with a maximum susceptibility ranging from 72-96 hrs. Because of: (1) the presence of conflicting data as to the duration of the period of sensitivity and (2) the lack of conflicting data as to the interference effects of tranquilizers on imprinted responses within the traditional critical period, the present study is designed to investigate the effects of a reserpine based tranquilizer (metoserpate hydrochloride), specifically designed for poultry, over a possible extended period of sensitivity. In addition, although the approach and following response has been traditionally employed in imprinting studies, these response measures are difficult to quantify. Consequently, the present

study will employ the methods of Roehling and Schulman (1973) and will measure strength of response in terms of start latencies and run speeds toward an imprinting stimulus located in the goal box of a straight runway.

METHOD

Subjects

The subjects were 150 White Leghorn chicks hatched from eggs obtained from the Poultry Science Department at Virginia Polytechnic Institute and State University. The eggs were hatched in a forced-air incubator maintained at a constant temperature of 37.65° C. with a humidity range of 60-70%. The eggs were turned three times a day. After hatching subjects were housed individually in suspended Hoeltge rat cages measuring 20.3 X 24.1 X 17.8 cm. Food and water were available *ad libitum* with a room temperature maintained at 98° F. All subjects were housed under a 24-hr. constant light condition.

Apparatus

Imprinting was tested in an open plywood runway measuring 127 X 35 X 20 cm. The floor of the runway was constructed of 0.25 in. hardware cloth. Opaque guillotine doors located 20 cm. from either end separated the runway from the goal and start boxes. An audiovisual imprinting stimulus was employed consisting of a toy bobbing bird paired with a 500 Hz. tone emitted from a speaker located behind the goal box, pulsing at a rate of 4 pulses per sec. with a pulse duration of 40 msec. This tone has been shown by Fischer (1972) and Sigman (1974) to be maximally attractive to young chicks. The imprinting stimulus was positioned behind a 20 $cm.^2$ Plexiglas window in the goal box. The intensity of the pulsing auditory stimulus measured at the midpoint of the runway was 71 db. Two Standard Electric timers recorded start latencies and run times. The start latency clock was activated by means of a microswitch mounted on the guillotine start box door and was stopped when a subject broke a light

beam located 2.5 cm. outside the start box door. The run time clock was activated simultaneously with the stopping of the start latency clock and was stopped with a second photocell unit positioned 2.5 cm. inside the goal box door. The sensitivity and operation of both photocells was controlled by a Hunter Photocell amplifier relay (Model Number 3355).

Drug

The drug used was metoserpate hydrochloride (Pacitran) obtained from E. R. Squibb & Sons, Inc. The tranquilizer was administered subcutaneously with a volume of 0.15 cc and a concentration of 0.053 gr. per 100 ml. This concentration and volume leads to a dosage of 2 mg/kg $\pm$.004 mg/kg in chicks of 1 to 5 days posthatch. This concentration has been reported by Gallup (1972) to be effective in reducing fear in domestic chicks. The placebo injection was an equal volume of distilled water.

Procedure

Upon hatching, subjects were randomly assigned to one of five experimental drug groups or one of the corresponding injected or non-injected control groups yielding a total of 15 groups in all (5 experimental, 5 injected control, and 5 uninjected control groups). One hour after injection or control manipulations, subjects were exposed to the imprinting stimulus for seven acquisition trials on one of the five days posthatch.

The subject was removed from its home cage and transported to the apparatus in a small opaque container which reduced the probability of accidental exposure to extra-experimental visual stimuli. Each exposure trial began 15 sec. after the subject was placed in the start box at which time the auditory stimulus was turned on and the start box door was raised. After the subject traversed the runway it was allowed 30 sec. in

the goal box before the auditory stimulus was terminated and the subject was removed from the goal box. The subject was immediately returned to the start box and, following a 15-sec. intertrial interval, the next trial commenced.

If the subject did not leave the start box within a 2-min. period the experimenter coaxed the bird with his hand to the middle of the runway and allowed an additional 2 min. to approach the stimulus. If the subject failed to approach, it was again coaxed toward the stimulus until it was inside the goal box door, where it remained for the 30-sec. box exposure. After the seven exposure trials the subject was replaced in its home cage, where it remained until it was to be tested.

Imprinting, for all subjects, was tested on Day 6. The procedure for the test trials was identical to the previous exposure trials except that no injection was administered.

On Day 7 extinction procedures were employed. The subject was placed in the start box, given 15 sec., and the start box door was raised. Neither the auditory nor the visual stimulus was present. Extinction criteria were as follows: (1) 2 full min. in the start box; (2) 1 full min. in the start box and 1 full min. in the runway; and (3) less than 1 min. in the start box and 2 min. in the runway. Each subject was given a maximum of 21 trials to reach extinction criterion.

