Pharmacology Biochemistry & Behavior, Vol. 16, pp. 943–947, 1982. Printed in the U.S.A.

Effects of TRH on Acquisition and Extinction of Shuttlebox-Avoidance Behavior in Fischer$_{344}$ Rats

YOSHITAKA TAMAKI

Faculty of Education, Shinshu University, Nagano 380, Japan

AND

YOSHIRO KAMEYAMA

Research Institute of Environmental Medicine, Nagoya University, Nagoya 464, Japan

Received 23 February 1981

TAMAKI, Y. AND Y. KAMEYAMA. *Effects of TRH on acquisition and extinction of shuttlebox-avoidance behavior in Fischer$_{344}$ rats.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 943–947, 1982.—Thyrotropin-releasing hormone (TRH) was injected intraperitoneally into male Fischer$_{344}$ rats in doses ranging between 1 mg/kg and 20 mg/kg to assess the effects on the acquisition and extinction of shuttlebox-avoidance behavior. Administration of 20 mg/kg TRH resulted in a rapid acquisition of avoidance behavior in early training trials. This enhancement did not involve changes in the occurrence of anticipatory responses to an inevitable shock but was correlated with an increase of concurrent intertrial-responses. Thus, the behavioral changes observed would be a reflection of TRH-induced changes on motor activity. TRH treatment did not alter the resistance to extinction of the avoidance response. This finding is corroborated by the fact that rats given the TRH treatment withheld the well-learned response to a warning signal, when this response was selectively punished after initial acquisition stage.

Thyrotropin-releasing hormone (TRH) Avoidance response Intertrial response Acquisition Extinction Punishment Shuttlebox

IN recent years, considerable interest has developed in neurobehavioral effects of the thyrotropin-releasing hormone (TRH). It is now clear that TRH has direct effects on the central nervous system unrelated to the control of endocrine functions of the adeno-hypophysis. For example, TRH potentiates behavioral changes following administration of pargyline and L-Dopa [18,19] and hyperactivity following tranylcypromine and L-tryptophan injections [8]. TRH also increases motor activity [1, 2, 14–16, 22] and antagonizes the locomotor depressive effect of pentobarbital and other centrally acting drugs [3] as well as that of α-methyltyrosine [12]. Moreover, TRH improves shuttlebox-avoidance behavior in rats when administered intracerebrally [17], and reverses the deleterious effect of α-methyltyrosine on lever-pressing avoidance response [12]. This functional effect of TRH on active avoidance behavior through the stimulation of central catecholaminergic mechanism is noteworthy, because TRH treatment can augment the release and turnover of catecholamines [5, 9, 10].

The present experiment provides further details on TRH treatment effects on shuttlebox-avoidance behavior in Fischer$_{344}$ rats. It is apparent that when rats were permitted to escape a shock the escape response tended to occur before the onset of shock. Other reports indicate that when the level of anticipatory responding is high avoidance acquisition is rapid [4, 13, 23]. On the basis of these findings, it is suggested that anticipatory responding in the avoidance situation plays a major role in determining avoidance response rate. Thus, if the effect of TRH on avoidance acquisition might be evaluated, this should be done within the context of the D'Amato procedure which involves the presentation of a shock at the end of the warning signal (WS)-shock interval on every trial, regardless of whether an avoidance occurs [6]. This procedure permits an assessment of a specific component of anticipatory responding which occurs during the WS-shock interval in the absence of a shock-avoidance contingency.

Because ACTH and similar peptides led to a delay in the extinction of pole-jumping avoidance behavior [7,24], a traditional extinction procedure permits evaluation of this TRH effect. As an alternative to such a paradigm, the shock is made contingent upon occurrence of an avoidance response [13]. Avoidance behavior in this procedure is selectively punished so that a WS predicts shock only when an avoidance occurred. Thus, failure to avoid (i.e., passive avoidance) results in no shock being delivered on that trial. Because initial active avoidance training will disrupt subsequent passive avoidance performance, it may be possible to

