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Authors
Cummins, RA
Budtz-Olsen, OE
Walsh, RN
et al.

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Testosterone, Early Experience and Behavioral Arousal in a Novel Environment

R. A. CUMMINS, O. E. BUDTZ-OLSEN, R. N. WALSH[1], and A. WORSLEY

Departments of Physiology and Psychology, University of Queensland, Brisbane, Queensland, Australia.

Rhode Island Red chicks were hatched either in isolation or in pairs. On the second day after hatching they were injected with either 0, 0.5, 1.5, 4.5, or 13.5 mg of testosterone. Two days later their emotional response to a novel environment was determined by counting the cheeping rate. It was found that, while low doses of testosterone elevated cheeping, the highest doses caused a depression back to, or below, control levels. The rearing environment also had an effect in that the isolated chicks cheeped more than their social counterparts for every dose except the 0.5-mg dose. It was concluded that testosterone can influence behavioral arousal in chicks but that the effect is dependent upon both dose and previous environmental experience.

Considerable attention has been focused recently upon the effect of testosterone on chick behavior. Observed behavioral changes have included the following: an increased persistence with which chicks organize their search pattern for a preferred food type (Andrew and Rogers, 1972), a decreased frequency of cheeping (peeping) in both familiar and unfamiliar environments (Andrew, 1963, 1969), a depression of carbachol-induced increases in locomotion (Andrew, 1969), and there has been a suggestion that testosterone-treated chicks show a decreased emotional response to a novel environment (Archer, 1973). These results suggest that testosterone can act as a central nervous system depressant, a rather surprising conclusion considering the hormone's importance in the arousal of male sexual and aggressive behaviors.

One factor common to all of these studies has been the use of pharmacological doses of testosterone; the lowest being 5 mg/chick (Andrew, 1972) while doses of up to 25 mg/chick have been more commonly used (Andrew, 1963, 1969; Andrew and Rogers, 1972; Archer, 1973). These doses

[1]Present address: Department of Psychiatry, Stanford University, Stanford, CA. 94305.

contrast markedly with the probable endogenous levels since plasma testosterone has been assayed at 0.12 μg/100 ml in 5-mo-old cocks; this value rising to only 0.24 μg/100 ml in sexually mature birds (Schrocksnadel, Bator and Frick, 1971). This suggested to us the possibility that the testosterone-induced depression might be a pharmacological effect and that the expected increase in behavioral arousal might accompany smaller doses.

Chick behavior can also be modified by environmental rearing conditions such that social isolation increases the emotional response to novel stimuli (Kruijt, 1962). The present study was designed to examine the interrelationships between such hormonally and environmentally induced changes in behavioral arousal using cheeping rate as the arousal index. Cheeping rate was used as the dependent variable for the following reasons: first because it has been used previously in this context (Archer, 1973), second because, from our observations, it seemed to represent the most obvious response to the novel environment, and third because cheeping represents a highly reliable form of behavioral quantification.

METHODS

Eggs from a Rhode Island Red strain were obtained after 18 days of incubation and hatched in a moderately lit room maintained at 37°C. Chicks were hatched in individual cardboard boxes and within 1 hr of hatching were transferred either singly (Isolated Condition, IC) or in pairs (Social Condition, SC) to their rearing environments. These environments consisted of solid-walled boxes measuring 25 X 20 X 23 cm, covered with lids containing 10 holes 3 cm in diameter and with a wood-shaving floor. In each box there were 50 ml jars of chick food and water which were replenished daily. For the first 2 days after hatching the environmental temperature was maintained at 37°C, thereafter it was lowered to 35°C.

On the second day after hatching chicks were injected with either 0, 0.5, 1.5, 4.5, or 13.5 mg of testosterone (Sustanon 250). Each dose was made up to 0.05 ml using arachis oil and 10% benzyl alcohol. Each dose was given intramuscularly into the thigh. Sustanon 250 contains a mixture of testosterone propionate, phenylpropionate, isocaproate, and decanoate; a mixture designed to maintain a constant blood testosterone level for some weeks.

