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Congenital hypothyroidism impairs response alternation discrimination behavior

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Abstract

The behavior of six congenitally hypothyroid and six normal control rats was assessed under forced alternation fixed-ratio, alternating lever cyclic-ratio (ALCR) and progressive-ratio schedules of reinforcement. Hypothyroidism was produced by adding methimazole (MMI) to the drinking water of pregnant dams from embryonic day 16 to postnatal day 25. There were no differences in behavioral performance between MMI-treated and control animals under the fixed-ratio and progressive ratio schedules. There were also no differences in circulating triiodothyronine levels between groups at the end of the study. Under the ALCR schedule, when alternation of responding was forced during the first three cycles but both levers (choice) were presented during the last three cycles (correct lever active), the entire control group reached a competency criteria in nine sessions. In contrast, only two MMI-treated animals reached criteria after 17 sessions, and the remaining four MMI-treated animals did not reach criteria by 30 sessions of training. These results suggest that congenital hypothyroidism impairs learning when a discrimination between correct and incorrect operanda is made available.

Keywords: Behavior; Thyroid hormone; Learning; Congenital hypothyroidism

1. Introduction

Hypothyroidism during human perinatal development results in profound alterations of mental capacities, neurological functions, and metabolic processes [24,29,60]. Mental retardation, motor deficits, deafness, lethargy, and slowed metabolism are all characteristic in subjects affected by congenital hypothyroidism [20,24,29,39,59,60]. It has been proposed that neurological features are affected by hypothyroidism during the second trimester of pregnancy, whereas postnatal hypothyroidism affects primarily metabolic pathways [5,6,15].

Perinatal lack of thyroid hormone action has been used to produce animal models of congenital hypothyroidism [4,48,49,53]. In the rat, the basic cortical and cerebellar maps and cell numbers are primarily established during embryogenesis but the elaboration of dendrites, formation of synapses, and refinement of cellular processes occur during postnatal development [34]. Most commonly, the hypothyroid state has been induced by administering antithyroid drugs such as propyl-thiouracil or methimazole (MMI) [10]. MMI is a potent antithyroid drug which acts by inhibiting the incorporation of iodide into thyroglobin, the thyroid hormone precursor protein [10]. However, since MMI administration blocks the biosynthesis of thyroid hormone but leaves the thyroid gland intact, the hypothyroidism induced is reversible by removal of the drug [11].

In the present study, MMI was administered to the dams of the experimental animals in order to investigate the effects of perinatal hypothyroidism on certain aspects of behavior in the rat. The behavioral procedure used was

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a modification of the cyclic-ratio schedule [18,19]. This modification has been previously utilized by Weldon et al. [63] to determine the effects of low doses of atropine sulfate, and by O'Hare et al. [46] to evaluate subtle behavioral changes following injection of aggregated β-amyloid into rat hippocampus.

2. Materials and methods

2.1. Animals

Twelve experimentally naive male Wistar rats (Harlan: Madison, WI) were used. The animals were 202–224 days old at the beginning of the experiment. The dams of six rats (experimental group) were treated with MMI (Sigma: St. Louis, MO) which was added to their drinking water (0.025%) beginning on embryonic day E16 and continued until postnatal day P25. The dams of the other six animals (control group) did not receive any treatment. 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 0700 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 seventh and ninth hour of the 12-h light cycle, and the daily allocation