Results

Because of the skewed distribution often associated with time measures, all latency measures were transformed to speeds (1/sec.). The mean acquisition start speeds are presented in Figure 1. Birds which were injected with distilled water showed faster speeds than either the

Figure 1. Mean acquisition start speeds (a) Drug conditions over Treatment day (b) Drug condition over Trials (c) Treatment day over Trials

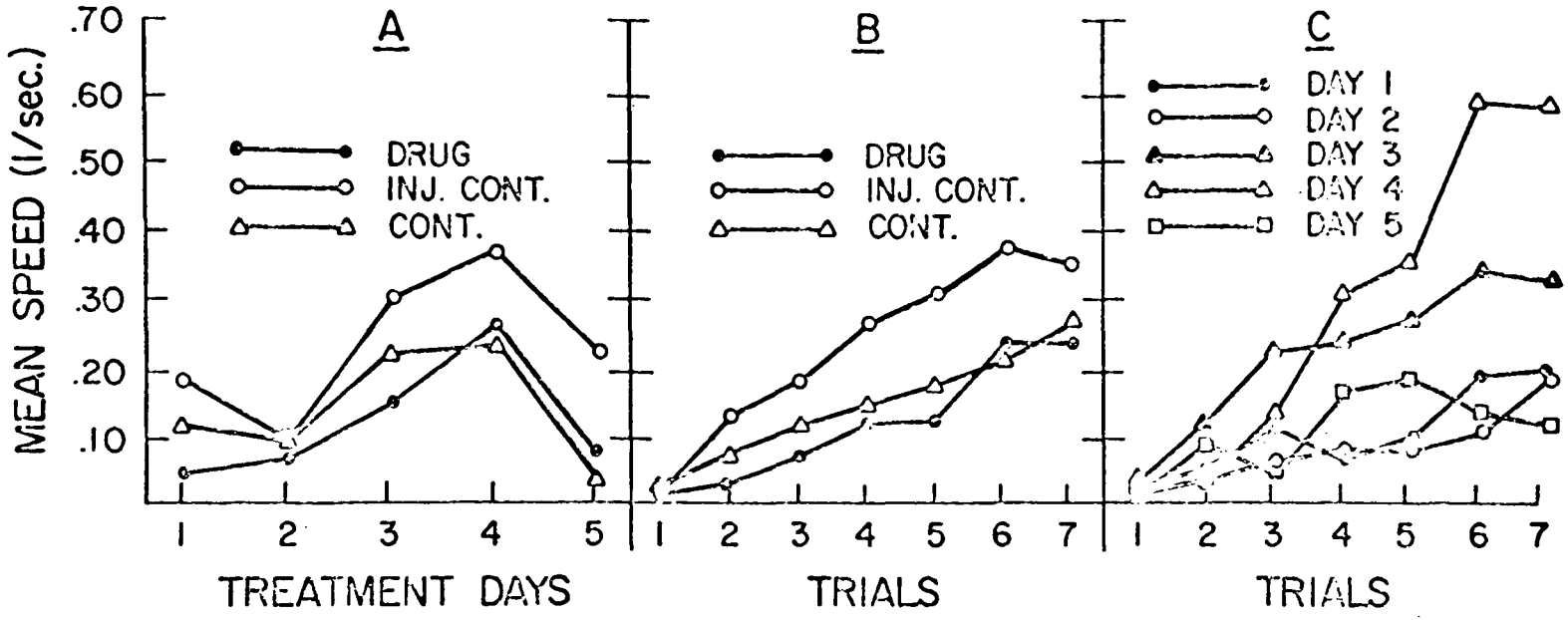

Figure 1. Mean acquisition start speeds (a) Drug conditions over Treatment day (b) Drug condition over Trials (c) Treatment day over Trials

non-injected control birds or the tranquilized birds (Figures 1a and 1b) except for birds injected on Day 2 (Figure 1a). Birds which were injected with metoserpate hydrochloride showed slower speeds than the non-injected control birds on Days 1, 2, and 3. This effect, however, is reversed for these two groups on Days 4 and 5. An analysis of variance of these data revealed significant differences for both the drug treatment and the day of treatment effects (Drug, $F = 8.737$, df = 2/135, p .01; Days, $F = 12.613$, df = 4/135, p .01).

In addition, there was an increase in start speed for all groups across the seven acquisition trials (Figure 1b). This decrease in speed over trials was significant ($F = 35.970$, df = 6/810, p .01). Figure 1c reveals that birds trained on Days 3 and 4 showed a greater rate of increase in speeds over trials than subjects run on Days 1, 2, and 5. The Trials X Days interaction is statistically reliable ($F = 6.075$, df = 24/810, p .01). No other interaction effects were found to approach statistically significant levels.

The mean acquisition run speeds are presented in Figure 2. These data indicate that birds injected with distilled water showed faster speeds than either the tranquilized or the non-injected groups except on Day 3 (Figure 2b). Additionally, birds injected with the tranquilizer showed considerably slower speeds than either the non-injected control group or the injected control groups on treatment Days 1 and 2. This effect, however, is not apparent on Days 3, 4, and 5 (Figure 2a). An analysis of variance of these data revealed significant differences for both the drug treatment and the day of treatment effects (Drug, $F = 8.702$, df = 2/135, p .01; Days, $F = 19.491$, df = 4/135, p .01).