Two days after the injection each chick was tested in an open field. This is a standardized apparatus used to examine behavioral responses to a novel environment. It consisted of a shallow metal cylinder 120 cm in diameter and 22 cm high with an off-white floor. Testing took place in a room adjacent to the rearing room with similar temperature and lighting conditions. Noise from the air-conditioning plant combined with sealed doors to make any vocalizations in the home room inaudible in the testing apparatus.

Testing involved placing the chick in the center of the open field and counting the total number of cheeps during each 30-sec interval over a 5-min period. This provided 10 consecutive 30-second intervals which subsequently constituted the factor of "intervals" in the experimental design. Testing took place on 3 consecutive days, at the end of which time the chicks were killed. Sex was determined postmortem. The cheep count included all audible vocalizations but seemed to be mainly composed of loud alarm calls.

Analysis was carried out by a 5-factor analysis of variance (ANOVA); the factors being home environment, dose, sex, interval of testing, and day of testing. This provided a $2 \times 5 \times 2 \times 10 \times 3$ factorial design with repeated measures on the last two factors. Due to the fact that sex could not be determined until after death, considerable variation occurred in the distribution of male and female chicks between the factors of environment and dose. Because of this, 60 chicks were used in each of three separate experimental runs conducted over a total period of 30 days. As a result of the combination of these three runs, the minimum number of subjects per cell was three. Therefore, the initial five-factor ANOVA was carried out with three scores per cell. Further ANOVAS, for the interpretation of significant interaction terms, used the scores from as many chicks as possible, with the restriction that all cells within each analysis contained equal numbers of subjects.

RESULTS

Results indicated significant main effects for dose ($F(4,40) = 6.302$; $P < 0.001$), environment ($F(1,40) = 10.125$; $P < 0.005$) and intervals ($F(9,360) = 5.282$; $P < 0.001$) but these results can best be interpreted by a consideration of the interaction found between all three ($F(36,360) = 2.648$; $P < 0.001$). This interaction is presented in Fig. 1, and was further analyzed by a series of two-factor ANOVAS, each using a dose factor at two levels and an intervals factor at 10 levels. The most prominent feature of this interaction is the elevated cheeping displayed by both SC and IC chicks under the lowest dose (0.5 mg/chick). A dose $\times$ intervals ANOVA between the 0.5 and 0 dose showed a main effect for dose in the SC group ($F(1,34) = 3.888$; $P < 0.06$) and a dose $\times$ intervals interaction in the IC ($F(9,252) = 2.233$; $P < 0.02$), indicating that irrespective of environment or sex the 0.5-mg dose of testosterone stimulated cheeping.

The significant environmental effect is also apparent from Fig. 1. With the exception of the 0.5-mg dose which stimulated chicks from the two environments equally, cheeping at all other dose levels was higher in the isolated chicks. The isolated controls cheeped more than the social (dose $\times$ interval; $F(9,414) = 2.124$; $P < 0.05$) and the depressive effect of all the higher doses was significantly counteracted by isolation. This was shown as a main

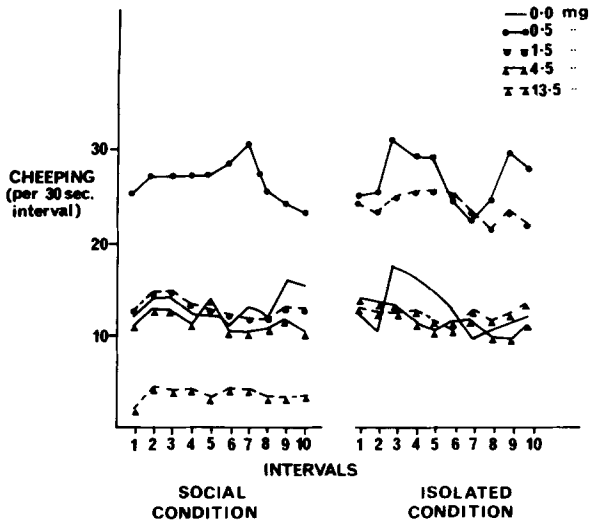

Fig. 1. The dose X environment X intervals interaction for open-field cheeping.