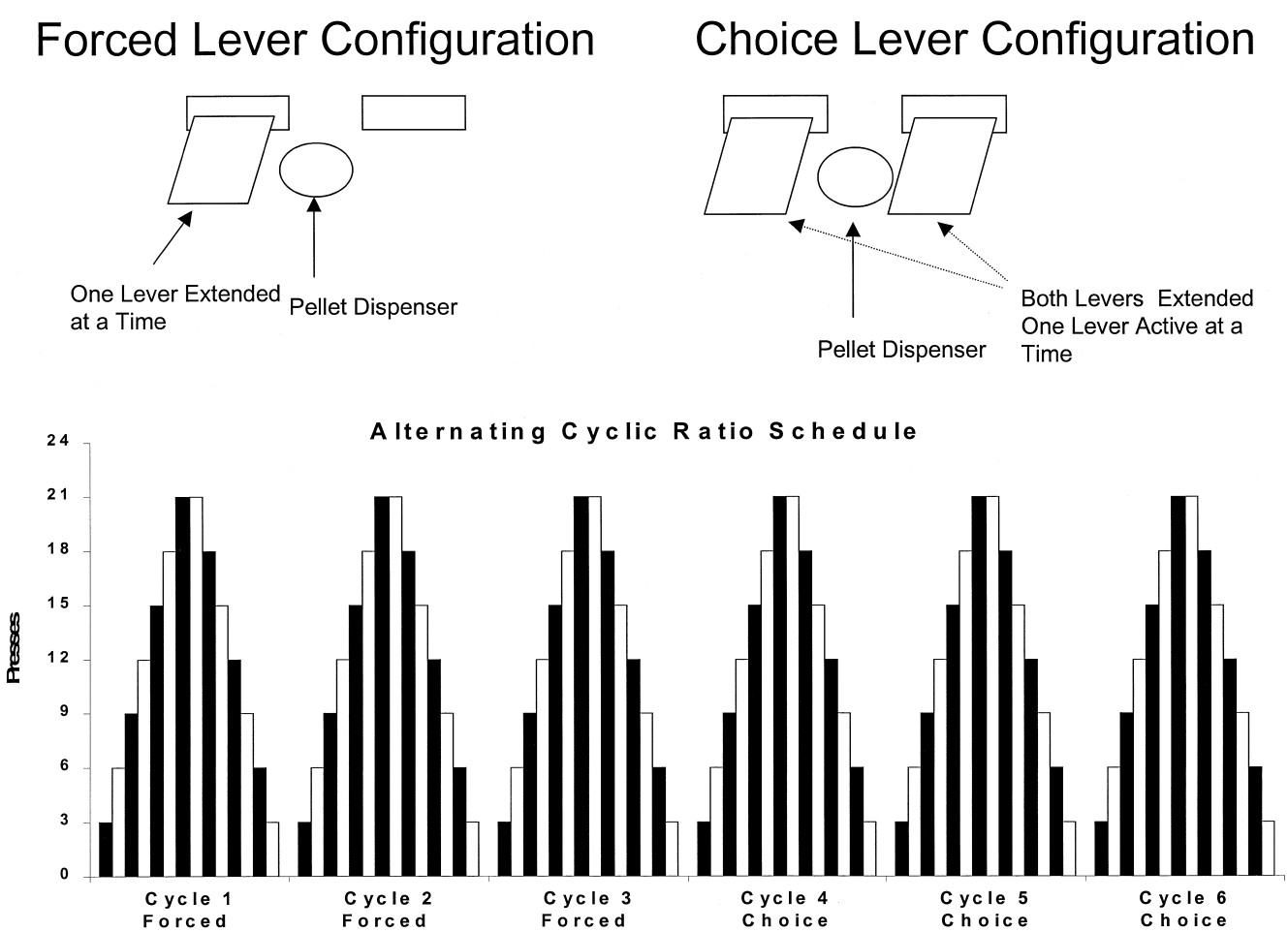


Fig. 1. Lever arrangement with the cyclic-ratio schedule. During forced cycles, only one lever is extended and active at a time. During choice cycles, both levers are extended continuously, but only one lever is active at a time. In the alternating cyclic ratio plot, black bars represent the left lever as active, and the white bars represent the right lever as active.