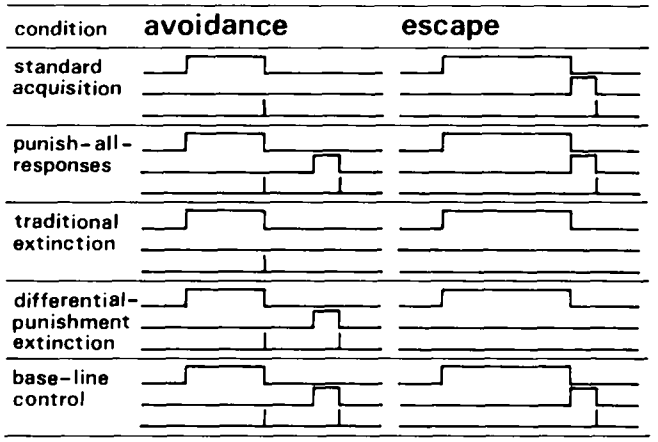

FIG. 1. An example of patterns of warning signal (WS) and shock both in avoidance and escape trials in 5 conditions during the 2nd half of the session. A top line refers to the onset and the offset of the WS, a middle line the shock location, and a bottom line the occurrence of the response.

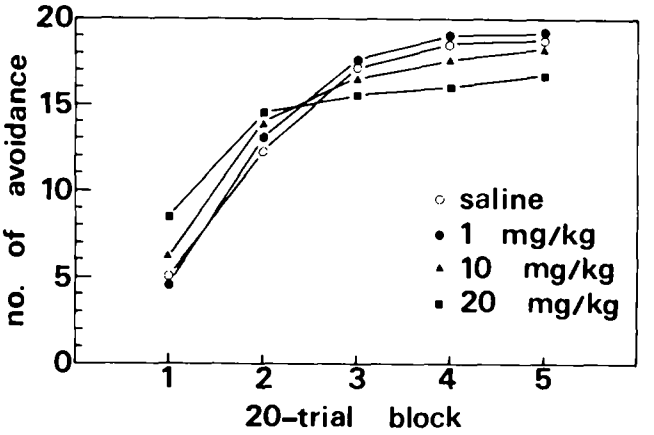

FIG. 2. The number of avoidance responses as a function of training trials in the 1st half of the session for 4 groups of 32 rats given standard acquisition procedure.

determine the source for any modification of the ability to withhold a well-learned avoidance response by TRH treatment.

METHOD

Animals

A total of 160 male Fischer$_{344}$ rats obtained from Charles River, Japan (CRJ COBS: F344/Du Crj, Kanagawa) were used. They were maintained in group cages with free access to food and water in a temperature controlled (21–23°C) room. The animals ranged in age from 71 to 88 days when tested, and were randomly assigned to experimental treatments.

Apparatus

Two identical BRS/LVE Model RSC-044 shuttleboxes were used. The shuttlebox, which measured 21.6×48.7×27.3 cm high, was partitioned into 2 compartments by an 6.3-cm-high aluminum barrier with Plexiglas side walls and tops, and aluminum ends. The floor consisted of 2-mm stainless steel rods spaced 11 mm center to center. This grid floor was arranged to tilt at about the mid-point. When a rat entered a compartment the floor tilted activating a microswitch located just outside the box. Each shuttlebox was lighted by 24-V bulbs on each of its end walls, and enclosed in a ventilated, sound-attenuating chamber. Mounted on the rear wall of this chamber was a speaker which permitted the presentation of a continuous background white-noise at 75 dB SPL. A WS was a 1000-Hz pure tone at 85 dB SPL delivered through the same speaker by a Bio-Medica, Osaka, Model BNA-88 audio/noise generator. The aversive stimulus was nominal 3-mA scrambled electric shock produced by a BRS/LVE Model SGS-003 shock generator delivered to whichever grid floor of the compartment a rat occupied.

All stimulus presentations and measurements of response were controlled by an on-line microcomputer system (Bio-Medica, Osaka, Model BICOM-8) located in an adjacent room.

Procedure

Twenty minutes before the start of the experiments, 4 groups of 40 rats were given intraperitoneal injections of physiological saline, 1 mg/kg, 10 mg/kg, and 20 mg/kg doses of TRH dissolved in physiological saline, respectively. Each injection volume was approximately 0.5 ml/rat.

After a 10-min habituation period in the shuttlebox, each rat was tested in a single training session of 200 trials with the fixed intertrial-interval of 30 sec. A tonal WS came on 10 sec before the shock onset and lasted just 10 sec unless an avoidance response occurred. Thus, WS and shock never overlapped. If a shock was administered after the WS-shock interval had elapsed, it could be promptly terminated by the response which would have avoided it. An avoidance response terminated the WS, regardless of other programmed consequences.

