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Effects of dysthyroidism in plus maze and social interaction tests

Josefina Sala-Roca[a,*], Maria Assumpció Martí-Carbonell[b], Adriana Garau[c], Sonia Darbra[b], Ferran Balada[b]

[a]*Department of Systematic and Social Pedagogy, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalonia, Barcelona, Spain*
[b]*Department of Psychobiology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalonia, Barcelona, Spain*
[c]*Department of Basic Psychology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalonia, Barcelona, Spain*

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Abstract

The aim of the present study was to determine the influence of thyroid hormones on the anxiety of male Wistar rats. Dysthyroidism was induced by adding 20 mg of methimazole (100 ml) to their drinking water or by adding 0.3 mg of L-thyroxine (100 ml) to their drinking water from the ninth day of gestation. After weaning, the drugs were administered to young rats until the end of the experiment. Anxious behavior was measured using the elevated plus maze and social interaction tests when the animals were 85 days old. Chronic methimazole administration produced a significant anxiolytic pattern in both tests. In the plus maze test, the methimazole-treated animals entered and remained more time in the open arms than the control animals. In the social interaction test, they spent more time in bodily contact, and did this more frequently than those in the control group did. Results from this experiment suggest that chronic thyroid deficiency produces an anxiolytic-like effect in both tests. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Hypothyroidism; Hyperthyroidism; Anxiety; Plus maze test; Social interaction test; Rat

1. Introduction

It has been described that two-thirds of patients with thyroid disease have psychiatric disorders. The most common disorders are panic attacks, anxiety, depression, phobias and obsessive compulsion (Placidi et al., 1998). Dysthyroidism has been also related to alcohol and other types of drug abuse and antisocial behavior (Alm et al., 1996; Stalenheim et al., 1998) and to traumatic stress (Newport and Nemeroff, 2000; Prange, 1999; Wang and Mason, 1999). In the last decades, different studies have analyzed the relationship between depression and hypothyroidism (for a review, see Baumgartner, 1993; Kirkegaard and Faber, 1998), whereas there have been far fewer studies of the relationship between hyperthyroidism and anxiety and stress, even though anxiety is a characteristic symptom of hyperthyroidism (Stern et al., 1996).

Studies of animals play a key role in the investigation of the mechanisms involved in normal behavior and pathology. The success of this approach depends on the extent to which the findings derived from animal models can be generalized to a clinical situation and also on the reliability and sensitivity of the methods used (Spear and File, 1996). Few investigations have used animal models to investigate the role of thyroid hormones in anxiety and depression. Several tests can be used to assess anxiety or anxious behavior in animals. Two of the most used tests are the plus maze and the social interaction test. However, these tests seem to measure different types of anxiety related to different biochemical and underlying neural mechanisms. File et al. (1993) found that animals put through the plus maze test had a decreased uptake of 5-HT in hippocampus and a decreased basal release of GABA in the cortex, whereas animals put through the social interaction test had an increased uptake of both 5-HT and GABA in the cortex. Furthermore, it has been observed that the social interaction test is sensitive to the effect of stressor controllability while the elevated plus maze is not (Grahn et al., 1995; Short and Maier, 1993).

* Corresponding author. Tel.: +34-93-581-31-88; fax: +34-93-581-14-19.
E-mail address: fina.sala@uab.es (J. Sala-Roca).

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Darbra et al. (1995) observed that adult rats treated perinatally with methimazole showed an anxiolytic pattern in the plus maze test. However, this test shows no effect when hypothyroidism is acquired in adulthood. In contrast, both adult hypo- and hyperthyroidism generated an anxiogenic pattern in the social interaction test (Darbra, 1994; Sala, 1999).

The aim of the present study was to determine the influence of thyroid function on the anxiety of male Wistar rats. Results from previous studies showed different effects when dysthyroidism was acquired in the perinatal period to when it was produced in adulthood. This study assessed the effects of chronic dysthyroidism on anxiety, measured by the plus maze and social interaction tests.