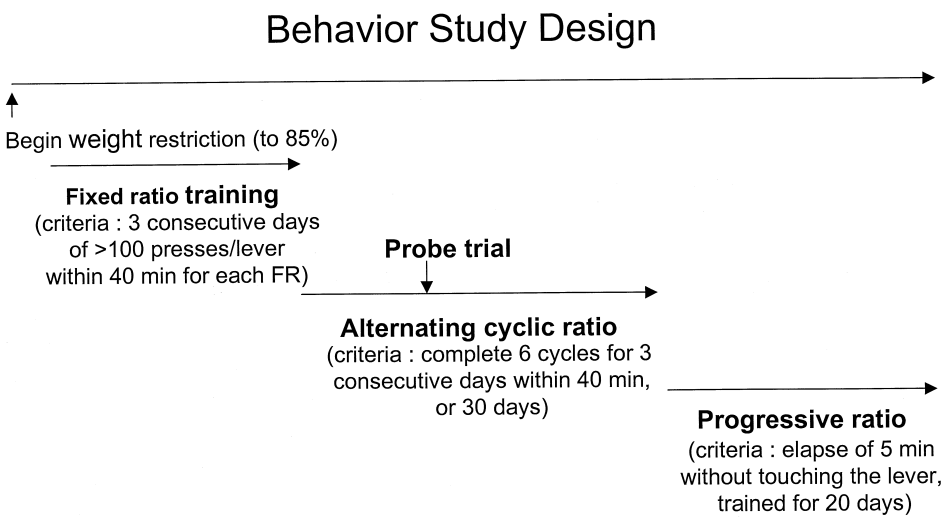


Fig. 2. Schematic of experimental design. Linear progression from beginning of weight restriction through completion of progressive ratio criteria shown.

of food was given approximately 30 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 FR-1), 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 40 min. In this way, animals were trained under FR-1, FR-3, FR-5 and FR-10 (10 responses per reinforcement). When they successfully completed three consecutive days of obtaining 100 reinforcers under FR-10, training under an arithmetic alternating lever cyclic-ratio schedule (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. During the first three cycles only the active lever was extended and the inactive lever was retracted, whereas during the last three cycles both levers were extended and the animal had to choose the correct lever (Fig. 1). Data were collected only for the last three cycles (choice cycles) of each session.

When testing finished under the ALCR, animals were tested using a progressive-ratio 3 schedule (PR-3) to assess their motivation for food [30,36]. In this schedule, only the left lever was extended and the third lever press was reinforced. The number of responses required to obtain a reinforcer was increased by three lever presses for each successive reinforcer. The session continued until 5 min elapsed without reinforcement. The number of responses emitted to get the last reinforcer was considered the break

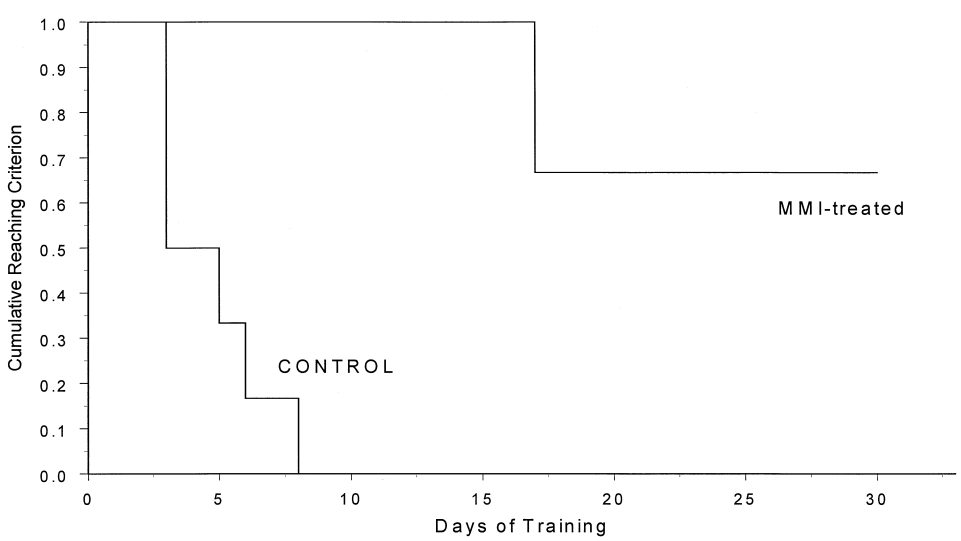


Fig. 3. Survival plot analysis. Control vs. MMI-treated group success in reaching alternating cyclic-ratio schedule criteria.

