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Imprinting Behavior: Pituitary-Adrenocortical Modulation of the Approach Response

Abstract. *Plasma corticosterone concentrations in newly hatched ducklings exposed to an imprinting model are inversely related to the strength of approach behavior. Injections of corticosterone before imprinting reduces following, whereas* α^{1-10}*-adrenocorticotropin or antiserum to corticosterone augments following behavior. The sensitive period for imprinting may be regulated by changes in the pituitary-adrenocortical axis.*

In his writings on the development of social behavior in birds, Lorenz (*1*) drew attention to the imprinting process in precocial species. Newly hatched chicks or ducklings will approach and follow the first moving object they see and develop an attachment or preference for this object. The strength of this behavior is important, since it brings and keeps the young bird in contact with a mother figure, thus permitting the learning or imprinting process to occur. I now report that the pituitary-adrenocortical system influences the filial approach and following behavior of imprinting.

Imprinting in young waterfowl and in newly hatched chicks occurs during a "sensitive" period shortly after hatching (*2*). During this early period, the plasma corticosterone concentration in Pekin ducklings increases rapidly (*3*). Because adrenocorticotropin (ACTH) and corticosteroids influence sensory function and learning processes (*4*), I began an investigation of the significance of this hormonal system in the imprinting context.

In these experiments, mallard ducklings were hatched in groups from eggs taken from a resident flock of game-farm mallards. The incubator was dark continuously, and birds were taken individually from the hatcher to the imprinting chamber in small lighttight containers. Ducklings used in the experiment were 15 to 24 hours old. Birds received intraperitoneal injections of either 1 I.U. of $\alpha_p{}^{1-39}$-ACTH (Schering) or 0.1 ml of 0.75 percent saline 20 minutes before testing (group 1); 10 μg of α^{1-24}-ACTH (Organon) or 0.1 ml of $ZnCl_2$ phosphate buffer 30 minutes before testing (group 2); 5 μg of α^{1-10}-ACTH or 0.1 ml of 0.75 percent saline 30 minutes before testing (group 3); 500 ng of corticosterone or 0.1 ml of propylene glycol, 0.75 percent saline, and ethanol (in the proportions 5:9:2) vehicle 15 minutes before testing (group 4); 500 ng of progesterone or 0.1 ml of the 5:9:2 propylene glycol vehicle 15 minutes before testing (group 5); or 0.1 ml of rabbit antiserum to a bovine serum albumin corticosterone conjugate or 0.1 ml of 0.75 percent saline 30 minutes before testing (group 6) (*5*). Each duckling was given the injection, color-marked, and returned to the hatcher until testing. The imprinting testing apparatus has been described (*6*). Briefly, the chamber consisted of a 1 by 5 m sand-covered runway or track along which moved either a blue ball (21-cm diameter) or a red cube (20 cm on side). These models each contained a loudspeaker that emitted the sounds "komm-komm" at fixed intervals; they were suspended from an overhead track by a Plexiglas rod and moved back and forth at a speed of approximately 11 cm/sec. The duckling was placed beside the appropriate model in the dark for 30 seconds before the chamber lights and model voice were activated; 30 seconds later the model began to move. The model moved back and forth in the runway for 10 minutes, and the experimenter recorded (i) the time from the onset of movement of the model until the duckling began to follow it (latency) and (ii) the number of seconds the duckling followed during the 10-minute training (following time). All ducklings were trained with the red cube rather than blue ball except the ducklings that received porcine ACTH and those subsequently used for the corticosterone determination. Ducklings in the latter group, which were exposed for 17 minutes to the blue ball, were decapitated 20 minutes after training; the trunk blood was centrifuged, and plasma was frozen and stored for later assay (*7*). Several trials of each injection experiment were run, each of which was accompanied by control injections of vehicle alone, as described above. Subjects in each trial were newly hatched birds. Because of differences in atmospheric pressure (*8*) and other variables, differences in amount of following are commonly encountered in imprinting studies. Therefore, the data were standardized as group mean variates and depicted as percentage of deviation from control medians. Statistical evaluation was with the Mann-Whitney U test or the product moment correlation coefficient.