2. Materials and methods

2.1. Animals and experimental groups

Fifty male Wistar rats bred in our laboratory were used in the research. During the experiment, the animals had food and water ad libitum. They had a light and dark circadian rhythm of 12 h (LP, 08.00–20.00 h) and the humidity was between 40% and 60%. These 50 male animals were taken from progenitors that were randomly assigned to three experimental groups. These experimental groups were: the thyroxine group ($n=15$; 4 dams), the methimazole group ($n=15$; 4 dams) and the control group ($n=20$; 10 dams). The control group had more dams so that there was always a control group parallel to each experimental group. Both treatments were administered via the drinking water. Dysthyroidism was induced by adding 20 mg of Methimazole (Sigma) to the drinking water (100 ml) or by adding 0.3 mg of L-Thyroxine (Sigma) to the drinking water (100 ml). Water intake was recorded throughout the experiment to make sure that all the animals had a similar intake. Methimazole is an antithyroidal compound of the tiourelines that blocks the organic binding of iodide and coupling of iodothyronines to form T4 and T3 without interfering with peripherical tissue deiodinase activity. We used this method because it is not as stressful as daily injections or thyroidectomy, which could affect the patterns analyzed. This method has been proved to be effective in the induction of dysthyroidism (Darbra et al., 1995).

2.2. Procedure

Treatments were administered to pregnant females from the ninth day of gestation via their drinking water. After weaning, the treatment was administered to the drinking water of young male rats until the end of the experiment. The behavioral tests started when the rats were 85 days old. After weaning, the animals were weighed every week until they were killed on day 89 to collect blood samples in order to analyze their T4 levels. In order to ensure that the animals were developing correctly, periodic veterinary controls were made.

The experimental protocol was in complied with the European Community Council Directive (EEC directive 86/609) for the care and use of laboratory animals and was, therefore, approved by the Ethical Committee on Animal and Human Experimentation of the Universitat Autònoma de Barcelona.

2.3. Behavioral tests

2.3.1. The elevated plus maze test

This test, standardized by Pellow et al (1985), is considered an ideal gauge of the effects of anxiolytic drugs (Pellow and File, 1986). The apparatus is a cross-shaped maze raised 50 cm above the ground. Lateral walls close two arms, while the other two are open (without walls). Animals are placed in the center for 5 min, facing an open arm. The number of times they go into the open and closed and the time expended, is registered. The animals had previously passed a Boissier test in order to stimulate activity as stipulated by Pellow et al. (1985).

2.3.2. The social interaction test

This test is sensitive to the effects of anxiolytic drugs (File and Hyde, 1978) and is performed in an open-field apparatus placed in an isolated chamber. The apparatus is a round platform (81 cm in diameter with 19 areas of equal surface), fitted with a timer and a white noise generator (60 dB). It is illuminated by a 100-W bulb located 120 cm above the center of the open field. For the 2 days before the test began, the animals were allowed to explore the apparatus individually for 4 min/day. In doing this, the animals were able to familiarize themselves with the apparatus but not with their partner. In this test, two nonrelated animals from the same experimental group were placed in the center of the apparatus. Their behavior was recorded for 10 min, and their behavioral patterns were later analyzed using a computer program that allowed us to measure a maximum of 11 variables and to analyze the time and frequency of each variable. Patterns considered in the analysis were the time and frequency of ambulation and exploration, self-grooming and immobility and social patterns: approximation and following, sniffing and social grooming, passive interaction, crawling over and crawling under, attacking and defending, escaping and avoidance (Grant and Mackintosh, 1963). To evaluate data from the social interaction test, the measures were grouped as active social behavior (approximation and following, sniffing and social grooming, crawling over, crawling under, escaping, avoidance), passive social behavior (passive interaction), activity (ambulation and exploration) and behavior that has been related to stress (freezing and self-grooming) (Moody et al., 1988; Kametani, 1988; Van Erp et al., 1994, 1995).