Four injection-dose groups were trained with the following 5 sets of conditions. The design was a 4 (doses) × 5 (conditions) factorial with 8 rats per cell. In these animals, 4 conditions were alike in acquisition within 100 trials but treated differentially for the next 100 trials (Fig. 1). In acquisition the standard procedure was used to establish avoidance behavior: shock was withheld whenever an avoidance response occurred in a given trial. In the 2nd half of the session, one group invariably continued standard acquisition trials prevailing for the 1st half of the session. This condition provided a ceiling control for the assessment of other groups' avoidance behavior. A 2nd group had the absence of shock-avoidance contingency removed by consistent presentation of a shock at 10 sec after WS onset, irrespective of the occurrence of an avoidance (better termed an anticipatory response to an inevitable shock). This was the punish-all-responses condition, in which an avoidance response was not effective in avoiding shock. A 3rd group trained under the traditional removal-of-all-shocks, i.e., extinction, condition. A 4th group had the absence of shock-avoidance contingency by making a shock contingent upon an avoidance response. Thus, the shock came on 10 sec after WS onset only in the trial where a rat performed an avoidance; hence this provided the differential-punishment extinction condition. A 5th group was tested for 200 trials to establish the baseline response rates in the absence of

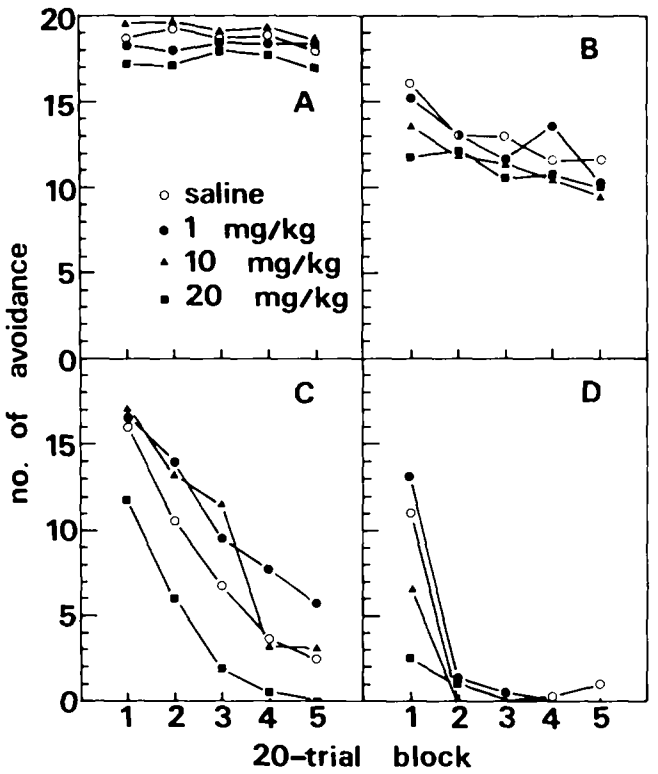

FIG. 3. The number of avoidance responses as a function of training trials in the 2nd half of the session for 4 groups of 8 rats treated with different conditions as follows: A, the standard acquisition condition; B, the punish-all-responses condition; C, the traditional extinction condition; and D, the differential-punishment extinction condition.

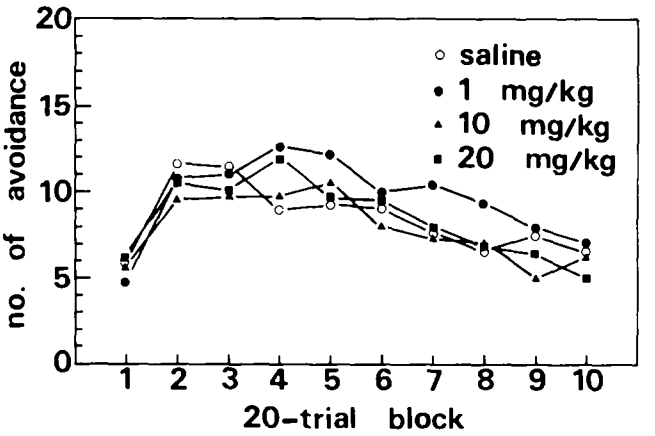

FIG. 4. The number of avoidance responses as a function of training trials for 4 groups of 8 rats given the base-line control condition.

shock-avoidance contingency by the presentation of a shock in every trial. That is, the condition which was abbreviated as the baseline control was run under the same in the punish-all-responses condition except over all trials.