effect for the 1.5-mg and 13.5-mg doses ($F(1,46) = 4.527$; $P < 0.05$ and $F(1,46) = 21.038$; $P < 0.001$), and as a dose X interval interaction for the 4.5-mg dose ($F(9,360) = 3.847$; $P < 0.001$). Further evidence of isolation-induced excitation was that in the social chicks, the dose X intervals ANOVA between the 0-mg and the 13.5-mg doses indicated a main effect for dose ($F(1,34) = 12.181$; $P < 0.005$) indicating a depression of cheeping, while no such effect was found for their isolated counterparts.

The factor of sex, while nonsignificant as a main effect, interacted with environment ($F(1,40) = 3.453$; $P < 0.05$). The females were unaffected by the rearing environments. The males were significantly depressed by the social condition and excited by the isolated condition (Fig. 2). No significant sex X environment X dose interactions were found.

DISCUSSION

The results of the analyses indicate that the effect of testosterone on chick cheeping in a novel environment is dependent upon dose of testosterone, sex, and rearing environment. Chick cheeping has commonly been regarded as a form of emotional behavior (Kaufman and Hinde, 1961; Sluckin, Fullerton, and Guiton, 1970) and the present effect of isolation to increase the response to a novel environment is consistent with previous works on chicks (Kruijt, 1962) and other animals (Fox, 1967; Sluckin *et al.* 1970). The changed emotional response caused by the rearing environments interacted

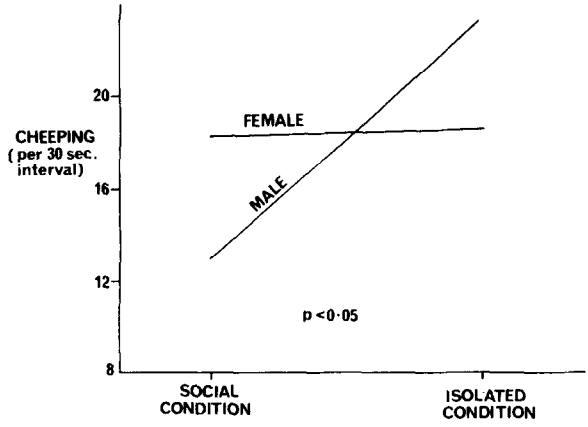

Fig. 2. The sex X environment interaction for open-field cheeping.

with the testosterone doses, the lowest dose increasing the cheeping of both environmental groups, whereas the depressive effect of high doses was counteracted in the previously isolated animals. Therefore, it would appear that the behavioral excitation produced by previous isolation has opposed the testosterone-induced depression.

Sex had little influence on the testosterone-induced changes as seen by the lack of any dose X sex interactions. This suggests that the cheeping rate of both male and female chicks is androgen sensitive; a result in contrast to previous reports that behaviors associated with food searching, copulation, and attack were influenced by testosterone in male, but not in female chicks (Andrew and Rogers, 1972). This discrepancy is possibly explained by the previous use of large doses combined with social rearing during the imprinting period (Andrew, 1972), a situation in which the depressive effects of testosterone might have been resisted by the more excitable female chicks (cf. Fig. 2).

Our general conclusions arising from this study are 2-fold. First, that the hormonal and environmental effects are interdependent and that this effect is probably a general one for all drugs altering the arousal state. Second, that the depressant effect of testosterone on chick behavior is the result of high pharmacological doses and that, within physiological ranges, the more likely response is that of behavioral arousal.

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