propylthiouracil (PTU) from the 18th day of gestation until weaning. The pups were weighed at regular intervals, and blood samples were collected by cardiac puncture under Nembutal anesthesia, 1 and 2 months after cessation of the anti-thyroid treatment, and the plasma samples were stored at −30 °C until hormonal measurements. Simultaneously with the 2nd blood sampling, the animals were ovariectomized in order to avoid interference with endogenous estrogens. 1 week later, the rats were killed by decapitation, blood was collected from the trunks, and pituitaries were quickly removed, blotted free of blood and chilled in ice-cold TEM buffer (10 mM tris, HCl, pH 7.4; 1.5 mM EDTA; 12 mM monothioglycerol).

The pituitary estradiol binding sites were measured at 2 °C by the protamine precipitation method described earlier[8,11]. Protein concentrations in the replicates were determined spectrophotometrically[12], and plasma thyroxine (L-T_4) concentrations were measured by radiocompetition against human thyroxine-binding-globulin[8].

One-way ANOVA according to BMDP statistical software was performed to detect significant differences between control and hypothyroid groups.

Results and discussion. Although neonatal hypothyroidism caused a marked and persistent delay in body growth, body weights started to increase two weeks after the cessation of the anti-thyroid treatment. The body weight increment then followed a similar pattern in hypothyroid and control animals from the age of 9–10 weeks on, which agrees with previous reports[13]. Similarly, plasma L-T_4 levels, which were previously shown to be nearly undetectable throughout the anti-thyroid treatment[8], had already returned to normal levels 1 month after the PTU withdrawal (fig. 1). At this stage they did not differ from L-T_4 titers in 13-week-old animals, a result in keeping with restoration experiments in adult rats[14]. Moreover, the subsequent ovariectomy led to a 40% rise ($p < 0.01$) of plasma L-T_4 levels in both controls and initially hypothyroid animals, suggesting that PTU-induced neonatal hypothyroidism had no apparent after-effects on ovary-thyroid interactions.

On the other hand, 2 months after cessation of the PTU treatment, the initially hypothyroid group still displayed a 75% reduction in the total amount of available EBS in the pituitary (fig. 2). It may tentatively be assumed that this lowered amount in pituitary EBS may, indeed, be part of the mechanism whereby neonatal hypothyroidism leads to persistent dysfunctions of the reproductive system. However, considering the actual concentrations in pituitary EBS as expressed in fmoles/mg protein, the 2 groups of rats were indistinguishable. Clearly, the long-lasting lack of pituitary EBS in rats suffering from perinatal hypothyroidism may be attributable to an impaired pituitary growth, rather than to decreased cellular synthesis of EBS. Consequently, the specific decrease of pituitary estradiol binding sites previously observed in hypothyroid rats during the early neonatal period[8] appears essentially as a transient and reversible postnatal effect.

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Corticoadrenal and behavioral response to open field in pairs of male rats either familiar or non-familiar to each other

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Summary. The effect of the presence either of a familiar or non-familiar conspecific animal on serum corticosterone and some behavioral responses in the open field was studied in male Sprague-Dawley rats. Animals tested in presence of a familiar animal showed a higher corticosterone response and a higher defecation rate. It suggests that rats experienced more emotional reactivity in presence of a familiar animal than in presence of a non-familiar one. Time spent in social interaction was higher in non-familiar pairs; however, ambulation and rearing were lower, suggesting competition between social investigation and novel environment exploration.

Using various behavioral measures of fear, many investigators have noted that fear experienced by rats in a stressful situation was reduced by presence of a conspecific animal[1–3]. However, contradictory results have been reported following work dealing with the effect of conspecifics on corticoadrenal response to stress[4–6]. This apparent lack of relationship between behavioral responses and corticoadrenal activity is not surprising since no correlation has been observed between defecation (the most often used index of fear in the rat) and serum corticosterone in response to

Corticoadrenal and behavioral response to open field in pairs either of familiar or non-familiar rats. It is represented means ± SD. In parentheses number of animals per group, except that for defecation data the boluses produced by both rats were counted together

Group	Serum corticosterone (μg/100 ml)	Defecation	Ambulation	Rearing	Active social interaction (sec)
Familiar rats	25.1 ± 4.4 (9)	10.4 ± 4.9 (5)	192 ± 37 (10)	75 ± 33 (10)	193 ± 45 (10)
Non-familiar rats	19.9 ± 3.4 (8)**	7.4 ± 4.0 (5)	143 ± 75 (10)**	40 ± 22 (10)**	433 ± 235 (10)**

** $p < 0.02$.

open field[7]. We have found the same corticoadrenal response to a novel environment in rats when tested alone and when in the company of non-familiar conspecific. However, this response was higher in pairs of familiar rats compared with both rats tested alone and rats tested with a non-familiar conspecific[5]. In the present report corticoadrenal response to an open filed in pairs of rats either familiar or un-familiar with each other was studied to refine previous findings. Furthermore, we have recorded some open field responses such as defecation, ambulation rearing and active social interaction with the aim of relating corticoadrenal and behavioral data.

