Pharmacology Biochemistry & Behavior, Vol. 19, pp. 599–604, 1983. © Ankho International Inc. Printed in the U.S.A.

Failure of Dexamethasone to Influence Sex Differences in Acquisition of Discriminated Lever Press Avoidance

ROB P. W. HEINSBROEK, HANS G. VAN OYEN, NANNE E. VAN DE POLL[1] AND GERARD J. BOER

Netherlands Institute for Brain Research, IJdijk 28, 1095 KJ Amsterdam, The Netherlands

Received 15 November 1982

HEINSBROEK, R. P. W., H. G. VAN OYEN, N. E. VAN DE POLL AND G. J. BOER. *Failure of dexamethasone to influence sex differences in acquisition of discriminated lever press avoidance.* PHARMACOL BIOCHEM BEHAV 19(4) 599–604, 1983.—Discriminated lever press avoidance was used to test the hypothesis that higher plasma levels of pituitary-adrenocortical hormones in female rats can be held responsible for the superior active avoidance of female as compared to male rats. Male and female rats were administered dexamethasone (500 microg/kg body weight) during 4 days of avoidance acquisition and 1 additional day of extinction. This treatment resulted in a strong suppression of the pituitary-adrenocortical activity in both sexes. The corticosterone plasma level was very low, the adrenal weight was significantly reduced, but the pituitary weight was not affected. In other words, animals treated with dexamethasone were exposed to the lever press avoidance situation without a reactive pituitary-adrenocortical system. Under these conditions, sex differences in behavior were not affected and, therefore, the hypothesis that sex differences in pituitary-adrenocortical hormone levels contribute to sex differences in active avoidance, was not confirmed.

Discriminated lever press avoidance Sex differences Dexamethasone

MALE and female rats have shown clear and significant differences in their reaction to aversive stimulation [1,2]. Female rats reacted more efficiently in situations where aversive stimulation can be minimized by adequate responding. For instance, during acquisition of shuttlebox avoidance females received fewer shocks [3, 13, 19] and reacted with shorter latencies in a lever press shock escape situation, as compared to males [10]. Males on the other hand, performed better than females [6, 12, 22] when suppression of a relevant response was demanded to avoid aversive stimulation. Sex differences in active and passive avoidance behavior are influenced by the action of gonadal hormones. Organizational effects of gonadal hormones during perinatal periods as well as activational effects of gonadal hormones during adulthood have been described [2, 20, 23, 24]. Thus far, little attention has been paid to the question which physiological mechanisms are involved in the manifestation of the influence of gonadal hormones on sexually dimorphic avoidance behavior.

Gray [16] has suggested that some of the sex differences in behavior might be related to the well-known sex difference in adrenocortical stress responses. Behavioral effects of gonadal manipulation would then be mediated by way of the immediate influence of the gonadal-axis on the pituitary-adrenocortical-axis. Davis *et al.* [10] have also refered to the pituitary-adrenocortical system as a mediating mechanism in an attempt to explain the sex dependent effects of castration on lever press shock escape behavior.

The sex difference in the pituitary-adrenocortical system can best be described as a difference in activity level, i.e., females having a larger pituitary and larger adrenals, a greater output of ACTH and corticosterone, and a faster turnover of corticosterone [9,17]. Under basal conditions as well as stress conditions hormone levels were found to be higher in females [15]. The avoidance learning is affected by pituitary-adrenocortical hormones and although a unitary theoretical explanation is lacking, it can not be denied that this system plays a role in avoidance learning. Activation of the pituitary-adrenocortical system as measured by the release of ACTH has been evident [21] during acquisition as well as extinction of a pole-jump test. Whereas a negative correlation was found between the number of avoidance responses and the ACTH plasma levels during acquisition, a positive correlation between these variables has been observed during extinction. The degree of activation therefore seems to be related to adequate responding. In turn, the release of pituitary-adrenocortical hormones is thought to improve acquisition and prolong extinction of active avoidance behavior [14]. As the activity of the pituitary-adrenocortical system is sexually dimorphic and affected by circulating gonadal hormones [17], it is indeed tempting to suggest that the sex differences in behavior and the behav-

[1]Requests for reprints should be addressed to N. E. Van De Poll.

ioral effects of gonadal hormones are mediated through the pituitary-adrenocortical-axis.