Table 1
Probe trial to assess motor ability
Comparison of MMI-treated versus control animal response rate as a function of cyclic ratio value. Mean response times increase for both groups with increasing ratio values. At the higher ratio values, the MMI-treated animals actually press faster than the controls, and significantly more so at the highest value. In all cases, $N = 6$ per group

Cyclic-ratio value	3	6	9	12	15	18	21
MMI-treated mean time	5.75	8.88	11.33	10.61	14.76	16.10	16.96
MMI-treated S.E.M.	0.66	1.41	2.51	0.80	2.63	2.15	2.75
Control mean time	9.38	10.06	14.88	13.50	11.30	21.50	20.30
Control S.E.M.	2.02	1.08	1.39	1.20	1.45	1.70	1.72
Probability	0.11	0.52	0.80	0.06	0.48	0.07	0.04

point, representing the maximum amount of work a subject would expend for one 45-mg reinforcer. A schematic of the experimental design is shown in Fig. 2.

2.4. Assessment of thyroid status

At the completion of behavioral testing, triiodothyronine (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 statistical methods were used to analyze the data. In addition, because animals were trained to specific criteria, the effect of treatment on performance was analyzed using survival analysis [13,37]. The effects of covariates (i.e., motivation and body weight) were assessed using Cox's proportional hazard model [12]. 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

The experimental group showed typical signs of congenital hypothyroidism [8,45,52,64] including reduced body weight and delayed eye opening. At the beginning of behavioral testing, the weight (mean ± S.E.M.) of the experimental group was 496 ± 8.7 g, as compared to 665 ± 21.4 g for the control group. Animals were maintained at 85% of original body weight throughout the testing period.

3.2. Behavior

Under the FR schedules, the mean number of sessions to reach criteria was 3.5 ± 1.2 sessions for the experimental group and 3.7 ± 1.0 sessions for the control group. No significant differences were found between the groups in terms of the number of sessions to reach criterion using survival analysis [13] (Generalized Savage, Mantel–Cox test, $P > 0.5$ for all FR schedules).

