



Varied duration of congenital hypothyroidism potentiates perseveration in a response alternation discrimination task

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Abstract

The behavior of five groups of rats (seven rats per group) made hypothyroid for varying lengths of time and one group of seven normal control rats was assessed under forced alternation fixed-ratio (FR1, FR3, FR5 and FR10), alternating lever cyclic-ratio (ALCR) and progressive-ratio (PR3) schedules of reinforcement. Hypothyroidism was produced by adding methimazole (MMI) to the drinking water of pregnant dams from embryonic day E16 to postnatal day P25. Four groups were given replacement thyroxine (T4) injections beginning at specific time points (P1, P7, P13, and P19). There were no differences in behavioral performance between control and experimental groups under the FR schedule, which indicates that the animals' sensorimotor abilities were intact. Under the forced ALCR schedule, all groups reached criteria similarly. However, under the choice lever ALCR schedule, control animals and those which received T4 replacement from early on (P1, P7, P13 groups) performed well and all had reached criteria by 11 sessions. In contrast, animals which did not receive any T4 replacement or received it late (P19 group) took longer to reach criteria and 5/14 animals had not reached criteria at all by 20 sessions. This deterioration in performance was paralleled by an increase in perseverative behavior as evidenced by an increased frequency of pressing the wrong lever when alternation of lever was required. This suggests that congenital hypothyroidism results in increased perseveration leading to a decrease in learning when a discrimination between correct and incorrect operanda is made available. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Congenital hypothyroidism in humans results in significant deficits in mental abilities, neurological functions, and metabolic processes (Glorieux and LaVecchio, 1983; Stein et al., 1991; DeLong, 1993; Goldey et al., 1995). It has been proposed that individ-

uals affected by congenital hypothyroidism can be loosely divided into two groups (Boyages, 1993; Boyages and Halpern, 1993; DeLong, 1993): those presenting with neurological features such as mental retardation, motor impairments and deafness as a result of prenatal hypothyroidism, and those presenting with altered metabolism as a result of postnatal hypothyroidism (Hetzl et al., 1988; Fuggle et al., 1991; Kooistra et al., 1994; Sher et al., 1998).

The fact that thyroid hormone influences brain development postnatally in the rat makes it an attractive

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animal model for the human condition of congenital hypothyroidism since experimental manipulations can be conducted during approximately the first three postnatal weeks without interventions in utero. Congenital hypothyroidism in rats is most typically produced through the administration of an antithyroid drug such as propyl-thiouracil or methimazole (MMI) (Cooper, 1984; Cooper et al., 1984) during the perinatal period, since rodent dendrite maturation and synaptogenesis occur primarily during postnatal development (Jacobson, 1991). MMI is a potent reversible antithyroid drug which acts by inhibiting the incorporation of iodine into the thyroid hormone precursor protein thyroglobulin (Cooper, 1984).

In a recent study (Mac Nabb et al., Brain Res., in press) detrimental effects of unopposed MMI administration throughout the first 25 postnatal days on alternating lever cyclic-ratio (ALCR) behavior were detrimental. In the present study, MMI was administered to dams of the experimental groups, along with thyroxine (T4) replacement in several groups beginning at different postnatal days to investigate the effects of perinatal hypothyroidism on certain behavioral tasks. Specifically, one was interested to determine the effect of T4 replacement therapy on performance, and, if so, at which day of initiation. For that purpose, the alternating cyclic-ratio schedule was used in both a forced lever and choice lever configuration. The alternating cyclic-ratio schedule has been previously utilized to determine the effects of low doses of atropine sulfate (Weldon et al., 1996), and to evaluate subtle behavioral changes following injection of aggregated β -amyloid into rat hippocampus (O'Hare et al., 1999). This behavioral task provided the opportunity to assess perseverative behavior in various groups of animals, and, possibly, associate it with performance.

2. Materials and methods

2.1. Animals

Experimentally naive Wistar rats were used. The parents consisted of two males and six females (Harlan: Madison, WI). The six dams were assigned randomly to the following six different treatments, one dam per treatment: (1) control (no treatment); (2) treatment with MMI without T4 replacement; (3) treatment with MMI and T4 replacement starting at P1; (4) treatment with MMI and T4 replacement starting at P7; (5) treatment with MMI and T4 replacement starting at P13; and (6) treatment with MMI and T4 replacement starting at P19. For the MMI-treated groups, MMI (0.025%; Sigma, St. Louis, MO) was added to the drinking water of the dams beginning at E16 and ending at P25; this way, each dam received approximately 7.5 μ g MMI

daily. T4 replacement (0.02 μ g/g BW) was administered to the pups via daily injections subcutaneously. The antithyroid effects of MMI were produced prenatally via the placenta which MMI crosses, and postnatally through the milk to which MMI is distributed (Weller et al., 1996). This protocol and dosage of MMI administration is typically used for the production of congenitally hypothyroid rats (Comer and Norton, 1982; Rice and Millan, 1986; Sack et al., 1995).