If the sex difference in avoidance behavior is mediated through the sex difference in the activity of the pituitary-adrenocortical system then eliminating the latter should result in a reduction of the sex difference in avoidance behavior. The synthetic glucocorticoid dexamethasone is known as a potent blocker of ACTH-release from the pituitary [5, 7, 11]. Scouten and Beatty [18] applied this method of functional suppression to investigate a possible relationship between the sex difference in avoidance learning and the sex difference in pituitary-adrenocortical activity. The results of that experiment, in which shuttle box avoidance was studied, did not support the hypothesis that sex differences in the adrenocortical system are functionally related to sex differences in avoidance behavior.

In the present experiment the effects of dexamethasone on the sex difference in avoidance learning were studied using a discriminated lever press avoidance procedure. Besides avoidance responses this procedure enables the recording of additonal dependent variables such as lever holding, intertrial responses and escape latencies. These may contribute to a better understanding of behavior controlled by an active avoidance situation [24]. It has already been shown that female rats learn a discriminated lever press avoidance task more quickly than male rats [24]. Females emit more avoidance responses, make fewer intertrial responses and spend less time holding the lever. It was the purpose of the present experiment to investigate whether dexamethasone differentially affects discriminated lever press avoidance behavior in male and female rats.

METHOD

Subjects

Twenty-four male and 24 female Wistar rats, obtained from Animal Supply House TNO, Zeist served as subjects. At the start of the experiment the animals were 140 days old and weighed 443 g (males) and 247 g (females). They were housed in a single-sex group of 4 animals. Lights were on from 12.30 p.m. till 0.30 a.m. Food and water were available ad lib except during experimentation. The animals had served as subjects in a one-way passive avoidance experiment when they were 100 days old. In this experiment some animals had received one or two injections of dexamethasone phosphate (500 microg/kg body weight) while others had received none. Results will be reported in a subsequent article (manuscript in preparation). The different treatment groups of that experiment were equally distributed over the two treatment groups of the present experiment. All experiments were conducted during the first 4 to 5 hours of the light period.

Apparatus

Four identical commercially available operant conditioning chambers were used (Grason Stadler 1111 double-lever rodent test chambers). The right lever was replaced by a metal cover. A force in excess of 0.25 Newton was needed to depress the lever. The chambers were placed in sound attenuating enclosures with fans providing air circulation. Electro-mechanical equipment from the same manufacturer was used to program the experimental events and collect the data. In addition, an on-line computer registration system was used to permit further data-analysis. Positive reinforcement consisted of the presentation of 45 mg food pellets (Noyes). Coulbourne Instruments shockers/distributors were used to administer shock (0.5 mA).

Procedure

Preceding the discriminated lever press avoidance all animals were first trained on a fixed interval schedule of reinforcement (F.I. 50 sec) during 11 days. Previous experience with lever pressing is considered to facilitate the acquisition of lever press avoidance [8,24]. During this stage of the experiment the animals were maintained on a 22 hours food deprivation schedule. Hereafter all animals were trained on lever press avoidance during 4 daily sessions of 50 trials each. A trial consisted of a safety period (80 sec) signalled by two colored lights and a warning period (maximum duration 20 sec) signalled by a white ceiling light and an intermittent tone (1 kHz, 0.2 sec on/0.5 sec off). If an animal did not respond during the warning period intermittent shock was administered (0.5 mA, 0.5 sec on/2.0 sec off). Shock presentation continued for 100 sec if the animal still did not respond. A lever press during the warning or shock period immediately produced another 80 sec safety period. Lever pressing during the safety period had no scheduled consequences. A lever press, initiated during the safety period but which continued during the warning period, was ineffective. On the fifth day of the experiment an extinction session was run. After 5 acquisition trials, the shockgenerator was turned off and 50 extinction trials followed. During the extinction session the "shockperiod" was fixed at 20 sec.

Males and females were divided in two groups (n = 12), one receiving daily injections of dexamethasone phosphate (Dex-males and Dex-females) the other receiving control injections of physiological saline only (Sal-males and Sal-females). Two hours before every acquisition session and before the extinction session the animals received subcutaneous injections of dexamethasone (500 microg/kg body weight) or an equivalent volume of physiological saline. Dexamethasone phosphate was obtained in capsules of 4 mg/ml (Decadron, Merck) and was diluted to 1 mg/ml with physiological saline. Animals were weighed every day after the avoidance session. Immediately after the last extinction session animals were decapitated and blood was collected for determination of the corticosterone concentration in the blood. Weight of the adrenals and the pituitary were also determined.