Method. Male Sprague-Dawley rats weighing approximately 350 g at the end of the experiment were used. On arrival, they were housed 5 per cage in a controlled environment (temperature 22 °C, light on from 06.00 h to 18.00 h) for 20 days before testing. Food and water were available ad libitum. Two rats, either 'familiar' (coming from the same home cage) or 'non-familiar' (coming from different home cages) were simultaneously placed in a circular white open field of 80 cm diameter). Tests were carried out in a room adjacent to, and with the same illumination as, the animal house. Total number of boluses discharged by both rats, individual squares crossed (ambulation), and rearings were recorded for 20 min. Time spent in active social interaction following File and Peet's criterion[6] was also measured. Between sessions boluses were removed and the apparatus cleaned.

Immediately after testing both rats were killed by decapitation in another room and trunk blood collected. Serum obtained was frozen (−20 °C). Corticosterone was analyzed by radioimmunoassay[8].

To assess reliability of behavioral data (especially defecation), the experiment was replicated using 32 additional male Sprague-Dawley rats weighing approximately 300 g. The rats were housed 3 per cage for 20 days and then exposed to open field for 5 min.

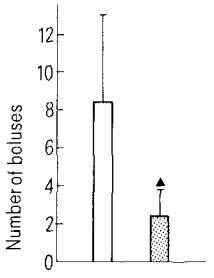
Statistical analysis was performed by Student's t-test, with prior log. transformation of data when they did not meet the requirements for such a test.

Results. After 20 min of exposure to open field, serum corticosterone was higher ($p < 0.02$) in pairs of rats familiar with each other than in pairs of 'strangers'. Although a trend toward an increase in both defecation and ambulation was observed in 'familiar' rats compared with 'non-familiar' ones, it did not reach statistical significance. Time spent in active social interaction was higher in 'non-familiar' animals ($p < 0.02$) whereas rearing decreased ($p < 0.02$). The results are presented in the table. The 2nd experiment gave similar behavioral results, the differences in defecation between both groups being significant ($p < 0.005$). (fig.).

Discussion. The assumption that familiarity was the most important factor in determining exacerbated corticoadrenal response to a novel environment in pairs of rats[5] is confirmed by the present results. The fact that 'non-familiar' rats spent more time in active social interactions than 'familiar' ones, could suggest that presence of a conspecific 'stranger' would act as a distracting stimulus reducing the stressful influence of the novel environment. However, this hypothesis does not explain the higher corticoadrenal response to a novel environment previously observed in pairs of familiar rats compared with singly tested animals[5]. We have hypothesized that changes in the environment in which a rat usually perceives its companion (open field vs home cage) could induce a higher emotional reactivity than the novel stimuli (including open field and novel conspecific) per se[5]. Thus, there is evidence suggesting that animals experience more fear in situations where familiar and novel elements are combined[9].

In behavioral experiments in which a reduction of fear induced by presence of a conspecific have been reported, the rats were housed in single cages for several days before testing[1–3]. In addition, familiarity was not controlled. These aspects could explain discrepancies between earlier reports and our results. In the present work, both defecation and serum corticosterone indicate that presence of a familiar conspecific could increase emotional reactivity in response to a novel environment.

Non-familiar rats spent more time in active social interaction than familiar animals. Both ambulation and rearing decreased in non-familiar animals. All these findings indicate that there was competition between exploration of the novel conspecific and exploration of the physical environment. Measures related to exploration should be discussed with caution in those studies in which pairs of rats are used. Especially, they should not be interpreted in terms of emotionality.



Effect of presence of a conspecific on defecation (means S.D.) in a 5-min open field test. Number of boluses produced by both rats in pairs of familiar (open bar) and non-familiar (closed bar) conspecifics are represented. Number of pairs tested in each group = 8. ▲ $p < 0.005$.

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