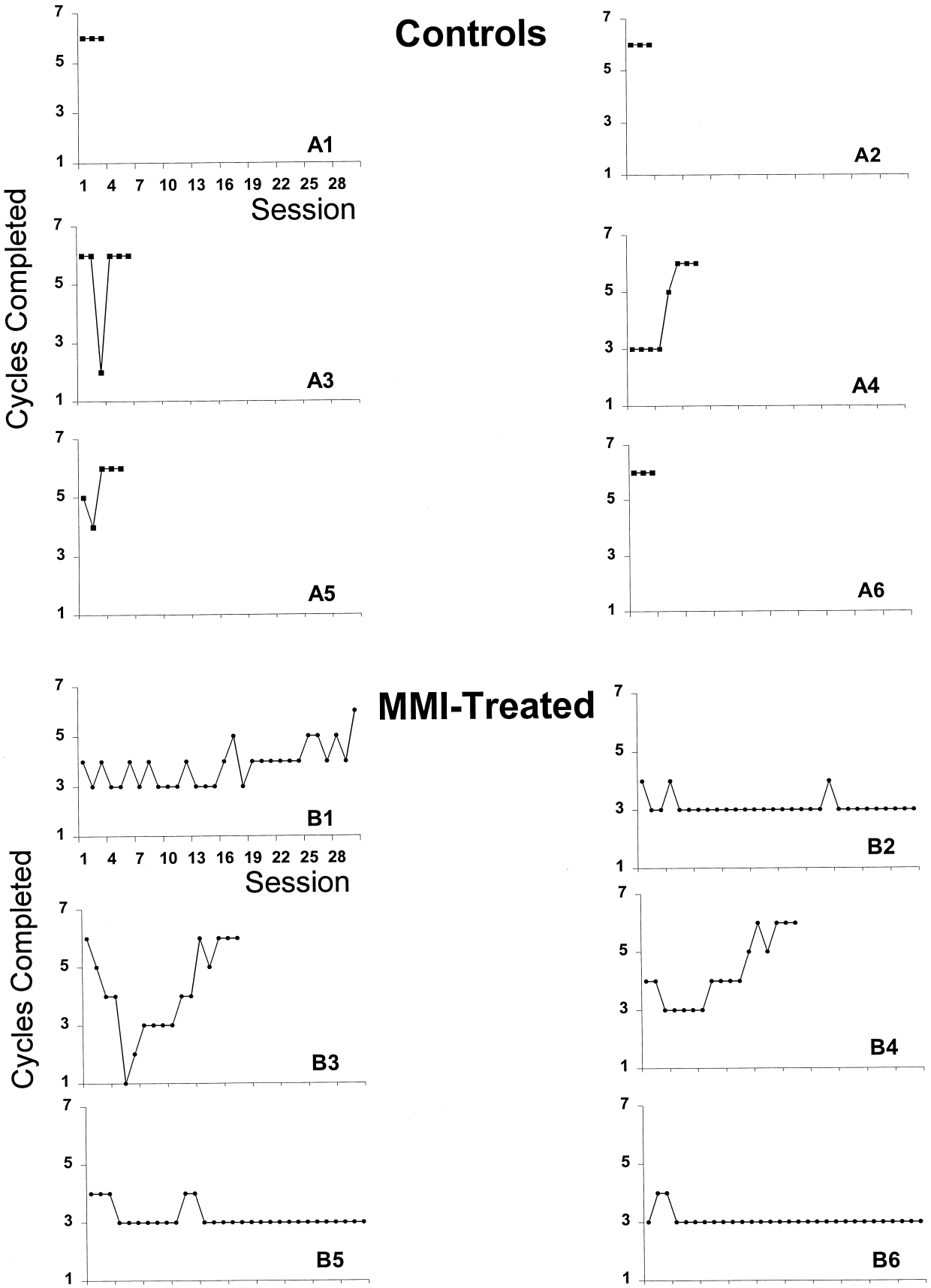
Under the ALCR schedule, the criterion for successful training was completion all six cycles of the cyclic-ratio schedule within 40 min. All animals in the control group reached criterion within nine sessions. In contrast, only two of the six rats in the experimental group reached criterion after 17 sessions, and the remaining four animals had not reach criterion even after 30 sessions (Fig. 3). The differences between control and experimental groups were statistically highly significant ($P = 0.0004$, Mantel–Cox test, survival analysis).

In order to test for potential motor impairments in the experimental group, a probe trial was conducted in which only the active lever was extended (and the inactive lever retracted) throughout all six cycles of the ALCR schedule. Under these conditions, all animals in the experimental group successfully completed the session, suggesting that the inability above to complete the schedule when both levers were extended during the last three cycles of the ALCR schedule was due to central rather than peripheral effects of congenital hypothyroidism. Mean times to complete each schedule component, S.E.M.s and probability levels (two-tailed *t*-test) are shown in Table 1.

It is noteworthy that the four MMI-treated animals which did not reach criterion, consistently responded during the first three cycles of the schedule, when only the active lever was extended into the chamber, indicating they were not motorically impaired. However, when required to alternate between levers with both levers were extended in the choice situation, half of the MMI-treated animals ceased responding (Fig. 4: B2, B5, B6). Animal B1 took 16 sessions to reach the sixth cycle, and only once successfully completed all the required cycles (Fig. 4). In contrast, all control animals reached criterion (top half, Fig. 4).