RESULTS

All animals learned to respond appropriately on the F.I.-schedule of reinforcement. There were no differences between the sexes, nor between the groups that were to be treated with dexamethasone or saline. The results of the discriminated lever press avoidance procedure are summarized in Fig. 1. These data were subjected to a three-way analysis of variance involving the factors sex, treatment and sessions (repeated measures). To increase homogeneity of variances, the square root transformation was applied to all data, except latencies which were normalized using a 10logx-transformation.

Avoidance Responses

Four consecutive days of training resulted in a marked improvement in avoidance responding, the main factor sessions revealing a significant effect, $F(3,132)=70.22$,

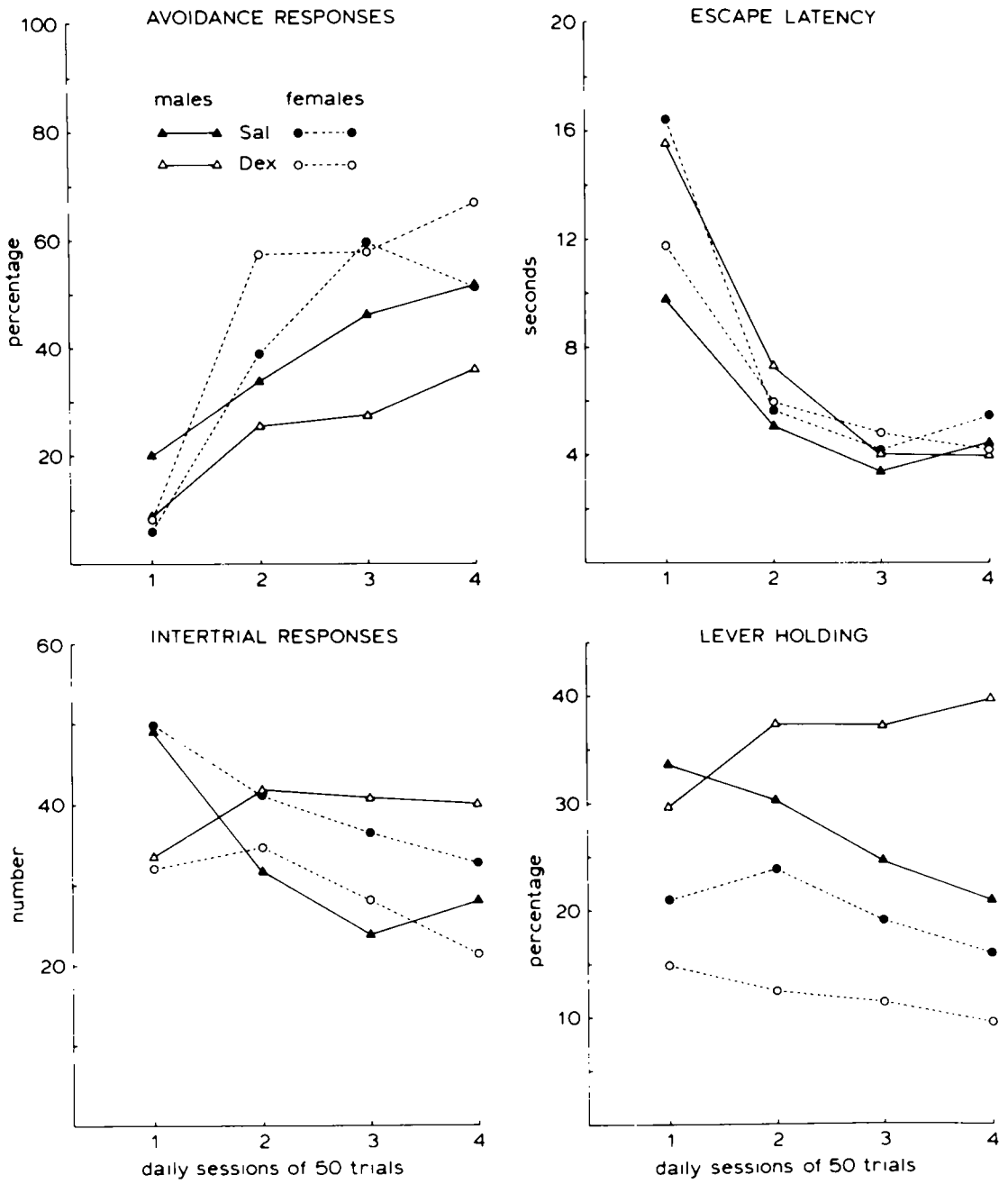

FIG. 1. Discriminated lever press avoidance in dexamethasone treated male and female rats (n=12). (Upper left) Acquisition of discriminated lever press avoidance during 4 consecutive days of 50 trials. Number of avoidance responses is expressed as a percentage of the total number of trials per session. (Upper right) Mean escape latency per session calculated for those trials in which no avodiance response was emitted. (Lower left) Mean number of responses emitted during the safe period when responding had no scheduled consequence. (Lower right) Mean percentage of time the animals spent depressing the lever. Percentage was calculated by dividing the time during which the lever was held down by the total session duration.

$p<0.001$. Females learned faster than males resulting in a significant sex × sessions interaction, $F(3,132)=10.40$, $p<0.001$. The hypothesis that dexamethasone should differentially affect female and male performance resulting in a smaller sex difference predicts a treatment × sessions × sex interaction. This interaction was not found to be significant, $F(3,132)=0.52$. No other factor nor any other interaction was statistically significant. Post-hoc analysis of the four days separately were conducted using a two-way analysis of variance with sex and treatment as factors. There was no sex

difference on the first acquistion day, on the second and third day significant sex differences, respectively: $F(1,44)=4.82$, $p<0.05$ and $F(1,44)=6.92$, $p<0.05$, were found. No significant sex difference was present on the fourth acquisition day, apparently on account of the curious decrease in responding of the Sal-female group. Neither treatment nor the interaction treatment × sex were significant on any of the four days.

Escape Latency

Response latencies during the shock period decreased rapidly after the first day of training and remained extremely short during the following sessions. Only the factor sessions turned out to be significant, $F(3,132)=81.79$, $p<0.001$.

Intertrial Responses