The effects of administering various hormones on approach behavior are summarized in Fig. 1. The administration of corticosterone inhibited the approach and following response (*9*). Ducklings waited longer before following the imprinting model and spent less time with the model during the 10-minute imprinting period. Those birds which received antiserum to corticosterone followed more quickly and followed longer during the training period than did controls (*10*). Ducklings receiving α^{1-10}-ACTH, a noncorticotropic (*11*) peptide that contains the first ten amino acids of ACTH, followed more quickly and longer than controls did. This result contrasts with those birds receiving the corticotropic porcine ACTH or synthetic corticotropin, α^{1-24}-ACTH (*11*). Neither of these substances significantly affected latency or following time. Progesterone, as well, had no effect on behavior.

Endogenous corticosterone concentrations are also inversely related to following time (Fig. 2). The data raise the question of whether differences in plasma corticosterone concentrations result from differences in the bird's reaction to the imprinting situation or whether they are the cause of these differences. My experimental manipulations of steroid and ACTH concentrations (Fig. 1) indicate that the hormonal differences cause the behavioral differences.

Data from the injection experiments (Fig. 1) suggest that ACTH and adrenocorticoids reciprocally modulate approach behavior, with the ACTH peptide facilitating the response and corticosterone inhibiting it. The action of corticosterone antiserum may be attributable to a reduction of corticosterone-induced inhibition and, possibly, to an increase in endogenous ACTH concentrations as well, since the hypothalamic-pituitary-adrenal

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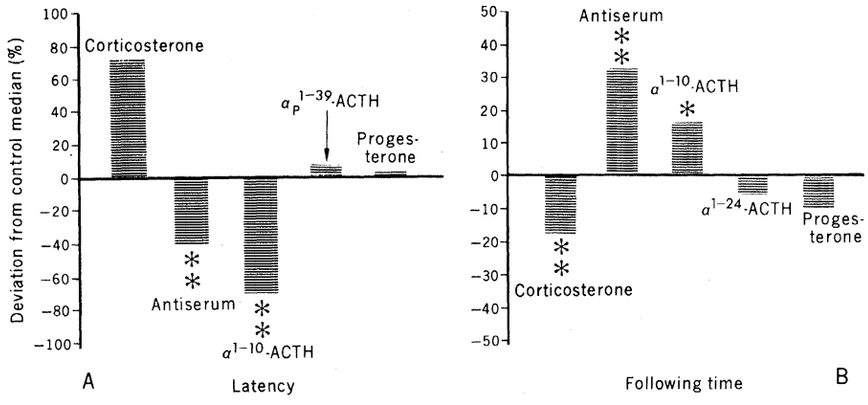

Fig. 1. Hormonal modulation of imprinting approach behavior in ducklings following an imprinting model during a 10-minute exposure period (18). (A) Following latency of experimental ducklings after onset of model movement as a percentage of the control latency. Ducklings were treated with corticosterone (N_{exp} = 18, $N_{control}$ = 17, U = 194, .05 < P < .10); corticosterone antiserum (N_{exp} = 21, $N_{control}$ = 24, U = 368.5, P < .01); α^{1-10}-ACTH (N_{exp} = 36, $N_{control}$ = 38, U = 923.5, P < .01); α_p^{1-39}-ACTH (N_{exp} = 18, $N_{control}$ = 19, P > .10); progesterone (N_{exp} = 5, $N_{control}$ = 5, P > .10). (B) Following time as a percentage of the control time. Corticosterone (U = 228, P < .01), corticosterone antiserum (U = 348, P < .01), α^{1-10}-ACTH (U = 851.5, P < .05), and α^{1-24}-ACTH (N_{exp} = 14, $N_{control}$ = 11, P > .10). Sample size was as in (A).

feedback system is functional in newly hatched birds (*12*). The failure of α^{1-24}-ACTH and porcine ACTH to affect behavior is presumably related to their action in increasing corticosterone levels, because the noncorticotropic α^{1-10}-ACTH fragment did facilitate the response. The data do not allow us to determine whether α^{1-10}-ACTH promotes following directly or whether it inhibits avoidance, thus permitting approach to occur. Landsberg and Weiss (*13*) reported that stress or an increase in corticosterone levels blocks retention of the imprinting experience. Their findings could be attributed to a reduction in following, and hence exposure (*6*), during training, and may not represent a direct effect on learning.