Seven male pups per dam were selected randomly from each litter. Therefore, 42 rats were made available for behavioral testing which began when they were 100–120 days old. At the onset of behavioral training rats were maintained at 85% of free-feeding body weights and housed individually with water continuously available in the home cage. The temperature in the vivarium was maintained at 23°C under a 12 h light/12 h dark cycle (lights on at 07:00 h).

2.2. Apparatus

Six two-lever Med Associates rat test chambers (model ENV-007, Med Associates, Georgia, VT), enclosed in sound attenuating compartments, were employed. The reinforcer was one 45 mg food pellet (F0021, Bioserv, Frenchtown, NJ), which was delivered into a tray situated midway between the levers. A Med Associates computer, programmed in MED-PC computer language, controlled the experiment and collected data.

2.3. Behavioral training

Sessions were conducted daily between the third and ninth hour of the 12-h light cycle, and the daily allocation of food was given approximately 15 min after the session. Animals were initially trained to respond alternately between both levers under continuous reinforcement, i.e. one response per reinforcer (fixed-ratio 1 or FR1), with the active lever being extended into the chamber and the inactive lever being retracted. The criterion for moving through successive stages of training was the delivery of 100 reinforcers in 20 min. In this way, animals were trained under FR1, FR3, FR5 and FR10 (ten responses per reinforcement).

When they successfully completed 3 consecutive days of obtaining 100 reinforcers under FR10, training under an arithmetic ALCR was begun. This schedule comprised an ascending, followed by a descending, sequence cycle of FR response requirements (3, 6, 9, 12, 15, 18, 21, 21, 18, 15, 12, 9, 6, 3) for each reinforcer. The animals were required to switch response levers after each FR was completed. Cycles of increasing, then decreasing, response requirements were presented six times during each session. Initially, all cycles were forced, meaning that only the active lever was extended

whereas the inactive lever was retracted. To reach criteria, animals were required to finish all six cycles of the ALCR within a 40-min period of time for ten consecutive sessions. Thereafter, the animals were trained for 20 sessions on a choice lever ALCR schedule in which both levers were extended and the animal had to choose the correct lever pressing pattern in order to receive reward within a 40 min time span. Data for each session were collected at the end of 40 min or at the completion of all six cycles.

When testing finished under the ALCR schedules, animals were trained on a progressive-ratio 3 schedule (PR3) for 2 days before moving to a PR5 schedule to assess their motivation for food reinforcement (Hodos, 1961; Hodos and Kalman, 1963; Jones et al., 1995). In this schedule, only the left lever was extended and the third (or fifth) lever press was reinforced. The number of responses required to obtain a reinforcer was increased by three (PR3) or five (PR5) lever presses for each successive reinforcer. The session continued until 5 min elapsed without reinforcement, or the animal spent 3 h in the chamber. The number of responses emitted to get the last reinforcer was considered the break point, representing the maximum amount of work a subject would expend for one 45 mg reinforcer. Data were collected at the end of each session, and all of the animals completed 20 sessions on the PR5 schedule. The performance of each animal was noted and a session average for each group was calculated from these individual performances. The grand average of the performance during the last five sessions in each group was calculated and used as a covariate in the Cox proportional hazards model (see below).

2.4. Assessment of thyroid status

At the completion of behavioral testing, triiodothy-

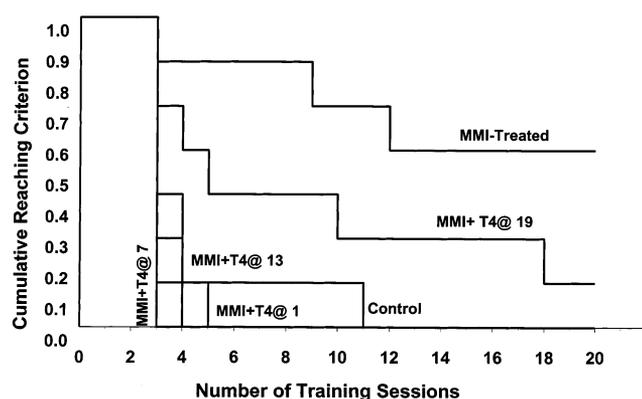


Fig. 1. Survival plot analysis of hypothyroid and control groups success in reaching criteria on the choice alternating cyclic-ratio schedule.

ronine (T3) levels were assayed. Serum was collected from each rat and assayed by the Minnesota Diagnostic Laboratory (St. Paul, MN) using a Coat-a-Count total T3 radioimmunoassay kit (Diagnostic Products, Los Angeles, CA).