During the safety periods males depressed the lever as often as females. The number of responses in the safe period decreased over the four sessions, $F(3,132)=5.18$, $p<0.005$, sex and treatment as factors not being significant. There was however a remarkable treatment × session interaction when males were analysed apart from females, $F(3,66)=3.57$, $p<0.05$. As can be seen in Fig. 1 Dex-males did not show the decline in intertrial responses like Sal-males, although an interaction between the factors sex, treatment and session did not reach significance, $F(3,132)=1.16$.

Product-moment correlation calculated for intertrial responses and avoidance responses revealed a marginal significant negative correlation ($R=-0.454$).

Lever Holding

Lever holding was calculated by dividing the total duration of all responses by session duration and multiplying the fraction by 100. Leverholding decreased over sessions, $F(3,132)=10.85$, $p<0.001$. Males showed more lever holding than females, $F(1,44)=4.48$, $p<0.05$. A small increase in lever holding during the 4 days of avoidance training was noted in Dex-males, although no significant sex × treatment × sessions interaction was established. An analysis of males separately failed to show a significant interaction between the factors treatment and sessions, $F(3,66)=1.78$, n.s. Again a marginal significant correlation was obtained between lever holding and avoidance responses ($R=-0.480$).

Extinction Session

During the last 5 acquisition trials that preceded the 50 extinction trials, females had a significantly higher number of avoidance responses, $F(1,44)=4.35$, $p<0.05$. The percentage of avoidance responses for the four groups was: Dex-males: 15%; Sal-males: 25%; Dex-females: 45%; Sal-females: 33%. The factor treatment was not significant. Figure 2 presents the following 50 extinction trials, these being divided in 5 blocks of 10 trials each. The number of avoidance responses decreased over trials, $F(4,176)=10.75$, $p<0.001$. A significant sex × treatment × extinction (blocks of 10 trials) interaction, $F(4,176)=6.05$, $p<0.001$ was found. No other factor nor any of the other interactions reached statistical significance. Post-hoc analysis of both males and females separately (two way analysis of variance) revealed in the group of females a significant interaction between the factors treatment and extinction, females: $F(4,88)=4.24$, $p<0.005$ and males: $F(4,88)=2.47$, $p<0.10$. Separate analysis of Dex-treated animals and Sal-treated animals showed a significant interaction between the factors sex and extinction in the Dex-treated group of animals, $F(4,88)=4.06$, $p<0.01$, but not in the Sal treated group, $F(4,88)=2.45$, $p<0.10$. In other words the interaction between the factors sex, treatment and extinction in the overall analysis of data is mainly a consequence of the comparatively rapid decline of avoidance responses found in the Dex-treated females. Analysing the number of intertrial responses during extinction did not reveal any significant effect.