Figure 2. Mean acquisition run speeds (a) Drug conditions over Treatment days (b) Drug conditions over Trials (c) Treatment Days over Trials

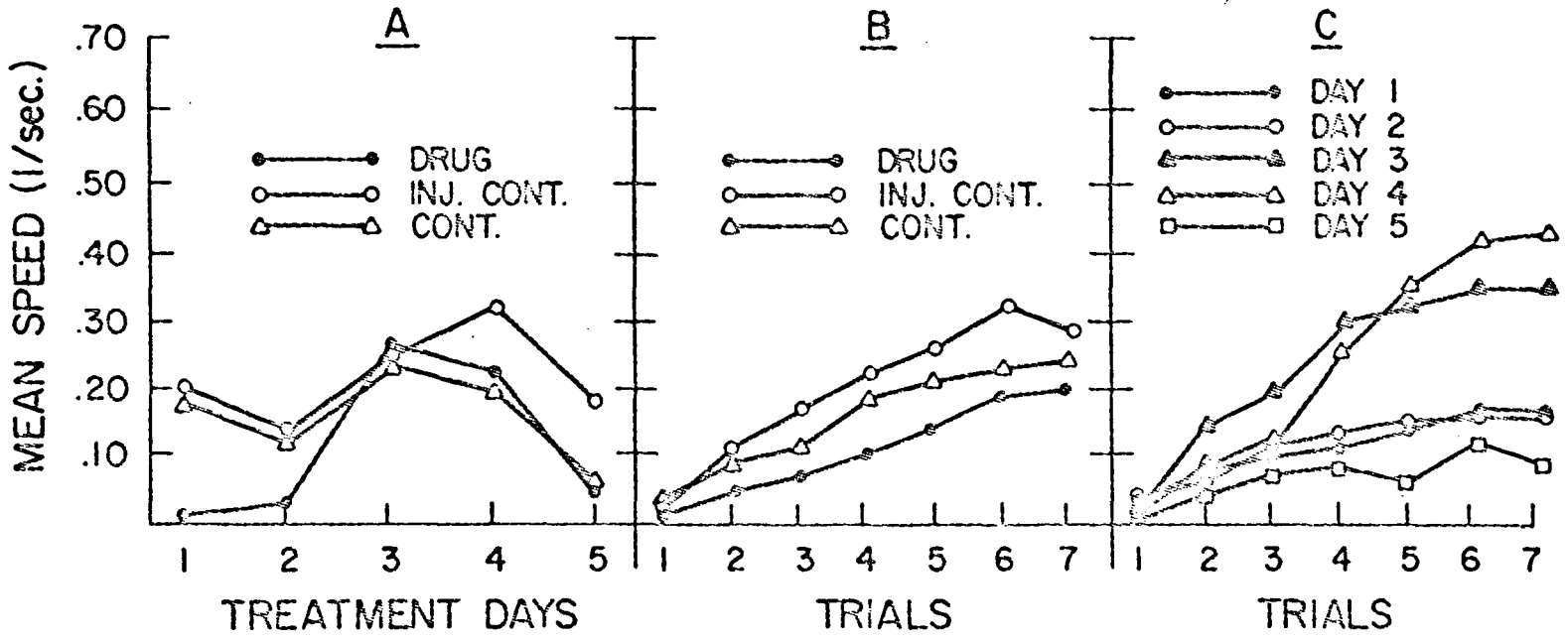

Figure 2. Mean acquisition run speeds (a) Drug conditions over Treatment days (b) Drug conditions over Trials (c) Treatment Days over Trials

An increase in acquisition run speed over trials was also observed (Figures 2b and 2c). Statistical evaluation proved this Trials effect reliable (F = 92.089, df = 4/135, p .01). As can be seen in Figure 2a the effects of all drug and control treatments vary considerably depending on the day of treatment. This effect is especially evident in the tranquilized birds receiving injections on Day 1 or Day 2 when the tranquilizer appears to suppress responding. This apparent suppression effect is reduced when subjects are injected on Day 3 or Day 4. Analysis of the differential effect of the drug treatment over the day of treatment generated a significant Drug X Day interaction (F = 2.131, df = 8/135, p .01). Figure 2b shows the mean run speeds for all Drug conditions across the seven acquisition trials. The gradual separation of groups over trials was expressed by a reliable Trials X Drugs interaction (F = 2.060, df = 12/810, p .05).

Figure 2c shows mean run speeds for the five treatment days across the seven acquisition trials. The rate of responding over trials was greater for subjects treated on Day 3 or Day 4 than for subjects treated on Days 1, 2, or 5. These differences are expressed by a significant Trials X Days interaction (F = 11.302, df = 24/810, p .01). The triple interaction, Trials X Drug X Days, was not significant. Individual comparisons of drug conditions on each of the acquisition days of both start speeds and run speeds using the Duncans New Multiple Range Test revealed significantly slower speeds for the tranquilized birds compared to the non-injected control groups on Days 1 and 3 in the start measure and Days 1 and 2 in the run measure (p .01). In addition, birds injected with distilled water showed significantly faster speeds than either the

tranquilized or the non-injected birds on Days 3, 4, and 5 in the start measure and Days 4 and 5 in the run measure (p .01).