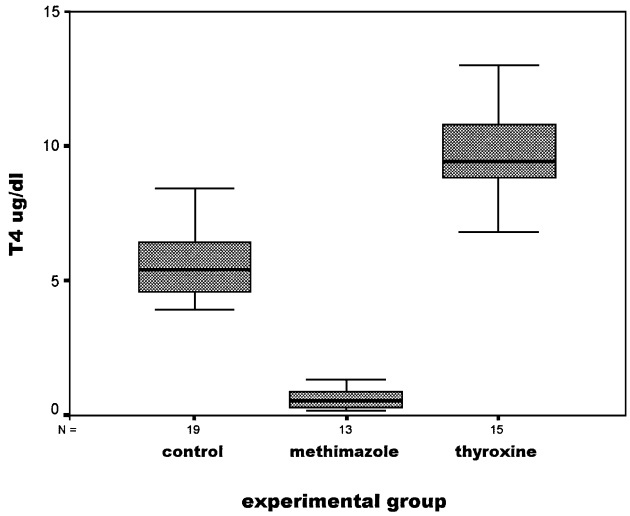

Fig. 1. Box and Jenkins diagram of the T4 plasmatic levels with quartiles (black line indicates median value). Methimazole group had reduced T4 levels ($P < .0001$) and the thyroxine group had increased levels ($P < .0001$).

2.3.3. Hormonal analyses

Serum levels of T4 were measured in order to establish the degree of dysthyroidism induced by our treatment. The animals were killed by decapitation when they were 89 days old, between 10.00 and 11.00 am. Blood samples were collected and centrifuged and serum was frozen and stored immediately at -40°C. Serum T4 was determined by radioimmunoassay (reference values 4.5–12.5 μg/dl). The kit was equipped according to standards with T4 values ranging from 1 to 24 μg/dl. The antiserum was highly specific for T4. The procedure was able to detect as little as 0.25 μg/dl. Each sample was duplicated to check the reliability. The percentage of recovery of the assays was within the standard, accepted limits for these measures. The coefficient of variation between duplicate samples was always $< 5\%$.

2.3.4. Statistical analysis

The data was analyzed using a commercial statistics package (SPSS/PC + Statistical package). Analysis of variance (ANOVA) was performed to detect differences in plasmatic T4 levels and orthogonal contrasts were applied to determine differences between groups. Differences in body weight were analyzed by ANOVA, using repeated measures and polynomial contrasts to measure the differences in weight increase associated to development between the three experimental groups. The effects of dysthyroidism on behavior were assessed using ANOVA to analyze individual patterns and multivariate analysis of variance (MANOVA) was used to analyze groups of patterns that were considered to measure the same characteristic. We chose this type of analysis because MANOVA considers covariation between patterns that measure the same attribute and provides more reliable results than a univariate analysis. Orthogonal contrasts were used to measure the differences between groups. In the analysis of plus maze measures, we also used MANOVA, but to analyze measures as percentages we used univariate analysis because some measures were linearly dependent, making multivariate analysis impossible.

We also analyzed the results of the social interaction test considering the pair-scores because as both partners received the same treatment, their behavior of one rat was not independent of that of the other.

3. Results

Serum from 47 animals (19 control, 13 methimazole, 15 thyroxine) was analyzed and plasmatic T4 levels were detected by radioimmunoassay. We did not have enough serum from the other three animals to analyze them. Differences in plasmatic T4 levels confirmed that the treatment was effective in inducing dysthyroidism [$F(2,44) = 38.28$, $P < .0001$). The methimazole group had reduced T4 levels ($P < .0001$) while those of the thyroxine group were increased ($P < .0001$) (Fig. 1).

The weight increase of 13 control, 14 methimazole-treated and 14 thyroxine-treated animals was analyzed. MANOVA excluded the other animals from the analysis because data for one of the 11 weight variables was missing. The increase in weight was affected by dysthyroidism ($F(20,380) = 106.5$, $P < .001$). Polynomial contrast revealed that the weight increase of the methimazole-treated group was lower ($P < .001$), and that of the thyroxine-treated group slightly higher ($P < 0.05$) than the control group's (Fig. 2).