The motivation level of the animals was assessed using the PR schedule. In this schedule, the three performance variables included the break point, the total number of level presses, and the number of pellets delivered. The average values of these measures from the last six sessions were calculated for each animal, and their differences tested between groups. There were no significant differences between the groups (Mann–Whitney rank sum test,

Fig. 4. Plots of individual animal's performance on the alternating lever cyclic-ratio schedule. Control animals are listed as A1–A6, and MMI-treated animals are listed as B1–B6. Each plot represents the number of completed cycles in successive sessions. Sessions ended after 40 min or when six cycles were completed. After 3 days of completing all six cycles within 40 min, the animal was considered to have reached criterion and moved off the ALCR schedule.


Controls
MMI-Treated
Cycles Completed
Session
A1
A2
A3
A4
A5
A6
B1
B2
B3
B4
B5
B6
1 4 7 10 13 16 19 22 25 28
7
5
3
1

$P = 0.1$) on any of the performance measures. As a further assessment of motivation, the mean number of reinforcers obtained during the last three days of the PR schedule, and mean body weights during the last 3 days of the cyclic ratio schedule were determined. Neither of these factors, alone or in combination, was statistically significant ($P = 0.71$ for PR-3 reinforcers; $P = 0.1$ for weight; and $P = 0.21$ for both combined; Global chi-square test in Cox proportional hazards model).

3.3. Thyroid status

The T3 levels (mean $\pm$ S.E.M.) were 91.2 ± 8.6 and 97.6 ± 5.3 ng/dl for the control ($N = 5$) and the experimental ($N = 6$) rats, respectively, at 328–350 days of age. These values did not differ significantly (t-test, $P > 0.5$).

4. Discussion

In human subjects, congenital hypothyroidism causes profound deficits in cognitive abilities and neurological function, and it appears that thyroid hormone is crucial for brain development during the last trimester of pregnancy [38,54,56]. Various methods have been employed to produce this condition in a variety of animal species including rat, mouse lamb, goat, and marmoset [4,48,49,53]. In the rat, the methods used to induce hypothyroidism include feeding low iodine diet during pregnancy, administration of antithyroid drugs perinatally, and destruction of the thyroid gland surgically or by irradiation at, or shortly after, birth. The fact that thyroid hormone influences brain development postnatally in the rat makes it an attractive 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. In addition, rat behavior has been studied extensively and quantitatively during this century, and a wealth of information is available on the basis of which to evaluate the effects of potential brain damage. Therefore, we chose the rat as our experimental model of congenital hypothyroidism and assessed the presence and severity of behavioral deficits using lever pressing and operant conditioning techniques.

Previous studies have been focused on spatial recognition, navigation, or motor tasks. In general, these studies have documented a decrease in perinatally hypothyroid animals' ability to learn and habituate to maze tests, and an increase in spontaneous activity. Such assessments have included the righting reflex [8,52,64], exploratory behavior [44,58,61,62], home orientation [35], maze learning [1,9,28], and avoidance learning [27,57,58]. Motor activities which have been characterized in the perinatal hypothyroid rat include delays in onset of locomotor activities [35,51,52], and hyperactivity, as assayed by open field exploration [1,27], enclosed automatic motion detectors [9,25,28], and running wheel performance [14,62]. The increase in locomotor 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 [9]. Finally, other experiments involving lever-pressing tasks have been focused either on adult onset hypothyroidism [21,22,50], or on simple FR schedules [14,58]. Schalock et al. [58] found no significant differences between control and perinatally hypothyroid rats, whereas Davenport and Hennies [14] reported hyperactivity and reduced fearfulness in perinatally hypothyroid rats using FR schedules.

In the present study, we investigated operant behavior based on memory-type functions of ''choice'' or switching rules. Throughout a series of FR schedules (FR-1, FR-3, FR-5 and FR-10) the performance of the hypothyroid animals did not differ significantly from that of control animals. Under these schedules, all rats were required to alternate responding from left to right lever, during forced responding conditions; that is, when only the active lever was extended into the operant test chamber. The predetermined criteria for advancing from one FR schedule to the next was met by both the control and the experimental groups, with no statistically significant differences between the two. However, the situation was very different under the ALCR schedule in which responding during the first three cycles was forced and responding during the last three cycles was open to choice between the correct (active) and incorrect (inactive) lever. In this case, the MMI-treated animals were severely impaired.