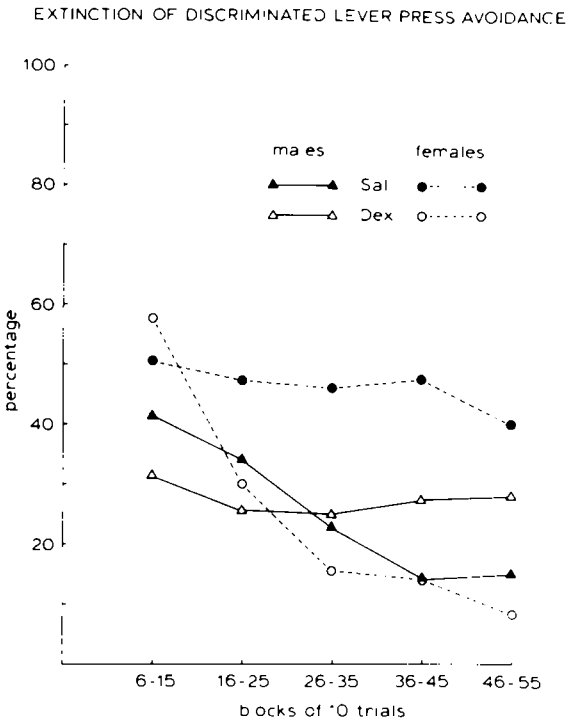

FIG. 2. Extinction of discriminated lever press avoidance during 50 trials in which the shockgenerator was turned off. Five acquisition trials preceded the 50 extinction trials (6–55). Percentage of avoidance responses during these 5 acquisition trials were: Dex-males 15%; Sal-males 25% Dex-females 45%; Sal-females 33%. "Avoidance responses" are expressed as a percentage of the total number of trials per block (10).

Pituitary Weights, Adrenal Weights and Corticosterone Concentration

Table 1 shows the absolute values of pituitary and adrenal weights. These data were used in a two way analysis of variance involving sex and treatment as factors. The female pituitary was significantly heavier, $F(1,44)=4.92$, $p<0.05$, than the male pituitary. The same was true with regard to the total weight of left and right adrenals, $F(1,44)=37.11$, $p<0.001$. Dexamethasone treatment did not affect the pituitary weight but there was a very strong effect of dexamethasone treatment on the total adrenal weight: the adrenal weight in Dex-animals was 23% lower than that of the Sal-animals, $F(1,44)=25.34$, $p<0.001$. No sex × treatment interaction was observed.

Corticosterone plasma concentrations appeared to be rather variable in the Sal-males and Sal-females groups. For

TABLE 1

MEAN WEIGHT OF PITUITARY AND ADRENALS AND MEAN CORTICOSTERONE CONCENTRATION (S.E.M.)*

	Pituitary (mg)	Adrenal (mg) left	right	total	Corticosterone concentration (ng/ml)
Dex males	13.1 (0.77)	17.3 (0.97)	16.3 (0.69)	33.6 (1.45)	7.29 (1.08)
Sal males	12.9 (0.80)	23.4 (1.46)	20.4 (1.58)	43.8 (2.36)	146.72 (75.54)
Dex females	14.1 (0.88)	23.3 (1.57)	22.8 (1.33)	46.1 (2.75)	20.28 (4.14)
Sal females	15.6 (0.91)	31.3 (1.05)	27.6 (1.65)	58.9 (2.31)	420.28 (213.75)

*These results were obtained at the end of 5 days of discriminated lever press avoidance during which dexamethasone treated animals received daily injections of 500 μg/kg body weight of dexamethasone phosphate and control animals an equivalent volume of physiological saline.

homogeneity of variances the 10logx-transformation was applied to these data. Both the main factors sex and treatment were significant, sex: $(F1,44)=17.65$, $p<0.001$ and treatment: $F(1,44)=122.03$, $p<0.001$. Plasma corticosterone concentration was much higher in females and dexamethasone treatment caused a strong decline in corticosterone concentration in both sexes. In Dex-animals the corticosterone concentration was no more than 5% of the corticosterone concentration in Sal-animals. No sex × treatment interaction was observed.