Individual comparisons of treatment days for the tranquilized groups showed that, in the start measure, subjects receiving the drug on Day 4 were significantly faster than birds receiving the drug on any other day (p .01). Birds receiving the drug on Day 3 differed significantly from birds receiving the drug on Days 1 and 4 (p .01); while birds receiving the drug on Days 1, 2, or 5 did not differ. When these same comparisons were made using the run measure it was found that birds receiving the tranquilizer on Days 3 and 4 differed significantly from the birds tranquilized on Days 1, 2, and 5 (p .01); whereas, there were no significant differences among birds receiving the tranquilizer on Days 1, 2, or 5.

Individual comparisons of treatment days for the injected control groups using the start measure revealed that birds injected on Day 4 were significantly faster than birds injected on Days 1, 2, or 5 (p .01); whereas, birds injected on Day 3 were significantly faster than birds injected on Days 1 or 2. These same comparisons using the run speeds revealed that birds injected on Day 4 were significantly faster than all other treatment days. Birds injected on Day 3 were significantly faster than birds injected on Days 2 and 5.

Individual comparisons of the treatment days for the non-injected control group showed that, in the start measure, birds receiving treatment on Days 3 and 4 were significantly faster than birds receiving treatment on Days 1, 2, or 5 (p .01); whereas, neither groups treated on Days 3 or 4 nor groups treated on Days 1, 2, or 5 differed significantly among themselves. These same comparisons using the run measure revealed that

subjects receiving treatment on Days 3 and 4 were significantly faster than subjects receiving treatment on Days 2 and 5 (p .01). Subjects receiving treatment on Day 1 were significantly faster than subjects receiving treatment on Day 5 only (p .01). In other words, irrespective of treatment, birds exposed to the imprinted stimulus on Days 3 and 4 tended to run faster than birds exposed on other days.

Figure 3 shows the mean start speeds during the test phase (i.e., no drug) of the study. These data indicate that subjects that were injected with distilled water in the acquisition phase of the study continued to show faster speeds than either the non-injected control or the previously tranquilized subjects (Figures 3a and 3b). In addition, the suppression effect exhibited by the groups tranquilized on either Days 1, 2, or 3 in the acquisition phase (Figure 1a) appears more pronounced when tested on Day 6. However, birds tranquilized on Day 5 in the acquisition phase failed to show suppressed running in the test condition.

An analysis of variance performed on these data revealed significant differences in test performance between the treatment and the day of acquisition effects (Treatment, F = 8.672, df = 2/135, p .01; Days, F = 11.904, df = 4/135, p .01). Additionally, there was observed an increase in speed for all groups across the seven test trials (Figure 3b) which was found to be significant (F = 22.257, df = 6/810, p .01).

Figure 3c shows mean start speeds for the seven test trials for each of the five days of treatment groups. Similar to the acquisition phase of the study, a significant Trials X Day interaction (F = 3.038, df = 24/810, p .01) is produced by a greater increase in speeds for subjects treated on Day 4 than subjects treated on Days 1, 2, or 5. No other

Figure 3. Mean start speeds under testing (a) Drug condition over Treatment day (b) Drug condition over Trials (c) Treatment day over trials