2.5. Data analysis

Standard display and statistical methods were used to plot and analyze the data (Snedecor and Cochran, 1989). In addition, because animals were trained to specific criteria, the effect of treatment on performance was analyzed using survival analysis (Kalbfleisch and Prentice, 1980; Cox and Oakes, 1984). The effects of covariates (i.e. perseveration and motivation) were assessed using Cox's proportional hazard model (Cox, 1972). The programs 1L and 2L of the commercially available statistical package BMDP/Dynamic (BMDP Statistical Software, Los Angeles, CA, 1992) were used for this analysis.

3. Results

3.1. General

At the beginning of the behavioral testing period, the weight (mean \pm S.D.) of the MMI group was 354 ± 34.9 g, MMI+T4 at P19 was 359 ± 15.9 g, MMI+T4 at P13 was 388 ± 16.2 g, MMI+T4 at P7 was 376 ± 26.9 g, MMI+T4 at P1 was 392 ± 28.3 g, and the control group was 507 ± 21.1 g. All groups were maintained throughout the behavioral testing at 85% of their original adult body weight.

3.2. Behavior

Under the forced ALCR schedule, when only one lever was available to press at a given time, all of the animals in each group completed the schedule within the allotted time for ten consecutive sessions.

However, when the animals were trained on the choice ALCR schedule, differences were observed among the treatment groups. As shown in Fig. 1, there was a gradation in performance of the groups to reach criteria, with the groups having been hypothyroid for the least amount of time completing the criteria in fewer sessions than the groups which were hypothyroid for longer periods of time. There was also an obvious grouping consisting of the control, MMI+T4 at P1, P7, and P13 groups and of the MMI+T4 at P19 and the MMI groups. The difference in performance between these two groups was statistically highly significant ($P = 0.0007$, Mantel-Cox test, survival analysis).