Body Weight Changes

Five days of avoidance learning resulted in a body weight loss of nearly 5% in the Sal-males and Sal-females. The Dexamethasone treated animals however lost about 13% of their body weight during the same period. A three way analysis of variance with sex, treatment and days as main factors revealed a highly significant effect of treatment, $F(1,44)=140.42$, $p<0.001$. The main factor sex however, and all interactions with this factor, were not significant. Males and females apparently lost an equal percentage of their body weight as a result of dexamethasone treatment and avoidance training.

DISCUSSION

It was the purpose of this study to investigate whether sex differences in avoidance learning can be related to the pronounced sex difference in the activity of the pituitary-adrenocortical system. Rats of both sexes were tested in a discriminated lever press avoidance procedure during four sessions of avoidance acquisition and 1 extinction session. Treatment with dexamethasone did not significantly affect avoidance learning in either sex showing that sex differences in hormonal levels of the piutitary-adrenocortical system do not contribute to the sexual dimorphism in avoidance learning.

During the four acquisition sessions the number of avoidance responses increased, accompanied by a sharp decline in shock escape latencies. Apparently animals first learn to escape during the shock period and then learn to avoid the shock by responding in the warning period. Lever holding and intertrial responses both decrease over sessions and negative correlations found between these two variables and avoidance responses show that rats that acquire the task properly eventually respond efficiently. These results are in accordance with results obtained in a previous experiment using this lever press avoidance situation [24]. Females show faster acquisition and spent less time depressing the lever in both studies [24]. The difference however, was less pronounced and the number of intertrial responses of females was not lower in the present study. These discrepancies may well be the result of a difference in pretest experience with food rewarded lever pressing. In the former study two months of food rewarded lever pressing preceded the avoidance experiment whereas in the present experiment only 11 days of food rewarded lever pressing were used. The magnitude of the sex difference in this situation therefore seems to depend on the amount of previous experience with lever pressing. Extensive experience facilitates the avoidance learning thereby exaggerating female superiority.

Administration of dexamethasone very obviously affected hormone levels in both males and females. Corticosterone levels were greatly suppressed and the adrenal weights were diminished. The pituitary weights were not affected by dexamethasone treatment. An important finding is the fact that these treatment effects were equally strong in males and females. If the sex difference in pituitary-adrenal system reactivity was an important factor leading to the sex difference in active avoidance learning, the above described suppression of this sytem should have had pronounced effects on the sex differences in lever press avoidance learning. As this did not occur, the hypothesis that the sex difference in active avoidance acquisition is the consequence of the sex difference in the pituitary-adrenocortical system activity was not supported by this study. This result is in accordance with the study of Scouten and Beatty [18]. Beatty *et al.* [5] also failed to find an effect of dexamethasone treatment on shuttle-box avoidance of male rats. ACTH treatment and adrenalectomy however, did affect the avoidance behavior [5]. In addition, hypophysectomy, an operation which has similar effects on the pituitary-adrenocortical system as dexamethasone treatment, also seriously interfered with avoidance acquisition [14]. This discrepancy between hypophysectomy and dexamethasone treatment remains unexplained.

Extinction of an active avoidance response should occur faster under dexamethasone treatment. It has been suggested that this is a result of both a direct influence of dexamethasone on the central nervous system and an indi-

rect effect via the inhibition of the ACTH-release from the pituitary [14]. Female rats have been found to be more resistant to extinction of an active avoidance response than males [4]. The results of the Sal-treated animals in the present experiment showed no significant sex difference although the same tendency towards slower extinction in females could be observed. Beatty *et al.* [4] suggested that the higher levels of ACTH in females are responsible for this sex difference. Dexamethasone treatment in the present experiment resulted in a comparatively rapid extinction in the females, as dexamethasone treatment did not affect the extinction in males. Our data therefore, might indeed be taken as a suggestion that a difference in ACTH levels between males and females leads to a sex difference in extinction.

In conclusion, this study presents clear evidence that the sex difference in active avoidance acquistion is not related to the sex differences in the pituitary-adrenocortical functioning. Concerning the sex difference in extinction of active avoidance, the present data do not exclude the possible importance of a sexual dimorphism in pituitary-adrenocortical functioning.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. F. van Haaren and Dr. H. H. Swanson for critical comments.