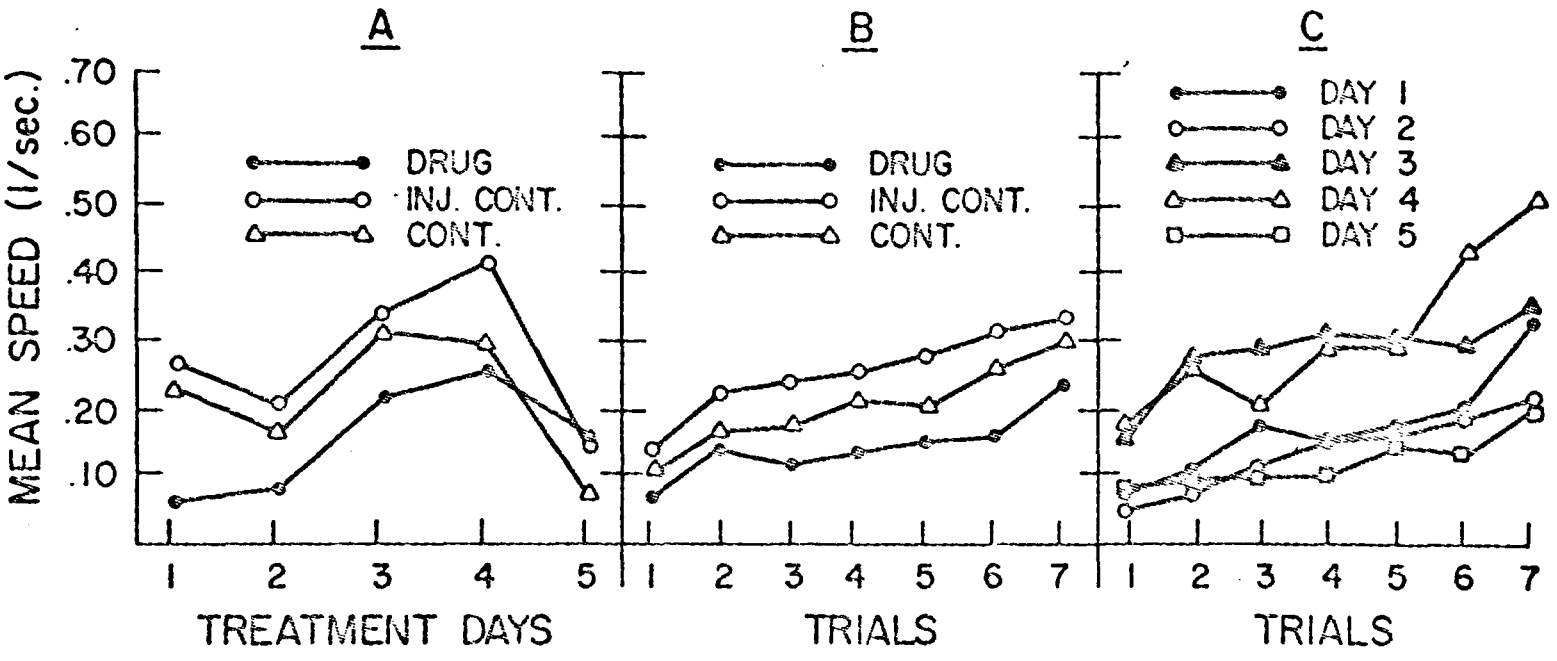

Figure 3. Mean start speeds under testing (a) Drug condition over Treatment day (b) Drug condition over Trials (c) Treatment day over Trials

interaction achieved statistical levels of reliability.

Figure 4 shows the mean run speeds during the testing phase of the study. Similar to previous results, animals injected with distilled water during the acquisition phase continue to show faster test run speeds than either the non-injected or the tranquilized subjects (Figures 4a and 4b) with the exception of those birds injected with distilled water on Day 3 (Figure 4a). The suppressed running of the tranquilized birds, observed during acquisition (Figure 2a) was even more pronounced when these same birds were tested under a no drug condition (Figure 4a).

An analysis of variance performed on these data revealed significant differences for both the drug treatment and the days of acquisition variables (Drug, $\underline{F} = 6.624$, df = 2/135, $\underline{p}$ .01; Days, $\underline{F} = 3.751$, df = 4/135, $\underline{p}$ .01). An increase in test running speeds was observed over trials (Figure 4b). This Trials effect was found to be statistically significant ($\underline{F} = 45.955$, df = 6/810, $\underline{p}$ .01). Figure 4c shows the mean run speeds for the five individual treatment days over trials during the testing (no drug) of the study. The significant Trials X Day interaction observed ($\underline{F} = 2.001$, df = 24/810, $\underline{p}$ .01) appears to be produced by the differential responding of those subjects treated on Days 1, 2, or 5.

Individual comparisons of start speeds during testing for the 15 groups revealed that there were no significant differences in test performance between the injected control and the non-injected control groups treated on Days 1, 2, or 3; whereas, both control conditions were significantly faster than the tranquilized subjects ($\underline{p}$ .01). However, the injected control group treated on Day 4, while continuing to be faster than the previously tranquilized birds, also showed significantly faster

Figure 4. Mean run speeds under testing (a) Drug conditions over Treatment day (b) Drug condition over Trials (c) Treatment day over Trials

