3000 pg per gland in the D stage at 17 h was observed. In all groups of rats there was a lack of estrogens and androgens, except in some groups investigated in the P stage. Estrogens concentration started in the P stage at 50 pg per gland at 10 h, reached a maximum at 14 h (about 60 pg per gland), and rapidly decreased during the following hours down to an undetectable level in the E stage at 14 h. The androgens level, undetectable in the P stage at 10 h, increased suddenly at 14 h (up to 50 pg per gland), reached a peak at 17 h (above 60 pg per gland) and afterwards fell sharply, to zero at 22 h.

From the present results it is evident that the progestagen level in the rat submaxillary gland homogenates during the estrus cycle was not constant but underwent fluctuations depending on the stage of the cycle and hour of the day. Progestagen content in this gland in the P stage was minimal in the morning, increased in the afternoon, and reached a maximum at night. Similar differences in progestagen concentration were found in proestrus in ovarian tissue[12-14] and also in ovarian venous plasma and peripheral blood[15,16]. In all these investigations preovulatory progestagens began to increase in the afternoon of the P stage and reached a maximum several hours later. The 2nd minimum of progestagen level in the submaxillary gland was found in the E stage at 14 h, while in the ovaries it was observed in the same stage just at 9 h[12]. The increase of submaxillary gland progestagen level in the afternoon in the E stage and the subsequent slow decrease of these hormones in the M and D stages correlate with progestagen concentration in peripheral blood plasma during those stages, but not with their concentration in ovarian vein blood[15]. Estrogen and androgen were undetectable in the rat submaxillary gland during most stages of the estrus cycle except the P stage when trace quantities were found with a maximum for estrogens at 14 h and for androgens at 17 h. Since plasma estrogen and androgen concentrations are low during the E and M stages, increase in the D stage and reach a maximum in the morning (estrogens) or in the afternoon (androgens) of proestrus[17-19], the content of these steroids in the submaxillary gland appears to correlate with this pattern.

In conclusion, steroid concentration in the female rat submaxillary glands in the consecutive stages of the estrus cycle is well correlated with the steroid level in the peripheral blood. This similarity strongly suggests the external origin of submaxillary gland hormones. On the other hand, the possibility cannot be excluded that there is some synthesis of progestagens in the submaxillary gland itself, although the rate is rather low[7,20].

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## Pineal indols and testosterone affect exploratory activity of male rats

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*Summary.* The testosterone level has an inverse relation to activity in the open-field test. This is more important in red light than in white light. Pineal indols do not disturb this action. Some of these results are consistent with the assumption that androgens play a role on the exploratory activity of adult subjects.

The pineal gland may play some role in the general motor and exploratory behavior of male rats following pinealectomy. Some investigators reports changes in the wheel-running score[1-3] or exploratory activity as studied in the open-filed test[4]. Treatment with pineal extract or with melatonin results in modification of exploratory activity[1,4,5]. The possible mechanism of action underlying these behavioral effects are not clear at this time. It is accepted that the pineal gland produces antigonadotropic agents[6,7] and that the gonadal hormones play an important part in exploratory activity. Female rats are more active and defecate less than males. But females, which have been exposed to androgens in early neonatal life, are comparable with males[8,9]. The purpose of this study was to investigate the possible role of melatonin and 5-methoxytryptophol on exploratory behavior in the open-field test and its relationship to testosterone action.

*Methods.* Subjects and treatment. 108 adult male Wistar rats were divided into 9 groups of 12 animals each. The animals were kept on a standard light-dark schedule (12:12). All the rats were castrated and 20 days afterwards each group received daily s.c. injections of testosterone propionate (TP), 10 µg, 50 µg or 500 µg, dissolved in peanut oil, for 10 days. At each level of testosterone a first group received 1 mg of 5-methoxytryptophol s.c., a 2nd group 1 mg of melatonin and a 3rd group the vehicle (absolute alcohol

0.858 M in saline solution 0.146 M). Melatonin and 5-methoxytryptophol (Sigma, St. Louis) were dissolved in the vehicle 10 days after the beginning of the treatment, all groups were given an open-field test.

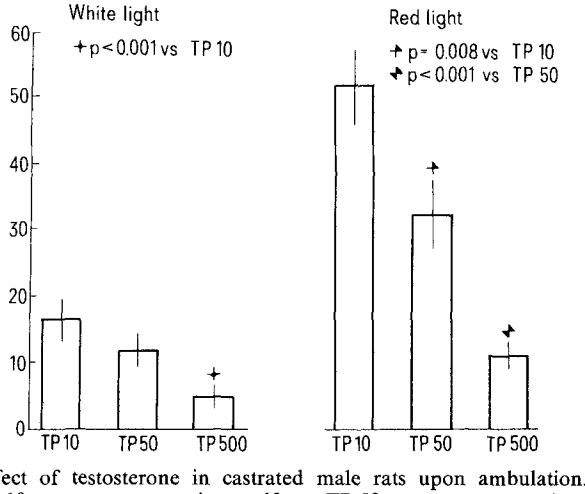
Apparatus and procedures. A circular open-field which had a floor marked into 21 segments divided by 2 circles into outer, inner and central regions was used. It was illuminated either with strong light white (150 W-floor lamp) or with dim red light (50 W-floor lamp). 10 days after beginning the treatment all groups were performed with red light. For each trial the animals remained in the apparatus for 2 min. The number of squares explored and defecation boluses were counted. Statistical analysis of data was carried out by means of ANOVA. A probability level of 0.05 or less was accepted as a significant difference.

