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Neonatal Treatment with TRH Affects Development, Learning, and Emotionality in the Rat[1,2]

LOIS O. STRATTON, CHRISTINE A. GIBSON

University of New Orleans, New Orleans, LA 70122

KATHRYNE G. KOLAR

Tulane School of Social Work, New Orleans, LA 70118

AND

ABBA J. KASTIN

Veterans Administration Hospital and Tulane University School of Medicine New Orleans, LA 70146

STRATTON, L. O., C. A. GIBSON, K. G. KOLAR AND A. J. KASTIN. *Neonatal treatment with TRH affects development, learning, and emotionality in the rat.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 65–67, 1976. Rats that received injections of TRH from Days 2–7 postnatally ran faster to a palatable food reward than controls at 33–37 days of age. Rats treated with TRH were less emotional and more active in an open field both as pups and as adults. There were few notable differences in developmental measures except that the teeth erupted earlier in control animals. Weights of glands and brain parts showed that ovaries, testes, pineal gland, and hypothalamus were heavier in treated animals. The results indicated that TRH given early in life may affect adult behavior by reducing emotionality.

TRH Neonatal Development Learning Emotions Rat

THYROXINE is important in the development of nervous tissue in young organisms [11]. An excess of thyroid hormone shortly after birth accelerates the appearance of several reflexes and other developmental signs in the rat [4,5]. However, early physical maturation is accompanied by impairment in learning at adolescent and adult ages (Ibid). Thyrotropin releasing hormone (TRH) activity is found in hypothalamic extracts of the rat brain as early as 18 days prenatally, and by 3 days of age it has reached 47% of adult levels [3]. In addition to the hypothalamus, substantial amounts of TRH are found in the thalamus, cerebrum, and brain stem of adult rats [9,17]. The appearance of TRH activity early in life and its pervasiveness in brain tissue suggests a role in neuronal, synaptic, or metabolic events which would affect behavioral and developmental processes. We decided that intervention at the earliest possible period of neonatal life would be most likely to reveal possible behavioral and physical changes due to administration of exogenous TRH.

METHOD

Animals

Thirty male and female pups from 6 albino Wistar male and female adult rats were used. The experiment was run in 2 sessions approximately 5 months apart, containing 16 and 14 rats, respectively. Since no significant differences were found between groups according to sessions, the results of individual sessions were combined and are reported here as one body of data. The control group contained 6 females and 9 males; the group which received TRH contained 5 females and 10 males. Rat pups were differentiated by ear marks so that it was possible to place nearly equal numbers from each litter into experimental and control groups. The data for 2 rats, 1 from each group, were eliminated from the study. An animal from the control group died at 12 days of age, and a rat from the TRH group failed to learn the maze due to experimenter error during pretraining. On Days 2–7 each rat was injected subcutaneously in the region of the hip with either 10 μg of TRH in a saline vehicle or 10 μg of saline. The experimenters did not know which solution contained the hormone.

Procedure

The following developmental data were tabulated: weight (Days 2–31), ear and eye opening, teeth eruption, swimming with the nose out of water (Days 5–7), and open

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[2] Requests for reprints should be sent to Lois O. Stratton, Department of Psychology, University of New Orleans, New Orleans, Louisiana 70122, or Abba J. Kastin, Endocrinology Section of the Medical Service, Veterans Administration Hospital, New Orleans, Louisiana 70146.

field activity including head, head and belly lifts, defecations, and wall clinging behavior. On the 33rd day the rats learned an 8 choice, 8 cul de sac Warden Maze for a palatable food reinforcement.

Open field activity was measured on 4 different occasions: Days 5–7, 12 14, 38–39, and 70–72. The first 2 tests were performed by placing pups in a 60 cm by 30 cm cardboard arena with 2.6 cm squares on the bottom. The third and fourth tests took place in a circular apparatus measuring 110 cm in diameter with radiating and circular lines forming odd shaped sections approximately 45 sq. cm.