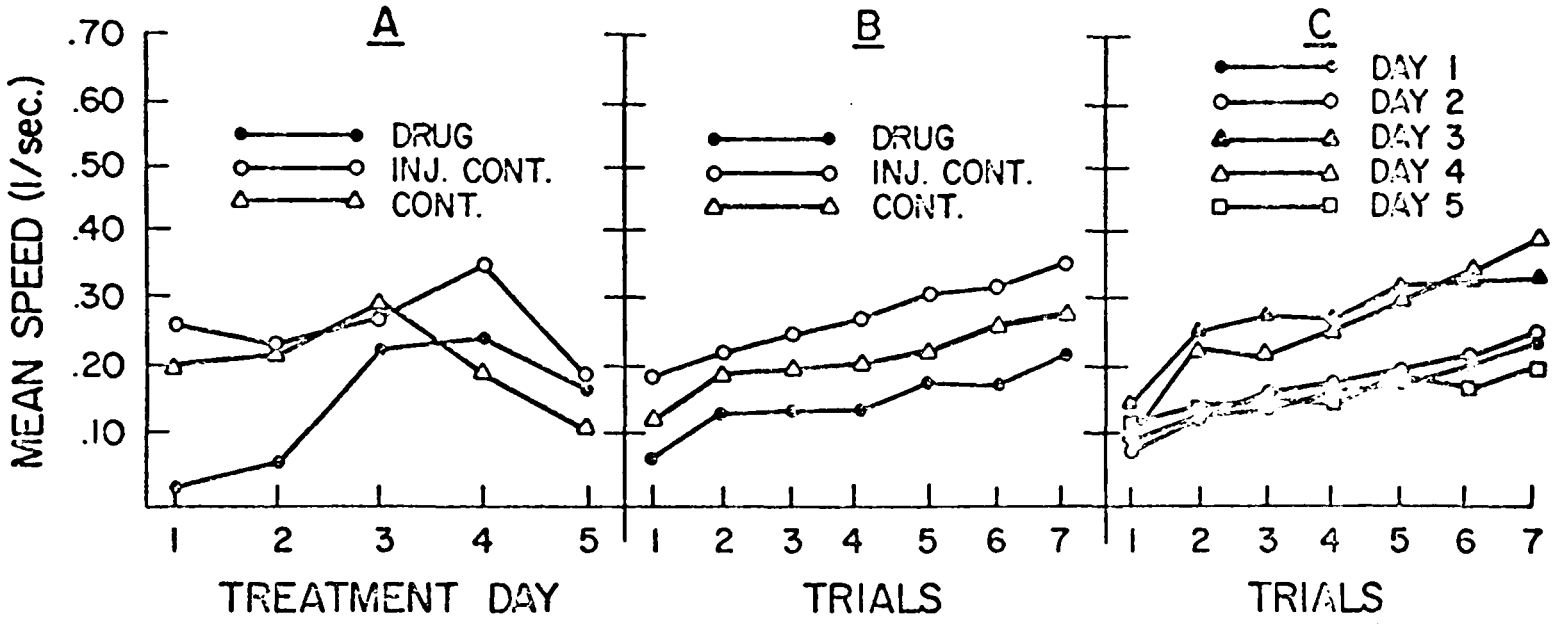

Figure 4. Mean run speeds under testing (a) Drug conditions over Treatment day (b) Drug condition over Trials (c) Treatment day over Trials

speeds than the corresponding non-injected control group (p .01). The Day 5 treated birds showed a reverse trend during testing. That is, the formerly drugged subjects are not reliably different from the injected control group, but both groups are significantly faster than the non-injected controls.

Individual comparisons of run speeds for the 15 groups during testing revealed that both non-injected and injected control birds treated on Days 1, 2, or 3 were faster than the corresponding tranquilized birds. These differences were reliable for treatment Days 1 and 2 (p .01). There were no significant differences in test performance between the two control groups treated on Days 1, 2, or 3. The injected control group treated on Day 4 ran fastest during testing, differing from both the corresponding non-injected and the previously drugged subjects (p .01). The injected control subjects treated on Day 5 differed significantly only from the non-injected controls (p .01).

Individual comparisons of the days of acquisition for tranquilized groups showed that in the test start measure subjects tranquilized on Day 4 were significantly faster than subjects tranquilized on Days 1, 2, or 5 (p .01). Subjects receiving the drug on Day 3 were significantly faster than subjects receiving the drug on Days 1 and 2 (p .01); whereas, neither Days 3 and 4 nor Days 1, 2, and 5 differed significantly among themselves. These same comparisons using the test run measure revealed that subjects receiving drug treatment on Days 3 and 4 were significantly faster than subjects receiving treatment on Days 1 and 2. Subjects receiving treatment on Day 5 differed only from subjects receiving treatment on Day 1 (p .01).

Individual comparisons of days of acquisition for the injected control subjects during the test phase using the start measure revealed that subjects receiving treatment on Day 4 were significantly faster than subjects receiving treatment on Days 1, 2, or 5 (p .01). Subjects receiving treatment on Day 3 were significantly faster than subjects receiving treatment on Days 2 and 5 (p .01); whereas, subjects receiving treatment on Day 1 differed significantly only from Day 5 (p .01). These same comparisons using the run measure revealed that birds treated on Day 4 were significantly faster than subjects receiving treatment on Days 2 and 5 (p .01). No other significant differences among Days were found in the injected control group using the run measure.

Individual comparisons of day of acquisition for the non-injected control groups now under testing conditions using the start measure revealed that subjects treated on Day 3 showed significantly faster speeds than subjects treated on Days 1, 2, and 5 (p .01). Subjects treated on Day 4 were significantly faster than subjects treated on Days 2 and 5 (p .01), while subjects treated on Day 1 were significantly faster than subjects treated on Day 5.

Figure 5 shows the mean number of trials to extinction for all drug conditions on each of the treatment days during the acquisition of the approach response. An analysis of variance on extinction speeds yielded no significant differences among groups. Additionally, X^2 performed on the number of subjects to leave the start box on the first trial of extinction and the number of subjects to complete the first trial of extinction revealed no significant differences between treatment groups.