*Results.* The animals which had been treated with 50 µg of TP had a lower mean exploratory activity score than any of the 10 µg of TP. The differences for total ambulation were more important ($p = 0.008$) with the red light test (fig.) The animals which had received 500 µg of TP had a lower average total ambulation in white light ($p < 0.001$) and red light ($p < 0.001$) than animals treated with a dosis of 50 µg (fig.). Testosterone treatment had no effect upon the number of defecation boluses at different levels of doses (table). Neither of the pineal indols administered affected significantly the exploratory activity or the number of defecation boluses (table).

Effect of testosterone, melatonin and 5-methoxytryptophol on the exploratory behaviour of male rats in the open-field test

| Treatment | Number total squares | | Defecation boluses | |
|---|---|---|---|---|
| | White light | Red light | White light | Red light |
| TP (10 µg) | | | | |
| Vehicle | 16.4 ± 2.8 | 51.6 ± 5.1 | 3.7 ± 0.7 | 1.3 ± 0.8 |
| 5-MTL | 18.5 ± 3.6 | 54.4 ± 5.2 | 3.7 ± 0.8 | 3.9 ± 1.3 |
| Melatonin | 14.2 ± 2.9 | 43.8 ± 6.2 | 5.0 ± 0.8 | 4.4 ± 1.1 |
| TP (50 µg) | | | | |
| Vehicle | 11.5 ± 2.2 | 32.1 ± 5.5** | 3.9 ± 1.0 | 1.8 ± 0.7 |
| 5-MTL | 16.6 ± 4.1 | 35.2 ± 8.1 | 3.6 ± 1.0 | 3.8 ± 0.7 |
| Melatonin | 10.2 ± 2.1 | 17.8 ± 3.9 | 4.1 ± 0.9 | 2.6 ± 0.7 |
| TP (500 µg) | | | | |
| Vehicle | 4.8 ± 1.8* | 10.9 ± 1.8*** | 1.8 ± 0.5 | 2.2 ± 0.7 |
| 5-MTL | 12.2 ± 2.6 | 19.5 ± 4.6 | 2.8 ± 0.8 | 3.1 ± 1.0 |
| Melatonin | 6.7 ± 2.6 | 17.0 ± 2.9 | 2.9 ± 0.6 | 2.5 ± 0.9 |

5-MTL: 5-methoxytryptophol. Probability values for ANOVA and Scheffe comparisons: *$p < 0.001$ vs TP 10; **$p = 0.008$ vs TP 10; ***$p < 0.001$ vs TP 50.




Effect of testosterone in castrated male rats upon ambulation. TP 10, testosterone propionate 10 µg; TP 50, testosterone propionate 50 µg; TP 500, testosterone propionate 500 µg.

*Discussion.* Gonadal steroids have, in the first days of life, a generally accepted influence upon the adult motor activity of both sexes. Female rats are more active and defecate less than males. Neonatal castration of males, especially if it is combined with prenatal exposure to cyproterone acetate, augments activity to levels displayed by females[10,11]. On the other hand, female rats exposed to androgens[8,9] or estrogens[12] in neonatal life, have lower activity scores than the control females and similar scores than males. The action of gonadal steroids in adult subjects is less known. An increase in the female motor activity has been found during estrous[14] or in rats with large estradiol benzoate treatment[15]; however, ovariectomy failed to affect open-field behavior[11,16]. As previous studies have reported, castration of adult male laboratory animals does not affect open-field behavior[13,17]. Some authors are of the opinion that the influence of gonadal hormones on adult motor activity is rather poor[13]. Our data, however, support the hypothesis that, in adult subjects, the plasma levels of gonadal steroids, specifically testosterone, may play an important role in motor activity response to stress. These data are in agreement with the relationships reported between physical exercise and testosterone plasma level in adult humans[18-20]. Defecation, a measure considered as a 'emotionality' response to stress[21], was not modified by testosterone in our study.

Several previous studies attribute to melatonin a depression of general motor activity[2,5]. More recent reports do not show any influence of melatonin on exploratory activity in the open-field test which is in agreement with the results reported here. If the pineal gland plays any significant role in this kind of behavior, as it has been suggested by some authors[1,3,4], neither melatonin nor 5-methoxytryptophol seems to be responsible.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