MANOVA was used to evaluate the effect of dysthyroidism on the covariation of patterns observed in the plus maze test. We, therefore, analyzed the number of times the animals went into the open and closed arms and the time they spent in the open arms. The analysis indicated that the treatment affected the evaluated variables [Wilks $(6,90) = 0.747$,

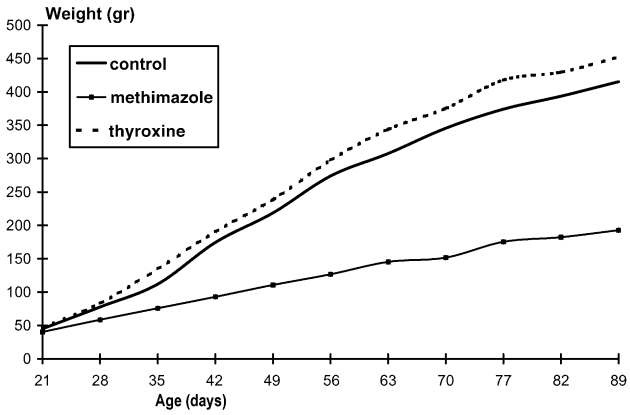

Fig. 2. Weight evolution from weaning until the end of the experiment. Measures were taken weekly. The methimazole group had lower ($P < .001$) and thyroxine group slightly higher ($P < .05$) weight increases than the control group.

$P < .05$]. The discriminant function generated by the analysis revealed that the methimazole-treated animals showed an anxiolytic pattern. The methimazole-treated group entered and remained in the open arms for more time than either the control group [Wilks (3,45) = 0.844, $P = .05$] or the thyroxine-treated group did [Wilks (3,45) = 0.798, $P < .05$] (Table 1).

We also analyzed the percentage numbers of times rats went into the open and closed arms along with the percentages time spent in the open and closed arms. The analysis indicated that the treatment affected the percentage of time spent in the open arms [$F(2,38) = 3.37$, $P < .05$] and the percentage of times rats entered the open arms [$F(2,38) = 4.01$, $P < .05$]. Orthogonal contrast indicated that the methimazole group stayed in the open arms for a longer time than the control group ($P = .05$) and the percentage number of times they entered the open arms was higher than that of the control group ($P < .05$). There were no significant differences between the thyroxine group and control group (Fig. 3).

Eight animals were excluded from the social interaction test because they did not have a partner that had received the same treatment. Therefore, 14 animals (seven pairs) from each experimental group were used. Results are summarized in Table 1. Attack and defense behavior were not observed. Multivariable analysis of active social behavior indicated that dysthyroidism affected these patterns. Standardized coefficients of the discriminant function generated by the multivariate analysis showed that the methimazole-treated group spent more time in bodily contact (sniffing and social grooming, crawling over and crawling under) and did so more frequently than the control group [Wilks (4,36) = 0.372, $P < .001$/Wilks (4,36) = 0.288, $P < .001$, respectively] and the thyroxine-treated group [Wilks (4,36) = 0.305, $P < .001$/Wilks (4,36) = 0.263, $P < .001$, respectively]. Passive interaction was also affected by dysthyroidism [$F(2,39) = 16.13$]. The methimazole-treated group spent more time in passive interaction than the control ($P < .001$) and thyroxine-treated

Table 1
Means, S.E.M. and standardized coefficients of discriminant functions generated by MANOVA of the behavioral pattern analyzed