Finally, because of the lack of significant differences between the

Figure 5. Mean number of trials to extinction for the three Drug conditions over Treatment day

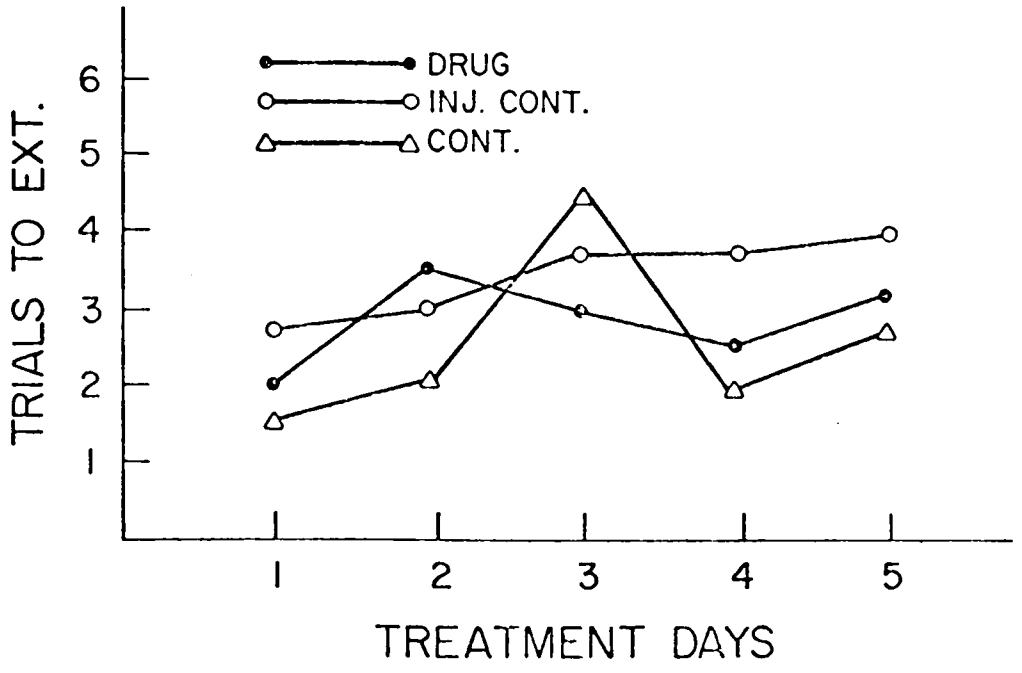

Figure 5. Mean number of trials to extinction for the three Drug conditions over Treatment day

non-injected and the injected control groups on acquisition Days 1 and 2 in the run measure (Figure 2a), and because of the apparent slower speeds of those subjects in the control groups treated on Day 2 compared to those control subjects treated on Day 1, a post hoc analysis was performed combining the two control groups to determine if a traditional significant drop in speeds over the first 48 hours was achieved. This difference between the two Treatment Days was found to be significant ($F = 5.502$, $df = 1/38$, p .05).

Discussion

The results of the present study, as seen in Figures 1a and 2a, show that those collapsed control groups receiving treatment on Day 2 showed a slower rate of responding than those collapsed control groups receiving treatment on Day 1. This decrease in responding is similar to the traditional view that the critical period for imprinting has terminated within the first 48 hours after hatching (Lorenz, 1935, 1937; Fabricius, 1951; Hess, 1962b, 1973). However, further examination of Figures 1a and 2a as well as Figures 1c and 2c reveals that the fastest approach speeds were achieved by subjects which received treatments either on Day 3 or Day 4. These data then appear to support data presented earlier indicating that the period of sensitivity, instead of ending within the first 48 hours after hatch, may extend well into the 96th hour (Hinde, Thorpe & Vince, 1956). Furthermore, it would appear that studies that support the position that the critical period is over by 48 hours after hatching (Hess, 1962; Kovach, 1963; Kovach & Hess, 1963) may have given up too soon.

Effects of Increased Emotionality on the Approach Response

In the present study injected control as well as non-injected control groups were employed. Figures 1a and 2a show the differences between the two control groups for each of the five treatment days during which the imprinted response was acquired. Assuming that an injection of distilled water has the effect of increasing the emotionality of the injected control birds as compared to the non-injected controls, it may be seen that, while the effects of this stimulation are negligible on Treatment Days 1, 2, and 3 in the run measure and Days 1 and 2 in the start measure, it significantly enhances the approach response of those birds treated on Days 4 and 5. These present findings coincide with the findings of Stein (1972), Havlena and Werboff (1963), and Hoffeld and Webster (1965) who report that a control injection procedure often produces long lasting stress effects which may cloud the subsequent evaluation of the effects of an experimental drug. Figures 1b and 2b show the differences between the treatment groups examined across the seven acquisition trials. Again, the enhancement effect of the injection procedure alone is evident. While no reliable differences were obtained on the first trial in the start measure and the first and second trials in the run measure, significant differences were obtained on all remaining trials. The control injection enhancement effect observed in the present study may be interpreted in light of the data presented by Barrett, Hoffman, Stratton, and Newby (1971) who demonstrated that, although punishment (response-contingent shock) suppressed the following response, response independent shock enhanced the imprinted response in birds which were imprinted within the first 24 hours after hatching. The enhancement effect in the present study is evident on Days

1 and 2, but it becomes significant on Days 3, 4, and 5 in the start measure and on Days 4 and 5 in the run measure. This finding is in opposition to the findings of Kovach and Hess (1963) who report that painful stimulation has a facilitative effect on birds at an early age (18 hrs.) and an almost complete interference effect at a later age (48 hrs.). Again, it must be noted that in the present study the injection control procedure did not have a facilitative effect until after 48 hours. Had Kovach and Hess (1963) continued their procedure using older birds, perhaps a facilitative effect, similar to that observed in the present study, would have re-emerged. It is also noteworthy that this facilitative effect of the injection procedure alone is carried over into the testing phase of the study (Day 6) when no injections were administered. These results suggest that an approach response acquired immediately following a stressful situation continues to be stronger during the testing of that response at least 24 hours later (as in the case for birds treated on Day 5 and tested on Day 6) and as long as six days (as in the case of subjects imprinted on Day 1 posthatch and tested on Day 6).