It is possible that this deficit in performance could be due to a motor defect in lever pressing and/or a reduced motivation to work for a reward. However, separate assessments of these two factors showed that the experimental animals did not differ from the control ones in either of these aspects. Specifically, the motor ability was tested in a probe trial in which responding during all six cycles of the ALCR schedule was forced (i.e., without choice) instead of having three cycles of two-lever choice. The MMI-treated animals had no difficulties with this task and all of them successfully completed the session. In fact, it can be seen in Table 1 that these rats generally completed individual schedule components at a higher rate than the control group and responded at a significantly higher rate during the highest schedule component. Finally, possible motivational differences between the MMI-treated and control groups were investigated by testing both groups under a PR-3 schedule. This schedule requires an ever increasing number of presses for each successive reinforcer delivered. The break point is the number of presses required for the next reinforcer which exceeds the value the animal will press for a reinforcer. This number is considered to be a measure of reinforcing value or motivational strength of the food reinforcer [30,31,36]. Under the

PR schedule there were no significant differences between the groups.

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. The experimental group demonstrated difficulty in acquiring lever switching behavior when both levers were made available under the ALCR schedule. It would appear that when an MMI-treated animal made a lever switching error, the animals perseverated on the incorrect lever until lever pressing extinguished. 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.

Traditionally, deficits of ''executive functions'' affecting choice behavior in human subjects have been associated with frontal cortex lesions. A classic task used to assess frontal lobe damage is the Wisconsin card sorting task in which subjects are rewarded for choosing certain cards based on criteria (like a color or a shape) and a rule for reward changes (i.e., from green to square) [26,47]. The key feature of this task is that the reward rule changes between blocks of trials. Subjects with frontal lobe damage typically perseverate and do not apply the new rule. 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 [16,17,23]. The results of the present study point to a qualitatively similar inability of the congenitally hypothyroid rats to adopt the new rule when given a choice. Therefore, damage of the prefrontal cortex and/or the basal ganglia may underlie the performance deficits. In fact in the rat, both the basal ganglia and the cerebral cortex have been shown to be affected by perinatal hypothyroidism. Changes in the caudate as a result of early postnatal hypothyroidism in the rat have included delays in neuronal proliferation, inhibition of dendritic arborization, and reduction of dendritic spines [41,42]. While a developmental growth spurt was seen between postnatal days 14–30, it was not enough to return hypothyroid animals to control levels of these parameters, and similar changes are found in the cerebral cortex. In rats made hypothyroid at postnatal day 10 by thyroidectomy and examined at postnatal day 80, it has been shown that the number of spines [2], apical dendrite density [32,55], and basal dendritic arborization [33] of layer 3 pyramidal cells from the visual cortex were significantly reduced as compared to control animals. In perinatal hypothyroid rats, the total volume of the prelimbic area of the medial prefrontal cortex was significantly reduced while there were no differences in the numbers of layer 3 pyramidal cells as compared to controls [43]. Finally, a developmental study of the effect of perinatal hypothyroidism on somatic sensory cortex has found no changes in thalamocortical topography or somatotopy, but that there were changes in dimensions of the barrel fields and postero-medial barrel subfields [7]. We, along with others, suggest that perhaps the main effect of congenital hypothyroidism on neural development is to reduce the amount of time in which the nervous system is exposed to agents coordinating developmental processes [3,7,40]. In this manner, arborization and synaptogenesis would be primarily affected because there would not be enough time to complete the process of normal development. In other words, the developmental ''window'' is open for a shorter time, but the brain template is not adjusted, so some things do not get the amount of time needed to develop normally.

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