REFERENCES

1. Archer, J. Rodent sex differences in emotional and related behavior. *Behav Biol* **14:** 451–479, 1975.
2. Beatty, W. W. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Horm Behav* **12:** 112–163, 1979.
3. Beatty, W. W. and P. A. Beatty. Hormonal determinants of sex differences in avoidance behavior and reactivity to electric shock in the rat. *J Comp Physiol Psychol* **73:** 446–455, 1970.
4. Beatty, W. W., P. A. Beatty and R. E. Bowman. A sex difference in the extinction of avoidance behavior in rats. *Psychon Sci* **23:** 213–214, 1971.
5. Beatty, P. A., W. W. Beatty, R. E. Bowman and J. C. Gilchirst. The effects of ACTH, adrenalectomy and dexamethasone on the acquisition of an avoidance response in rats. *Physiol Behav* **5:** 939–944, 1970.
6. Beatty, W. W., K. C. Gregoire and L. L. Parmiter. Sex differences in retention of passive avoidance behavior in rats. *Bull Psychon Soc* **2:** 99–100, 1973.
7. Beatty, W. W., C. W. Scouten and P. A. Beatty. Differential effects of dexamethasone and body weight loss on two measures of activity. *Physiol Behav* **7:** 869–871, 1971.
8. Cicero, T. J. and R. D. Meyers. Facilitation of discriminated avoidance learning by prior operant training. *Psychol Rep* **22:** 1273–1276, 1968.
9. Critchlow, V., R. A. Liebelt, M. Bar-Sela, W. Mountcastle and H. S. Lipscomb. Sex difference in resting pituitary-adrenal function in the rat. *Am J Physiol* **205:** 807–815, 1963.
10. Davis, H., J. W. Porter, J. Burton and S. Levine. Sex and strain differences in leverpress shock escape behavior. *Physiol Psychol* **4:** 351–356, 1976.
11. De Kloet, E. R., J. van der Vies and D. de Wied. The site of the suppressive action of dexamethasone on pituitary-adrenal activity. *Endocrinology* **94:** 61–73, 1974.
12. Denti, A. and A. Epstein. Sex differences in the acquisition of two kinds of avoidance behavior in rats. *Physiol Behav* **8:** 611–615, 1972.
13. Denti, A. and J. A. Negroni. Activity and learning in neonatally hormone treated rats. *Acta Physiol Lat Am* **25:** 99–106, 1975.
14. De Wied, D. Piutitary-adrenal system hormones and behavior. In: *The Neurosciences*, edited by F. O. Schmitt and F. G. Worden. Cambridge, MA: M.I.T. Press, 1974, pp. 653–666.
15. Dunn, J., L. Scheving and P. Millet. Circadian variation in stress evoked increases in plasma corticosterone. *Am J Physiol* **222:** 402–406, 1972.
16. Gray, J. A. Sex differences in emotional behavior in mammals including man: endocrine bases. *Acta Psychol* **35:** 29–46, 1971.
17. Kitay, J. I. Pituitary-adrenal function in the rat after gonadectomy and gonadal hormone replacement. *Endocrinology* **73:** 253–260, 1963.
18. Scouten, C. W. and W. W. Beatty. Adrenocortical function and sex-differences in acquisition and extinction of active avoidance behavior in the rat. *Psychol Rep* **29:** 1011–1018, 1971.
19. Scouten, C. W., L. K. Grotelueschen and W. W. Beatty. Androgens and the organization of sex differences in active avoidance behavior in the rat. *J Comp Physiol Psychol* **88:** 264–270, 1975.
20. Sfikakis, A., C. Spyraki, N. Sitaras and D. Varanos. Implication of the estrous cycle on conditional avoidance behavior in the rat. *Physiol Behav* **21:** 441–446, 1978.
21. Van Dijk, A. M. A. Determination of radio-immunoassayable ACTH in plasma and/or brain tissue of rats as influenced by endocrine and behavioral manipulation. Doctoral Thesis, Utrecht, 1979.
22. Van Oyen, H. G., N. E. van de Poll and J. P. C. de Bruin. Sex, age and shock-intensity as factors in passive avoidance. *Physiol Behav* **23:** 915–918, 1979.
23. Van Oyen, H. G., N. E. van de Poll and J. P. C. de Bruin. Effects of retention interval and gonadectomy on sex differences in passive avoidance behavior. *Physiol Behav* **25:** 859–862, 1980.
24. Van Oyen, H. G., H. Walg and N. E. van de Poll. Discriminated leverpress avoidance conditioning in male and female rats. *Physiol Behav* **26:** 313–317, 1981.