Effects of a Tranquilizer on the Approach Response

The apparent interference produced by the tranquilizer with the performance of the approach response on Days 1 and 2 of acquisition confirms the previous literature (e.g., Hess, 1957; Bradford & McDonald, 1969) which demonstrated an interference effect during the first 48 hours in birds exposed to an imprinting stimulus while under the influence of a tranquilizing drug. A further examination of Figure 2a reveals that this tranquilization effect continues to be present on all treatment days except for those subjects tranquilized on Day 3 where no differences were

found between any of the treatment groups. It is also noteworthy that after Day 3 there is a significant separation between the uninjected control and the injected control groups, and the effect of the tranquilizer is to reduce responding to equal that of the uninjected controls on Days 4 and 5.

In addition, if the tranquilizer is injected into very young birds (up to 48 hrs. old) an interference effect is observed during acquisition which continues to be evident at 144 hours old. On the other hand, if the tranquilizer is injected into birds 72-96 hours of age, the effect of the tranquilizer is minimal. This latter effect is also evident on test conducted at 144 hours of age.

These data lend support to Bradford and McDonald's (1969) findings which show no differences in subjects switched from a drug to a no-drug condition during testing.

One final point concerning the test data is the apparent drop in speeds from the last trial of acquisition to the first trial of testing. This would suggest that the seven trials of acquisition on a single day are not sufficient to produce a strong imprinted bond. This interpretation is consonant with the findings of Christopher and Hale (1968) who demonstrated that repeated exposures over days were necessary for stable imprinting preferences to be established.

Although profound differences were found between the three treatment groups and between the five different treatment days during acquisition as well as an almost complete carry-over of these differences into testing on Day 6, no differences were found when these same groups were subjected to extinction procedures on Day 7. This suggests that the extinction

procedure is not sensitive to the experimental manipulations employed in the present study.

A possible source of interpretive confusion in the procedure of the present study is that the post acquisition-pretest interval is not held constant for all groups. That is, differences found between groups during testing could possibly be a product of the different lengths of time between acquisition and testing. A remedy for this confound, obviously, is to keep this pretest interval constant. Then one must confront the problem of either testing those birds treated on Day 1, sometime within the peak of the critical period, or testing those birds treated on Day 5, sometime well into the 10th or 11th day. The former leads to problems involving the strong possibility of reacquisition during testing; the latter involves problems of chicks who may have passed the developmental period during which filial responses might reasonably be expected to occur under test conditions employed. The problem then lies in the fact that in any developmental study it becomes extremely difficult to separate the effects of age from those of experience.

SUMMARY AND CONCLUSIONS

An attempt was made to study the effects of a tranquilizer (meto-serpate hydrochloride), specifically designed for poultry, on the neonatal approach response of domestic chicks. Acquisition of this response was accomplished in a straight alley on birds ranging in age from 24 to 120 hours old. Testing of this response was done when all birds were 144 hours old under a no-drug condition. At 168 hours of age all subjects were subjected to extinction procedures. The strength of the response was measured in terms of latency to start as well as time spent traversing the alley. Resistance to extinction was measured in terms of the latencies mentioned above as well as number of trials to extinction. The following results were obtained:

1. The peak of imprinting susceptability was found to lie between 72 and 96 hours after hatching.
2. Injection control procedures have an enhancement effect on the approach response, especially in groups exposed on Days 4 and 5.
3. There was almost a complete carry-over effect from acquisition to testing. Birds that responded with the fastest speeds during acquisition also responded the fastest during testing. Birds that performed with the slowest speeds during acquisition performed with the slowest speeds during testing.
4. There were no differences between groups in resistance to extinction.

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THE EFFECTS OF METOSERPATE HYDROCHLORIDE ON THE NEONATAL APPROACH BEHAVIOR OF DOMESTIC CHICKS

by

Arthur N. Roehling

(ABSTRACT)

An attempt was made to study the effects of a tranquilizer (metoserpate hydrochloride), specifically designed for poultry, on the neonatal approach response of domestic chicks. Acquisition of this response was accomplished in a straight alley on birds ranging in age from 24 to 120 hours old. Testing of this response was done when all birds were 144 hours old under a no-drug condition. At 168 hours of age all subjects were subjected to extinction procedures. The strength of the response was measured in terms of latency to start as well as time spent traversing the alley. Resistance to extinction was measured in terms of the latencies mentioned above as well as number of trials to extinction. The results of the present study show a peak in imprinting susceptability between 72 and 96 hours after hatching. The tranquilizer had a traditional interference effect except for those birds exposed on Day 3 while the injection control procedure had an enhancement effect on birds treated on Days 4 and 5. In addition, there were no differences observed during extinction between groups.