	Methimazole (mean ± S.E.M.)	Control (mean ± S.E.M.)	Thyroxine (mean ± S.E.M.)	Coefficient discriminant *f*
Plus Maze [MANOVA: λ Wilks = 0.75, P < .05. Methimazole group differs from control and thyroxine groups (P ≤ .05)]				
No. of entries close arms	2.67 ± 0.33	2.25 ± 0.35	3.07 ± 0.46	0.715
Time open arms	82.53 ± 17.40	42.40 ± 9.54	42.00 ± 9.51	− 0.450
No. of entries open arms	4.53 ± 0.78	2.30 ± 0.51	2.53 ± 0.53	− 0.862
Social interaction				
Active social behavior (time) [MANOVA: λ Wilks = 0.25, $P < .001$. Methimazole group differs from control and thyroxine groups ($P < .001$)]				
Crawl over and crawl under	16.97 ± 2.30	2.72 ± 0.74	2.36 ± 0.70	0.786
Sniffing and social grooming	71.24 ± 7.34	50.51 ± 5.10	40.25 ± 5.64	0.506
Approximation and follow	33.08 ± 6.13	51.54 ± 5.29	58.5 ± 6.92	− 0.581
Escape and avoidance	7.77 ± 2.54	13.72 ± 2.27	19.24 ± 3.82	− 0.110
Active social behavior (frequency) [MANOVA: λ Wilks = 0.19, $P < .001$. Methimazole group differs from control and thyroxine groups ($P < .001$)]				
Crawl over and crawl under	9.79 ± 0.92	2.07 ± 0.45	1.71 ± 0.46	0.902
Sniffing and social grooming	28.14 ± 2.81	36.29 ± 3.14	26.21 ± 3.29	0.225
Escape and avoidance	6.00 ± 1.53	13.79 ± 1.94	15.93 ± 3.10	− 0.336
Approximation and follow	24.50 ± 4.15	43.71 ± 3.86	39.29 ± 4.93	− 0.552
Activity (time)[MANOVA: λ Wilks = 0.45, P < .001. Methimazole group differs from control and thyroxine groups (P < .001)]				
Exploration	245.37 ± 15.76	316.66 ± 13.01	311.53 ± 9.47	0.907
Deambulation	30.47 ± 5.34	54.38 ± 4.99	47.82 ± 4.72	0.848
Activity (frequency) [MANOVA: λ Wilks = 0.44, P < .001. Methimazole group differs from control and thyroxine groups (P < .001)]				
Exploration	68.86 ± 5.34	114.64 ± 4.52	101.71 ± 4.46	1.001
Deambulation	26.36 ± 4.16	55.14 ± 4.57	47.07 ± 4.80	− 0.001
Behavior related to stress [MANOVA: λ Wilks = 0.874, P=.078. ANOVA: time F = 4.44, P < .05; frequency F = 5.24, P < .05]				
Time self-grooming	12.79 ± 3.31	10.95 ± 2.10	30.19 ± 10.47*	
Frequency self-grooming	2.00 ± 0.43	3.14 ± 0.59	5.29 ± 1.62*	
Time immobility	0.59 ± 0.13	0.36 ± 0.07	0.56 ± 0.12	
Passive social behavior [ANOVA: time passive interaction F(2,39) = 16.13; P < .001]				
Time passive interaction	181.74 ± 18.00**	99.17 ± 7.91	89.55 ± 9.55	
Frequency passive interaction	61.43 ± 2.10	65.64 ± 3.74	55.29 ± 5.43	

Coefficients represent the weight and direction of each variable in the discriminant function. Passive social behavior and behaviors related to stress were analyzed by ANOVA.

* Thyroxine-treated group differs from the control group ($P < .05$).

** Methimazole-treated group differs from both the control and thyroxine-treated groups ($P < .001$).

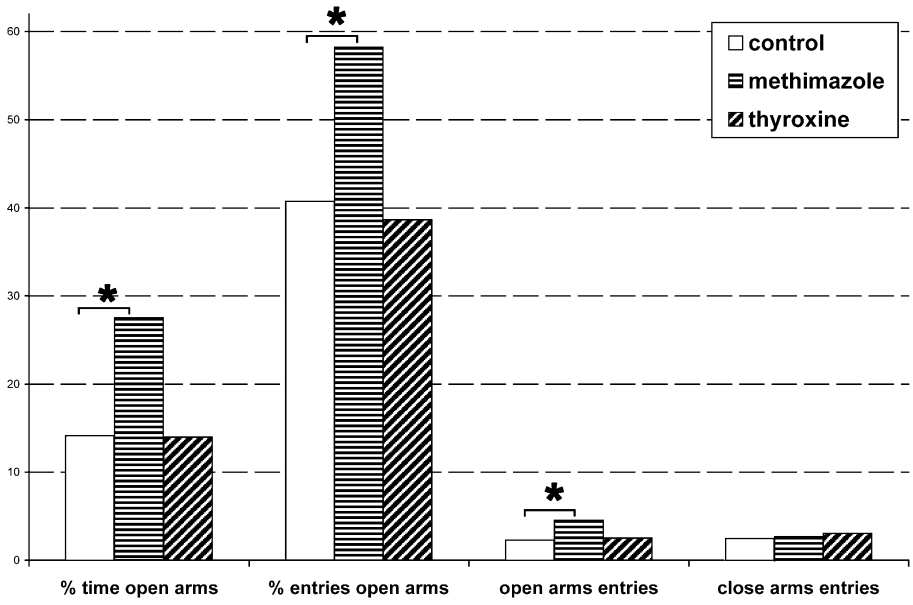

Fig 3. Percentage of the time and entries in open arms and number of entries in open and closed arm of the Plus Maze test (* $P \leq .05$).