On the basis of the data presented here, I propose that the timing of the sensitive period for imprinting in ducklings is at least partly determined by the activity of the pituitary-adrenocortical axis. This system is already active at hatching (*12*), and during the early posthatch period the blood-brain barrier of birds matures rapidly (*14*). This increasing competence of the blood-brain barrier would be expected to increasingly limit movement of peptides from plasma to brain. Such a reduction in ACTH in the brain may be important in ending the sensitive period. Comparing the sensitive periods of mallards and Pekin ducklings provides support for this hypothesis. Gottlieb has suggested that the mallard has a more sharply defined sensitive period than its domestic counterpart, the Pekin duck (*15*); he has shown that, contrary to evolutionary expectations, domestic Pekin ducklings are more inclined to follow an imprinting model than wild mallards are. Correspondingly, I have shown that wild mallards have much higher plasma corticosterone (and presumably plasma ACTH) concentrations than Pekins shortly after hatching (*3*). The mallard corticosterone concentrations drop rapidly during the first day after hatching, whereas the Pekin concentrations increase during this time and remain elevated for 2 to 3 days.

How this hormonal system acts on the nervous system of the newly hatched duckling is not known. In mammals, however, corticosterone affects sensory processing by the brain by raising the detection threshold (*4*). Of particular significance for my results is the recent finding that the developing avian brain possesses a high-affinity, limited-capacity binding system for glucocorticoids in the optic tectum (*16*). Koranyi, Endröczi, and Tamasy (*17*) have reported (i) a decrease in the amplitude of tectal evoked potentials of young chicks after α^{1-10}-ACTH administration and stimulation of the optic chiasma and (ii) an increase in potentials after injections of cortisol. The amount of glucocorticoid or ACTH-like peptide present in this area may modulate the input to higher brain centers and thus influence the behavioral excitability or arousal of the young bird after exposure to the imprinting object.

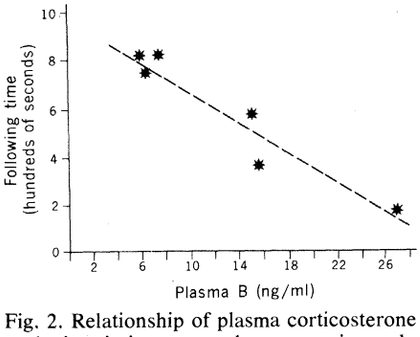

Fig. 2. Relationship of plasma corticosterone to the imprinting approach response in newly hatched ducklings (N = 6, r = −.9547, P < .01); blood was collected by decapitation 20 minutes after a 17-minute exposure to the imprinting model. Plasma corticosterone was also correlated with approach latency (r = +.89, P < .05).

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19. I thank R. E. Phillips for encouragement and support and P. P. G. Bateson for his comments on the manuscript. W. H. Burke donated the antiserum; H. M. Greven and Organon (Oss) donated the α^{1-10}-ACTH and α^{1-24}-ACTH; Schering AG (Berlin) contributed the $\alpha_p{}^{1-39}$-ACTH. Supported by National Institute of Child Health and Human Development postdoctoral fellowship HD 00495-02.

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Schizophrenic Symptoms Improve with Apomorphine

Abstract. *Eighteen chronic schizophrenic patients received subcutaneous doses of apomorphine, a dopamine receptor agonist, and of placebo in separate trials. A significant improvement in psychotic symptoms occurred after apomorphine compared to placebo. The results are interpreted as a consequence of presynaptic dopamine receptor activation by apomorphine with a subsequent decrease in dopamine-mediated neural transmission.*

Much recent research has suggested that alterations in neural transmission mediated by dopamine (DA) may contribute to the development of schizophrenic symptoms (*1*). Support for this hypothesis derives mainly from the following pharmacologic observations. All clinically effective antipsychotic agents share the ability to block DA receptors. Indeed, the antipsychotic potency of neuroleptics in general parallels their DA receptor binding affinity (*2*). The antipsychotic effect of neuroleptic drugs is potentiated by α-methyl-*p*-tyrosine, a drug that blocks the DA-synthetic enzyme tyrosine hydroxylase (*3*). Furthermore, many drugs that augment DA transmission in brain, such as amphetamine or methylphenidate, can cause or exacerbate psychotic symptoms (*4*). In accord with these observations which underlie the DA theory of schizophrenia, apomorphine, a potent, centrally active DA receptor agonist (*5, 6*), should exacerbate psychotic symptoms.

Recent preclinical observations suggest, however, that at certain doses apomorphine might exert exactly the opposite effect. Dopamine receptors reside on DA cell bodies (*7*), and DA agonists appear to activate presynaptic DA receptors to inhibit DA synthesis and release (*8*). Accordingly, DA receptor agonists that preferentially affect presynaptic receptor sites may inhibit, not facilitate, DA-mediated transmission. Since apomorphine, when administered at relatively low doses, appears to mainly influence presynaptic receptor sites (*9*), beneficial rather than deleterious effects might accrue in schizophrenic patients. Here we report a significant improvement in psychotic symptoms in patients with chronic schizophrenia given apomorphine at a dose of 3 mg subcutaneously.