The response latency from WS onset or shock onset, and the number of concurrent intertrial-responses for each trial were measured.

RESULTS

Avoidance Response

There were no significant differences among 4 injection-dose groups of 40 rats in the number of trials required to achieve the 1st avoidance response. (To comply with the assumption of the homogeneity of variance, we performed an analysis of variance on square root (X + 0.5) transformation of these scores [11].)

Figure 2 presents the number of avoidance responses for 100 trials in 4 injection-dose groups of 32 rats with a standard acquisition condition, excluding the baseline controls. A mixed analysis of variance of acquisition scores was performed for 4 doses with 4 conditions varied in the 2nd 100 trials and 5 trial-blocks treated as a repeated measure. The main effect of blocks was significant, $F(4,448)=337.86$, $p<0.001$. The interaction of doses × blocks was also significant, $F(12,448)=5.30$, $p<0.001$. This reflected a difference in the development of avoidance responses among 4 groups given different doses. When a pooled error term was used for comparison of individual block means, group differences were significant at Block 1 and 4, $p<0.001$ and $p<0.005$, respectively. Subsequent Scheffé tests indicated that the 20 mg/kg group avoidance was superior to that of other groups at Block 1, but inferior to that in groups with saline and 1 mg/kg at Block 4. Although TRH treatment failed to alter avoidance responses significantly at any dose in Block 5, $p<0.10$, the 20 mg/kg group also showed slightly lower avoidance than other groups. The avoidance response of the 20 mg/kg group was acquired more rapidly than with other groups, but declined to a lower level of occurrence as training progressed. None of other main effects or interactions were significant, all $Fs<1.00$

A mixed 4 (doses) × 5 (blocks) analysis of variance was performed on avoidance behavior in the 2nd 100 trials for each of 4 different conditions, also excluding the baseline controls. For continued standard acquisition (Fig. 3A), analysis showed only the significant effect of groups, $F(3,28)=3.92$, $p<0.05$. Thus, the 20 mg/kg group had lower avoidance behavior than the 10 mg/kg group, indicating that the 20 mg/kg group could not avoid very well during the maintenance stage. Nevertheless, it proved partially reliable because its avoidance performance was slightly but not significantly inferior to that of saline group. For the punish-all-responses condition (Fig. 3B), in which the shock-avoidance contingency was not in effect, no significant differences were observed among 4 groups in the occurrence of avoidance responses, $F(3,28)=1.97$. Only the effect of blocks was significant, $F(4,112)=14.30$, $p<0.001$, indicating that block means differed except among Blocks 2, 3, and 4.

The 20 mg/kg group which underwent a traditional extinction procedure appeared likely to extinguish the avoidance response more rapidly than other groups (Fig. 3C). However, the effect of groups could not approach a conventional level of the significance, $F(3,28)=2.64$, $p<0.10$, due to the relatively higher within-cell variance. The significant effect for blocks, $F(4,112)=61.61$, $p<0.001$, but not for the interaction, $F<1.00$, reflected the general decline in avoidance behavior across blocks. This result appears to be consistent with numerous earlier findings as to the extinction process. As shown in Fig. 3D, in general, rats which received the differential-punishment extinction procedure made few avoidance responses prior to the 1st failure to respond (plus, of course, a number of unpunished

avoidances just before this condition began), and then acquired rapidly other behavior, i.e., typically freezing, rather than crossing, as an new alternative avoidance response. Stated another way, once immobility began to occur in the presence of the WS, it resulted in virtual absence of avoidance responses because rats would not get a shock if not responding to the WS. These behavioral aspects were noticeable in all groups, so the effect of groups was not significant, $F<1.00$.

For the baseline control condition (Fig. 4), group differences were not significant in avoidance behavior, $F(3,28)=1.04$. Only the effect of blocks was significant, $F(9,252)=16.21$, $p<0.001$, indicating the reverse U-shaped occurrence of avoidance.