($P < .001$) groups, but the frequency of this behavior did not differ. On the contrary, when activity was analyzed, the methimazole-treated group spent less time in exploration during the social interaction test and did so less frequently than the control [Wilks (2,38) = 0.490, $P < .001$/Wilks (2,38) = 0.460, $P < .001$, respectively] and thyroxine-treated [Wilks (2,38) = 0.576, $P < .001$/Wilks (2,38) = 0.624, $P < 0.001$, respectively] groups. The computer program used to measure behavioral patterns calculated the percentage of the total time (10 min) that the animals spent doing each analyzed behavior. Considering this, the methimazole-treated animals spent less time exploring because they spent more time interacting with their partner in the social interaction test. The methimazole-treated group spent 51.8% of the time in social interaction patterns and 45.97% in individual activity, while the control group spent 37.11% of the time in social interaction patterns and 61.84% in individual activity, and the thyroxine-treated group spent 34.98% of the time in social interaction patterns and 59.89% in individual activity (Table 2).

Table 2
Percentage of time spent in behaviors that imply bodily contact (sniffing, social grooming, crawling over, crawling under and passive interaction), social behaviors without bodily contact (approximation, following, escaping and avoidance), activity (ambulation and exploration) and behaviors related to stress (self-grooming and immobility)

	Social behaviors that implies body contact (%)	Social behaviors without body contact (%)	Activity (%)	Behaviors related to stress (%)
Methimazole	44.99	6.81	45.97	2.23
Control	26.23	10.88	61.84	1.89
Thyroxine	22.03	12.96	59.89	5.12

In the plus maze test, the thyroxine-treated group was no different to the control group. In the social interaction test, thyroxine-treated animals spent less time in patterns such as passive interaction, sniffing and social grooming, but these differences did not reach a significant level. The multivariate analysis indicated that the thyroxine-treated group spent more time self-grooming or immobile than the control group. Although this effect did not reach a significant level, there was a tendency to be significant [Wilks (2,38) = 0.874, $P = .078$]. Therefore, we also considered the univariated index of self-grooming where this difference reached a significant level, both in time [$F(1,39) = 4.44$, $P < .05$] and frequency [$F(1,39) = 5.25$, $P < .05$].

Since both partners received the same treatment and the behavior is, therefore, not independent of the partner's behavior, we analyzed the results of the social interaction test considering the scores in pairs. The results of this analysis were similar to the results obtained using scores individually.

4. Discussion

Plasmatic thyroxine levels and the body weight curve confirmed that the treatments used were effective. Plasmatic levels of thyroxin were markedly reduced in the methimazole-treated group whereas in the thyroxine-treated group they were nearly twice as high as the control group. Despite the important effects on body weight of methimazole, there were no major physical alterations of the animals. Veterinary controls did not report health problems. These were striking observations because literature indicates that neonatal or chronic hypothyroidism provokes severe neurological defects. In fact, our previous studies (Darbra et al., 1995) did

not show serious defects in animals perinatally drinking a methimazole solution. These results suggest that this treatment, even when chronic, could be considered mild.

Behavioral results indicated that methimazole treatment has an anxiolytic effect, both in the plus maze and the social interaction tests. Meanwhile, it seems that thyroxine treatment does not have a significant effect on these tests, all we observed was a slight effect on self-grooming.

The anxiolytic pattern of the methimazole-treated animals was also observed in adults when they were treated perinatally with methimazole from the ninth day of gestation and for 21 postnatal days (Darbra et al., 1995). It is clear that the observed anxiolytic effect does not seem to be influenced by animal weight because when methimazole was administered perinatally animals reached a normal weight in adulthood (Darbra et al., 1995).