Perseverative Behavior- Group Averages

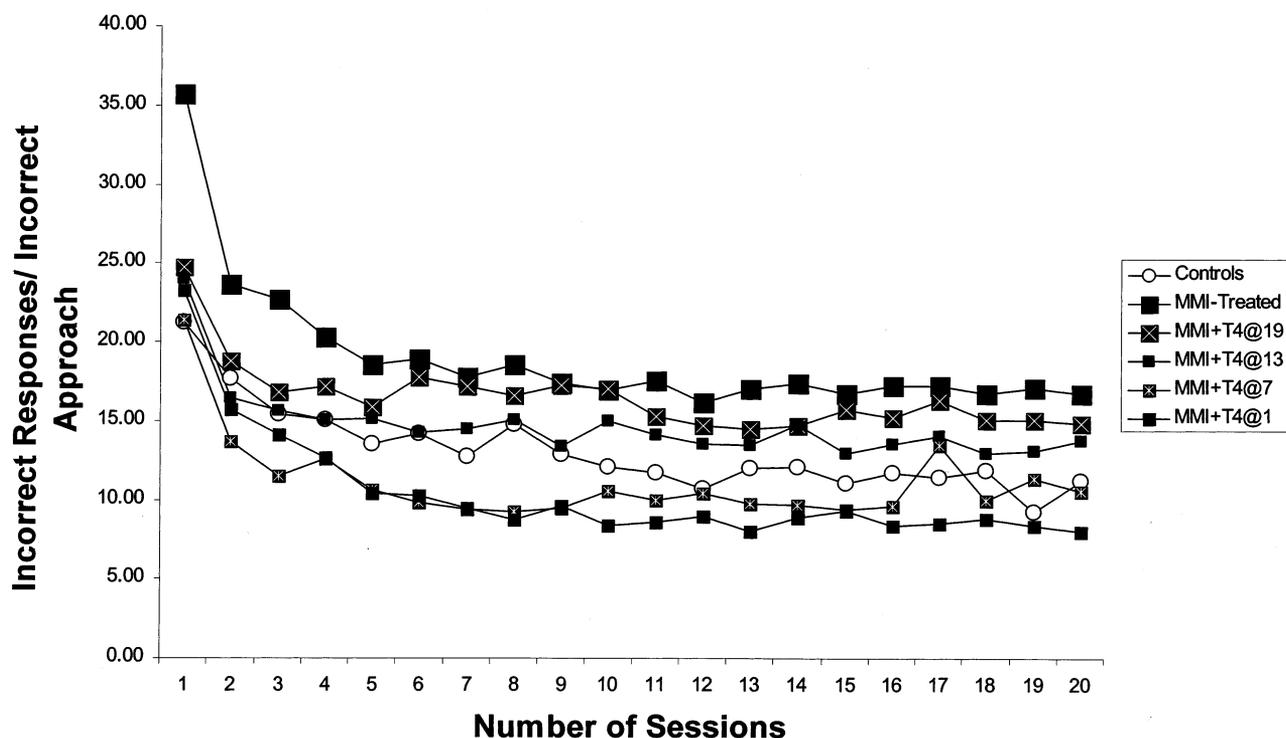


Fig. 2. Linear plot of the average group perseverations as a function of the session. For each session, averages of a given group's number of incorrect responses were divided by the number of incorrect approaches and plotted.

Group averaged data indicated a relationship between perseverations and the length of hypothyroidism, as shown in Fig. 2. Animals hypothyroid for longer periods also made more errors in lever pressing than animals hypothyroid for shorter durations. The amount of perseverative behavior was a significant covariate in the differences seen in the choice ALCR schedule survival plot ($P=0.0017$, Cox proportional hazards model). A strong linear correlation between perseverance and day at which T4 treatment began is shown in Fig. 3.

Finally, there were no significant differences in performance during any of the FR training schedules (Mantel–Cox test, data not shown). Also, there were no significant differences among the two groups with respect to the PR schedule (motivation; see Section 2 above) used as a covariate in the survival analysis above (Cox proportional hazards model).

3.3. Thyroid status

Thyroid status was assessed by calculating the ratio of whole T3/BW. None of the MMI-treated groups (with or without T4 replacement therapy) were hypothyroid, as compared to the control group.

4. Discussion

Previous studies of congenital hypothyroidism in the rat have focused on spatial recognition, navigation, or motor tasks. In general, these studies have documented a decrease in the ability of perinatally hypothyroid animals to learn and habituate to maze tests, and an increase in spontaneous activity (Morgan and Eimon, 1976; Comer and Norton, 1982, 1985; Tamasy et al., 1986; Rice et al., 1987; Akaike et al., 1991; Weller et al., 1996). This increase in spontaneous activity in transiently hypothyroid rats, as compared to control animals, may be due to the fact that control animals habituate to their test surroundings after a few trials, while hypothyroid rats maintain the same level of exploratory and spontaneous movement over time (Comer and Norton, 1985).

Previous experiments involving lever pressing tasks have focused either on adult onset hypothyroidism (Fundaro et al., 1985; Rial et al., 1987; Fundaro, 1989) or on simple FR schedules (Davenport and Hennies, 1976; Schalock et al., 1979) in which either no differences were found between groups (Schalock et al., 1979), or hyperactivity was indicated in the perinatally hypothyroid group (Davenport and Hennies, 1976).

In the present study, operant behavior was investigated based on memory-type functions of ‘choice’ or switching rules. Throughout a series of FR schedules (FR1, FR3, FR5 and FR10) the performance of the any of the hypothyroid groups did not differ significantly from that of the control group. This indicated that there was no motor impairment and that all animals could learn to press the available lever. Additionally, all groups performed similarly under the forced ALCR schedule. A common feature of both schedules (FR and forced ALCR) is that there is no choice in pressing the lever as only one lever is presented at a time. Now, under the choice ALCR schedule, significant differences were observed among groups, both in terms of the animals’ ability to reach criteria, and in the amount of their perseveration. The duration of the hypothyroid state directly influenced both the number of sessions taken to reach criteria in the choice ALCR schedule, and the number of perseverations by a given group.

The results of this study suggest that congenital hypothyroidism does not affect motor activity or motivation. Similarly, circulating T3 levels, as assessed by serum T3 assay, were also unaffected. However, perinatal hypothyroidism did affect learning. Successful performance on the ALCR schedule at minimum requires the motor ability to press, the motivation to press, and the ability to remember/know to switch levers after receiving a reward. The perinatally hypothyroid rats in this study had normal motor abilities and motivation levels, but they were severely impaired in the execution of the ALCR schedule.