On Day 26 of life the pups were weaned and placed in cages containing either 1 or 2 other pups of the same sex. Starting on Day 29, they were food deprived for 4 days until their weight reached 85% of the predeprivation level. On Days 30–32 the rats were fed wet mash (sugar, milk, and ground pellets) in the goal box of the maze for 2 minutes followed by supplemental food in the home cage. On Days 33–37 the rats were placed in the start box and time and error scores recorded until they reached the goal box.

At 75 days of age the 14 rats from the second session were sacrificed. Total brain weight and the weight of brain parts plus several endocrine glands were tabulated.

RESULTS

Several of the rating scales yielded rank order on categorical data; in addition, several ratio scaled measures resulted in skewed distributions. For these reasons nonparametric statistics were applied to the results. The Wilcoxin test for unpaired replicates [15] was used for ordered data. The significance of difference between proportions was used to analyze the categorical data [2].

Developmental measures. Weights taken from Days 2–32 and during the maze task showed no differences between experimental and control groups. There were no significant differences in the time at which the eyes and ears opened; however, the eyes of control group rats tended to open sooner. Teeth eruption and swimming ability was retarded in rats receiving TRH. At 7 days of age 6% of experimental rats and 38% of controls had teeth ($p<0.001$). At 8 days the percentage rose to 44% for animals receiving TRH and 66% for controls ($p<0.05$). Control rats had a superior ability to swim with their heads out of water on Day 7 ($p<0.05$).

Open field activity. The first open field test, performed on Days 5 7 of life, showed that rats receiving TRH during that period crossed more lines than controls on each day ($p<0.01$). Lifting the head off the floor of the small arena was more common for the group receiving TRH ($p<0.01$). On Days 12 and 13, after all injections were completed, the group that received TRH continued to cross more lines than controls ($p<0.05$; both days), and produced more head and belly lifts ($p<0.01$; both days). In the large open field on Day 38, the TRH group was more active ($p<0.05$), defecated less ($p<0.05$), and showed less wall clinging behavior than controls ($p<0.05$). During the second large open field test the TRH group was more active on Day 70 ($p<0.001$) and defecated significantly less on Days 71 ($p<0.01$) and 72 ($p<0.05$). Wall clinging occurred more often in control animals on Day 72 ($p<0.05$). On days when the differences were not significant there were trends in a similar direction.

Maze learning. Body weights taken on the first, third, and fifth day of learning corresponded with the earlier finding that no weight differences were observable. An error was recorded when rats entered or reentered any cul de sac beyond three quarters of its body length. Time for a rat to run from start box to goal box was calculated for each trial. The analysis of error scores revealed that fewer errors were made by the TRH group. Significant differences between groups occurred on Day 3 ($p<0.01$) and Day 5 ($p<0.05$), and a nonsignificant trend in the same direction occurred on Day 4. Time difference scores showed that the group that received TRH ran the maze in shorter times than controls on all days ($p<0.01$). Daily differences can be seen in Fig. 1.

Brain and gland weights. There were no differences in total brain weight, or weights of occipital cortex, posterior cortex, striatum, thalamus, hippocampus, pons-medulla, cerebellum, or thyroid gland. The ovaries and testes, pineal gland, and hypothalamus were heavier for animals that had received postnatal TRH (n = 14; $p<0.05$ in all cases). The adrenal glands of rats in the TRH group weighed less (n = 14; $p<0.05$).

DISCUSSION

Rats receiving TRH postnatally and tested shortly after weaning ran more rapidly and efficiently for a food reward than control animals. While experimental rats showed no superiority in terms of error scores early in training, their