Nevertheless, in two previous experiments, we analyzed the effects of methimazole and thyroxine treatment on 60- (Darbra, 1994) and 40-day-old (Sala, 1999) rats using plus maze and social interaction tests. No anxiolytic effect on methimazole-treated animals was observed in either experiment. In contrast, an anxiogenic pattern was observed in these groups, namely, these animals showed less social grooming, approximation to their partners, crawling over and crawling under and passive social behaviors than those in the control group, while they showed more self-grooming and freezing than animals in the control group.

It is possible that some of the effects observed in the methimazole-treated group may not be related to thyroid status, but we believe that this is improbable. The effect of methimazole is highly specific to thyroid activity, which is why several studies have used methimazole to induce hypothyroidism (i.e. Barradas et al., 2000; Comer and Norton, 1982; Dakine et al., 2000; MacNabb et al., 2000; Maran et al., 2000; Parija et al., 2001; Weller et al., 1996).

The anxiolytic effect observed in perinatal and chronic methimazole-treated groups may well be the result of changes in the neurotransmitter systems involved in anxiety, such as GABA and the serotonin system. Different experiments have shown that thyroid hormones regulate GABA receptor activity (Abe et al., 1992; Martin et al., 1996; Sandrini et al., 1991) and the serotoninergic system is depressed by hypothyroidism (Rastogi and Singhal, 1976, 1974; Savard et al., 1984). But the latter effect seems to be limited to the first 20 days after delivery (Rastogi and Singhal, 1974). This could be the reason why the anxiolytic pattern is not observed when treatment starts after this period. If this effect on the serotonin system were irreversible, it might explain why the anxiolytic patterns induced in the perinatal period continue in adulthood even though the opposite effect was observed in adult methimazole treatment.

In contrast, the plus maze test did not reveal effects on behavior in thyroxine-treated animals. However, in the social interaction test, these animals spent more time self-grooming than the control group animals did. Even though File does not validate self-grooming as a measure of anxiety, this behavior has been used as a measure of anxiety in different experiments (i.e. Burchuladze et al., 1994; Escorihuela et al., 1999; Ferre et al., 1995; Kumar et al., 1999; Kantor et al., 2000; Schino et al., 1996; Steimer et al. 1998; To et al. 1999, Van den berg et al., 1999). Moreover, self-grooming has also been related to environmental stress (Kametani, 1988; Moody et al., 1988; Van Erp et al., 1994, 1995). Therefore, the increase of this behavior in the thyroxine-treated group can be related to an increase in anxiety or in vulnerability to stress. In agreement with this observation, we observed that these group spent less time in patterns, such as passive interaction, sniffing and social grooming. However, these differences did not reach a significant level. In previous experiments, thyroxine treatment did not cause any effects when it was administered perinatally in a plus maze test (Darbra et al., 1995) or in adulthood (Sala, 1999; Darbra, 1994). However, an anxiogenic effect was observed in the social interaction test when thyroxine was administered after 60 (Darbra, 1994) or 40 days (Sala, 1999), namely, that thyroxine treated animals made less social contact and more self-grooming behavior.

Different studies suggest that the gabaergic system is involved in the mechanisms of grooming behavior (Barros et al., 1994; De Barioglio et al. 1991; Osborne et al. 1993; Souza Spinosa et al., 2000) and as was mentioned earlier, thyroid hormones regulate GABA receptor activity. Thyroid hormones could behave as neurotransmitters or neuromodulators of the GABA receptor (Martin et al. 1996).

Finally, we must take into account that the effects of deficiency or excess are not necessarily opposite. In fact, thyroid receptors, in addition to ligand-dependent gene activation, can repress basal transcription in the absence of ligand (for review, see Aranda and Pascual, 2001). Therefore, hormone thyroid levels can produce quantitative and qualitative transcriptional changes and these could influence behavior.

In conclusion, the results of this experiment suggest that chronic thyroid deficiency produces an anxiolytic-like effect. These results, in the light of our previous experiments, suggest that the effects of perinatal dysthyroidism are organizational effects, since different effects were observed depending on whether dysthyroidism is acquired in the perinatal period or adult period, and the first effects prevail over the latter throughout chronic dysthyroidism.

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