Intertrial-Response

The number of concurrent intertrial-responses was analyzed to assess the effect of TRH treatment on avoidance behavior. Looking at the intertrial-responses within 100 trials under the standard acquisition condition, an analysis of variance which was an identical type of avoidance behavior showed the significant effect of blocks, $F(4,448)=63.06$, $p<0.001$, as well as the interaction of groups × blocks, $F(12,448)=1.99$, $p<0.05$. By a follow-up analysis of this significant interaction, group differences were reliable only in Block 1, $p<0.005$, indicating that the 20 mg/kg group had intertrial-responses higher than other groups.

DISCUSSION

The present findings show that intraperitoneal administration of 20 mg/kg TRH lead to a rapid acquisition of shuttlebox-avoidance behavior, but has no effect on the anticipatory responses to an inevitable shock under the baseline control condition. An enhancement of avoidance acquisition does not involve the possible effect of TRH on the information value of the WS, related to the motivational factor, presumably because early avoidance responses are generated by the essential factor as a reaction to shock [6]. However, it entirely coincides with a significant increase of concurrent intertrial-responses. The effectiveness of TRH appears to modify avoidance by increasing the propensity for response initiation. It is thus presumed that facilitatory acquisition of avoidance is a consequence of transient hyperactivity. This interpretation is corroborated by our present observation in a free-operant avoidance schedule that TRH in dose of 20 mg/kg increased the rate of burst responding despite little contribution to the time in which rats were free of shocks (unpublished data).

The available evidence to the catecholaminergic mechanism indicated that TRH either causes a release of dopamine indirectly or somehow modulates the dopamine receptor site. A rapid avoidance acquisition accompanied by an increase of intertrial-responses appears to be due to the dopaminergic stimulant action of TRH, because hyperactivity induced by TRH is probably mediated by the anatomical dopamine pathway [15]. It is noteworthy, however, that the concentration of TRH necessary to produce this effect is considerably high (20 mg/kg), because only a small amount of administered TRH is likely to reach the brain compared to the amount that penetrate into the pituitary [21]. Accordingly, TRH in doses less than 10 mg/kg did not affect avoidance acquisition. These results corroborate our preliminary findings with TRH doses of 2 mg/kg and 5 mg/kg, and previous findings in lever-pressing [12] and in shuttlebox conditioning [17,20].

Under the differential-punishment extinction condition where an avoidance response was punished after the initial acquisition stage, TRH treatment lead to the quick development of avoidance suppression. Accordingly, no substantial changes in resistance to extinction of avoidance behavior are observed at any TRH dose during traditional extinction. These findings do not confirm the suggestion that TRH would delay extinction of the avoidance response possibly as a function of its structural relationship with ACTH analogues [25].

It should be noted that rats had an appreciable level of anticipatory responses for long periods of time despite the absence of any apparent reinforcement (Figs. 3B and 4). One must not, of course, ignore the problem that the anticipatory response to an inevitable shock suffers some degree of punishment. For example, anticipatory responses made late in the WS-shock interval quickly resulted in a shock which may have served as a response-contingent punishment. However, this is not the important factor because avoidance responses vanished if the responses were selectively followed by a shock as in the differential-punishment extinction condition.

ACKNOWLEDGEMENTS

This study was supported in part by a grant from the Ministry of Health and Welfare of Japan to Y. K. The authors thank T. Kanai for his assistance in the running of animals. Requests for reprints should be sent to Y. T., Faculty of Education, Shinshu University, Nishinagano, Nagano 380, Japan.