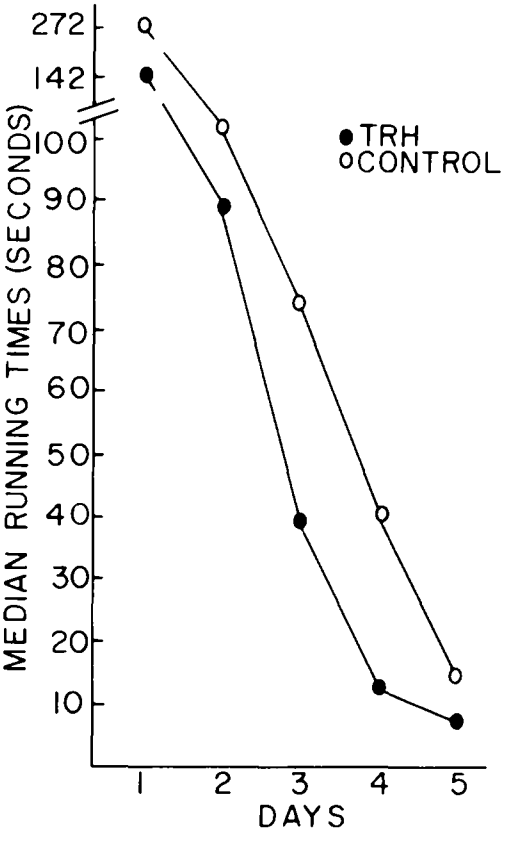

FIG. 1. Median daily running speed in an eight cul de sac Warden maze for rats that received TRH during the neonatal period and for controls.

time scores were superior beginning on the first day. Thus differences between groups were most discernable when comparing temporal measures suggesting that performance, rather than learning, was most affected by the early treatment. In a related study, α-melanocyte stimulating hormone (MSH) and an MSH release inhibitory factor (MIF-I) had a similar beneficial effect upon running scores in a complex maze when injected before acquisition trials [13]. Increased motivation or attention, and decreased anxiety have been considered as performance variables that could explain the beneficial results of MSH and fractions of ACTH [6].

Animals receiving TRH were more active during all phases of open field testing; at the same time they were also less emotional in terms of number of defecations and wall clinging behavior. It is plausible to assume that the greater activity of experimental rats in novel and open areas reflected reduced fear of testing situations. In the maze task reduced emotionality might discourage retracing of alleys and cause less freezing and displacement activity in the alleys, thus improving time scores. The results indicate that early treatment with TRH provides some protection against negative emotional reactions to novel environments both before and after rats reach maturity. The finding that experimental animals had smaller adrenals is consistant with the idea that they experienced less stress during the time period prior to 75 days of age when they were sacrificed.

Hormones such as MSH and the fragment MSH/ACTH 4-10 which are commonly associated with stress or adaptation seemed to have paradoxical fear reducing characteristics in the following studies. When placed into tonic immobility (death feign), lizards injected with MSH or MSH/ACTH 4-10 recovered sooner than those receiving corticosteroids or saline [14]. Rats receiving MSH appeared less fearful of a light previously associated with foot-shock. This so called conditioned-emotional response was attenuated in experimental animals in as much as they continued to press a lever for food while the fear-related light was on (unpublished data). TRH, MSH, and MSH/ACTH 4-10 all seem to reduce measures of emotionality although MIF-I had no effect in one study [13]. The data from studies with human subjects [7,12] provided evidence of a reduction in state anxiety following injections of MSH/ACTH 4-10.

In the present study the effects of TRH appeared dissimilar to those found in neonatal studies with thyroxine. Recently, Bakke, Lawrence, Bennett and Robinson [1] found that neonatal treatment with thyroxine reduced the concentration of serum TRH reaching the pituitary. They suggested that abnormalities found in adulthood might result from impaired secretion of TRH. Retardation of teeth eruption and poorer swimming scores occurred on the sixth and the last day of TRH injection; activity scores were enhanced during the same period. This could be due to the fact that development of peripheral structures such as teeth and reflexes, influenced by thryoxine, may be functionally separable from direct effects of TRH on the brain. Separation of central and peripheral effects of TRH has been demonstrated by Plotnikoff, Prange, Breese and Anderson [10] who showed that TRH potentiated DOPA equally well in thyroidectomized or hypophysectomized rats and controls. In another study TRH potentiated the effects of the antidepressant drug, imipramine [9]. The present study using rats indicated that early treatment with TRH reduced emotionality and facilitated learning of a complex maze. One study reported that normal human females treated with TRH experienced reduced tension and increased mental clarity [16]. Confirmation of the apparent similarity of findings in rats and human beings together with related effects on brain psychological processes would be encouraging.

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