Eighteen patients (twelve males and six females) were studied. They were diagnosed as having chronic schizophrenia by the research diagnostic criteria of Spitzer and Endicott (*10*); in six, the disease was the paranoid type, in seven, the undifferentiated type, and in five, the schizoaffective type. Each patient had prominent psychotic symptoms despite ongoing treatment with neuroleptic medication. All patients received two separate drug trials, apomorphine (3 mg) and placebo (3 ml) in a double-blind, placebo-controlled design. The order of drug administration was randomized. No nausea or vomiting occurred because of the anti-emetic effect of the neuroleptic medication. Drug effects appeared within 20 minutes and lasted up to 60 minutes. Psychotic symptoms were scored using a modification of the New Haven schizophrenia scale (M-NHSS) (*11*). This scoring instrument records data elicited in a semistructured interview that is designed to demonstrate the thought patterns of psychotic subjects. The scale uses explicit criteria for noting a symptom and rating its intensity. In subsequent studies we have found that the M-NHSS items correlate with the thought disorder items on the Brief Psychiatric Rating Scale, but the M-NHSS is expanded and more detailed and the items are defined. Furthermore, it is designed to rate psychotic thought disturbances at intervals as frequent as 20 minutes. Reported scores derive from the consensus ratings of two interviewers who did not know what treatment had been given.

Apomorphine significantly decreased schizophrenic symptoms in these patients. The reduction (mean ± standard error of mean) in psychosis ratings with placebo was 1.67 ± 0.9, compared with a decrease of 6.22 ± 0.4 with apomorphine ($P < .02$). Paired data points from each patient during placebo and apomorphine treatment (Fig. 1) illustrate the reduction of 20 to 50 percent in psychotic symptoms in nine of the schizophrenics. In the responding patients, the decrease in psychotic symptoms, although transitory, was clinically remarkable. Certain patients temporarily stopped hallucinating, and others lost their delusions. A typical example of the symptomatic relief with apomorphine administration is provided by a 22-year-old male who suffered continual auditory hallucinations and thought control: "The voices are quite strong; they swear they are going to make me suffer, burn me, freeze me for seven years; I wish [the voices] were my mother and father, but they are an exodus." After apomorphine administra-

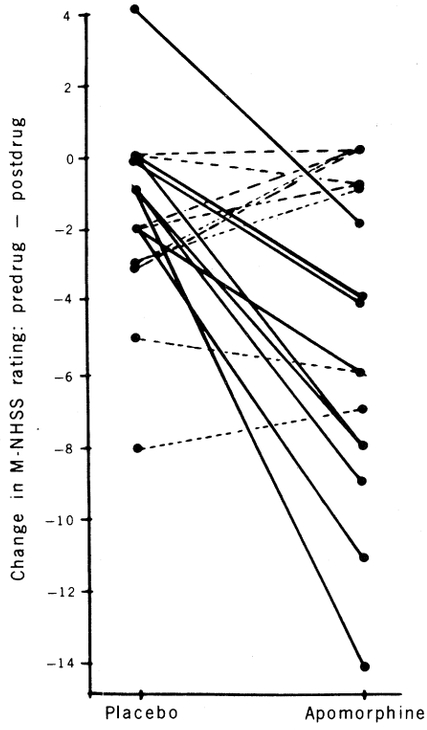

Fig. 1. Decrease in psychosis ratings in 60 minutes following placebo and apomorphine administration to each of 18 patients. Scores on the modified New Haven schizophrenic scale (M-NHSS) were determined before drug administration (baseline) and 30 and 60 minutes after drug or placebo was given. Change scores were calculated as (30-minute − baseline) + (60-minute − baseline). Significance was tested with Student's *t*-test. Solid lines represent the change in psychosis scores of the apomorphine-responding patients; the dotted lines represent the apomorphine nonresponders. Change scores of responders decreased 9.8 ± 0.02 points (mean ± standard error of mean) more after apomorphine than after placebo. For nonresponders the corresponding value was an increase of 2.0 ± 0.7. The decrease in psychosis, although transient, was 20 to 50 percent when it occurred.

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