REFERENCES

1. Agarwal, R. A., R. B. Rastogi and R. L. Singhal. Enchancement of locomotor activity and catecholamine and 5-hydroxytryptamine metabolism by thyrotropin releasing hormone. *Neuroendocrinology* **23:** 236–247, 1977.
2. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange, Jr. and M. A. Lipton. Antagonism of ethanol narcosis by thyrotropin releasing hormone. *Life Sci.* **14:** 1053–1063, 1974.
3. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange, Jr., M. A. Lipton and N. P. Plotnikoff. Effects of thyrotropin-releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. *J. Pharmac. exp. Ther.* **193:** 11–22, 1975.
4. Campenot, R. B. Effect of amygdaloid lesions upon active avoidance acquisition and anticipatory responding in rats. *J. comp. physiol. Psychol.* **69:** 492–497, 1969.
5. Constantinidis, J., F. Geissbühler, J. M. Gaillard, T. Hovaguimian and R. Tissot. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone: Evidence by fluorescence histochemistry. *Experientia* **30:** 1182–1183, 1974.
6. D'Amato, M. R. Role of anticipatory responses in avoidance conditioning: An important control. *Psychon. Sci.* **8:** 191–192, 1967.
7. Donovan, B. T. The behavioral actions of the hypothalamic peptides: A review. *Psychol. Med.* **8:** 305–316, 1978.
8. Green, A. R. and D. G. Grahame-Smith. TRH potentiates behavioral changes following increased brain 5-hydroxytryptamine accumulation in rats. *Nature* **251:** 524–526, 1974.

9. Horst, W. D. and N. Sprit. A possible mechanism for the antidepressant activity of thyrotropin releasing hormone. *Life Sci.* **15:** 1073–1082, 1974.
10. Keller, H. H., G. Bartholini and A. Pletscher. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. *Nature* **248:** 528–529, 1974.
11. Kirk, R. E. *Experimental Design: Procedure for the Behavioral Sciences*. Belmont, CA: Brooks/Cole, 1968, pp. 63–67.
12. Kulig, B. M. The effects of thyrotropin-releasing hormone on the behaviour of rats pretreated with α-methyltyrosine. *Neuropharmacology* **14:** 489–492, 1975.
13. Lovely, R. H., N. E. Grossen, S. A. Moot, R. H. Bauer and J. J. Peterson. Hippocampal lesions and inhibition of avoidance behavior. *J. comp. physiol. Psychol.* **70:** 343–352, 1971.
14. Masserano, J. M. and C. King. TRH increases locomotor activity in rats after injection into the hypothalamus. *Eur. J. Pharmac.* **69:** 217–219, 1981.
15. Miyamoto, M. and Y. Nagawa. Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. *Eur. J. Pharmac.* **44:** 143–152, 1977.
16. Mora, S., A. Loizzo and V. G. Longo. Central effects of thyrotropin-releasing factor (TRF): Interaction with some antipsychotic drugs. *Pharmac. Biochem. Behav.* **4:** 279–282, 1976.
17. Mora, S., A. G. Nasello and L. Fieschi. TRH on rat conditioned avoidance behavior: Interaction with brain catecholamines. *Pharmac. Biochem. Behav.* **13:** 137–139, 1980.
18. Plotnikoff, N. P., G. R. Breese and A. J. Prange, Jr. Thyrotropin releasing hormone (TRH): Dopa potentiation and biogenic amine studies. *Pharmac. Biochem. Behav.* **3:** 665–670, 1975.
19. Plotnikoff, N. P., A. J. Prange, Jr., G. R. Breese, M. S. Anderson and I. C. Wilson. Thyrotropin releasing hormone: Enchancement of Dopa activity by a hypothamic hormone. *Science* **178:** 417–418, 1972.
20. Plotnikoff, N. P., A. J. Prange, Jr., G. R. Breese, M. S. Anderson and I. C. Wilson. The effects of thyrotropin-releasing hormone on Dopa response in normal, hypophysectomized, and thyroidectomized animals. In: *The Thyroid Axis, Drugs, and Behavior*, edited by A. J. Prange, Jr. New York: Raven Press, 1974, pp. 103–113.
21. Stumpf, W. E. and M. Sar. ^{3}H-TRH and ^{3}H-proline radioactivity localization in pituitary and hypothalamus. *Fedn Proc.* **32:** 1, 1973.
22. Taché, Y., M. Lis and R. Collu. Effects of thyrotropin-releasing hormone on behavioral and hormonal changes induced by β-endorphin. *Life Sci.* **21:** 841–846, 1977.
23. Tamaki, Y. and M. Inouye. Avoidance of and anticipatory responses to shock in prenatally x-irradiated rats. *Physiol. Behav.* **22:** 701–705, 1979.
24. deWied, D., A. Witter and H. M. Greven. Behaviourally active ACTH analogues. *Biochem. Pharmac.* **24:** 1463–1468, 1975.
25. deWied, D., A. Witter and H. M. Greven. Behaviourally active ACTH analogues. *Expl Brain Res.* **23:** 52, 1975.