Thyroid hormone has been associated with the regulation of neural differentiation processes such as dendritic arborization, synaptogenesis and myelination (Ipina and Ruiz-Marcos, 1986; Ipina et al., 1987; Rodriguez-Pena et al., 1993; Oppenheimer and Schwartz, 1997). Earlier work in humans and in rats has indicated that there is a critical window during which replacement thyroxine can ameliorate the damage resulting from hypothyroidism during brain development (DeLong, 1993; Kooistra et al., 1994; Weller et al., 1996). In humans, this window has been roughly localized to the third trimester of gestation and continuing in to the early postnatal period (Raiti and Newns, 1971; DeLong, 1993; Kooistra et al., 1994). In the rat, the sensitive period has been defined from shortly prior to birth until the end of the third week postnatal (Morreale de Escobar and Escobar del Rey, 1983; Nathaniel et al., 1988; Weller et al., 1996; Oppenheimer and Schwartz, 1997), which coincides with the period of neuronal arborization and maturation in the cerebral cortex (Morreale de Escobar and Escobar del Rey, 1983; Oppenheimer and Schwartz, 1997). The results concur and localize the critical time to occur between P13 and P19. During this window, the animals cease to benefit substantially by T4 replacement treatment with reference to the choice behavioral task. It is interesting that a similar window was identified in a study of the number of spines in pyramidal cells in the visual cortex of rats thyroidectomized at P10: if the rats were given T4 replacement beginning at P12, spines developed normally, whereas if the T4 replacement was started at P20, pyramidal spines developed abnormally as as-

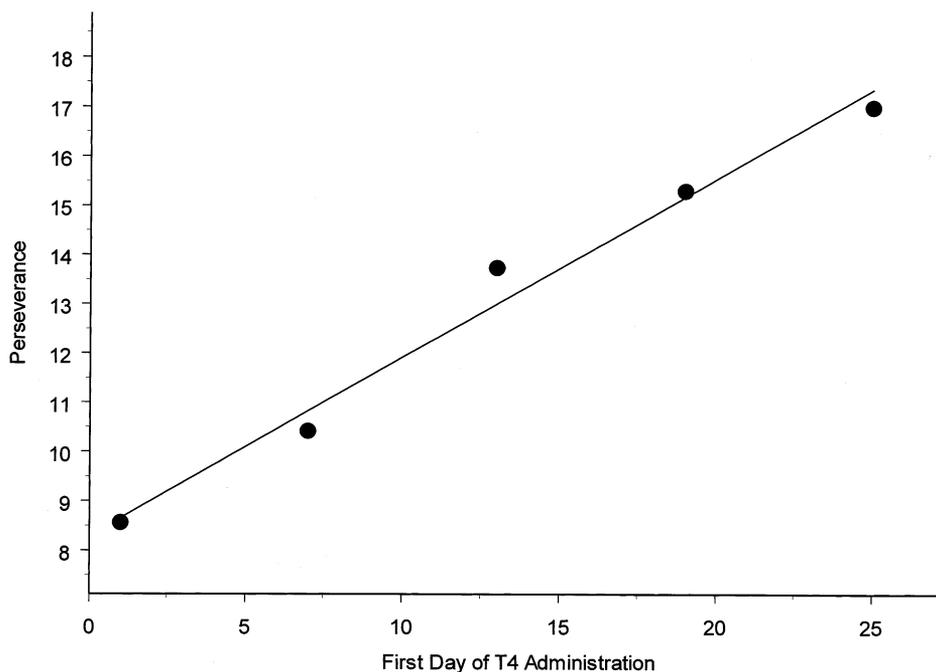


Fig. 3. Plot of the amount of perseverance as a function of day when thyroxine (T4) replacement was initiated.

sessed both from the number and their distribution along the apical dendritic shafts (Ruiz-Marcos et al., 1979).

Finally, the cortical areas the damage of which might be reflected in the results obtained could be hypothesized on. First, it is reasonable to assume that the damage is widespread in the cortex, as it has been found in areas other than the visual cortex (Calikoglu et al., 1996). Second, the task used in this study involved choice between two alternatives. Now, it is a landmark deficit of frontal lobe lesions that human subjects tend to persevere when the reward rule changes in a choice task (Grant and Berg, 1948; Owen et al., 1993). A similar deficit has been identified in monkeys with lesions of specific regions of the prefrontal cortex located around the principal sulcus. Such lesions, as well as lesions in the basal ganglia, result in deficits in delayed response and delayed alternation tasks (Divac, 1971, 1972; Fuster, 1981). Since the MMI-treated rats in this study also perseverated in the ALCR choice task, it was hypothesized that their deficit might similarly reflect frontal lobe damage. Of course, additional experiments using morphological, biochemical, and other approaches are needed to test this hypothesis more completely.

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