Effects of Housing Conditions and 5-HT2A Activation on Male Rat Sexual Behavior

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BROTTO, L. A., B. B. GORZALKA AND L. A. HANSON. Effect of housing conditions and 5-HT2A activation on male rat sexual behavior. PHYSIOL BEHAV 63(4) 475–479, 1998.—Adult male rats were housed individually or in groups for a period of 39 days. In Experiment 1, the effects of housing conditions on sexual behavior and concurrent spontaneous "wet dog shaking" (WDS) were investigated. Individual housing significantly impaired male sexual behavior and resulted in a trend toward increased WDS. In Experiment 2, the effects of housing conditions were examined following administration of the serotonergic type 2A (5-HT2A) agonist DOI. Individual housing significantly increased DOI-induced WDS. The implications of these findings are discussed in the context of stress-induced corticosterone secretion and the possible regulatory effect on 5-HT2A receptors. © 1998 Elsevier Science Inc.

5-HT2A receptors Isolation Serotonin Sexual activity Stress

ALTHOUGH there have been numerous studies addressing the effects of individual housing of the prepubertal rat on adult sexual behavior, relatively few have been concerned with individual housing of the postpubertal rat. In general, the former have concluded that the observed decrements in male sexual behavior after individual housing are caused by a lack of social experience during development [e.g., (8,26)]. However, this theory was weakened by evidence that postpubertal individual housing of male rats, even those reared in a social environment, also exhibited deficits in adult sexual behavior (7). It seems reasonable that individual housing per se, rather than individual housing during a critical period, constitutes a stressor which produced the observed deficits in adult sexual behavior.

Individual housing or social isolation has been shown to produce marked physiological disturbances. As early as 1963, clinical symptoms of chronic stress were reported in 100% of experimental rats that had been housed in isolation for 4–6 weeks (14). These symptoms, which included caudal dermatitis, increased adrenal and thyroid weights, lighter spleen and thymus, and an array of other distinct physical conditions, can be attributed to hyperfunction of the adrenal cortex (14,15). The physiological response to individual housing conditions appears to mimic the response to chronic stress exposure. Later studies revealed that short-term housing of adult rats in isolation elevates plasma corticosterone levels (2,19) and is sufficient to constitute a stressor.

Recent studies have provided increasing evidence for a relationship between central serotonin (5-HT) and mechanisms mediating chronic stress (4,21). For example, the discovery that glucocorticoid receptors are present on almost all 5-HT neurons and that plasma corticosterone levels can influence 5-HT neurotransmission seems to suggest a functional interdependency between the hypothalamo–pituitary–adrenocortical (HPA) axis and the 5-HT system (4,10). The relationship between the HPA axis and the 5-HT2A receptor has received particular attention. 5-HT2A receptors do not follow classical rules of receptor regulation (25). They are resistant to upregulation following a decrease in serotonin availability (3) and following the inhibition of serotonin synthesis (18). This is unlike most neurotransmitter systems that upregulate postsynaptic receptors after a depletion or a decrease in the neurotransmitter. Therefore, alternative mechanisms may be involved in 5-HT2A regulation that are independent of serotonin levels. Glucocorticoids may have regulatory effects on 5-HT2A receptors although the evidence is somewhat conflicting. Glucocorticoid removal by adrenalectomy has no effect on 5-HT2A receptor density in the frontal cortex of the rat (5,16), yet chronic corticosterone treatment increases 5-HT2A receptor density in the frontal cortex (16). Furthermore, adrenalectomy was shown to significantly increase 5-HT2A receptors in the hippocampus but not in the hypothalamus (20). Although glucocorticoids appear to be implicated in the regulation of 5-HT2A receptors, the conflicting results render this suggestion equivocal.

Chronic corticosterone treatment significantly increases the display of wet dog shakes (WDS) following the administration of the selective 5-HT2A agonist DOI (16). WDS are a behavioral response that consist of a rotational shudder of the head, neck, and trunk (1) and have been successfully employed as a noninvasive measure of 5-HT2A receptor activity in vivo (31). An inverse relationship exists between frequency of male copulatory activity and spontaneous WDS (28). This suggests that increased 5-HT2A activity mediates an inhibition of male sexual behavior (11) and a...
facilitation of WDS behavior. In support of this, brainstem injection of minute quantities of DOI increases WDS and inhibits sexual behavior (29).

The available evidence suggests that individual housing of the male rat results in elevated corticosterone levels and impaired sexual performance. Recent reports that corticosterone may be involved in the regulation of 5-HT2A receptors provide the impetus for the present study. By measuring behavioral changes in WDS and male sexual behavior, we are employing an in vivo reflection of underlying 5-HT2A activity; therefore, it may be possible to assess, albeit indirectly, the effects of individual housing on 5-HT2A activity in a noninvasive manner. The possibility exists that the effects of individual housing on sexual behavior are mediated, in part, by the upregulation of 5-HT2A receptor density in response to increased corticosterone secretion.

EXPERIMENT 1

Method

Subjects. Forty-five male Wistar rats were obtained from Charles River Canada Inc., Montreal, at 70 days of age. Males were housed in groups of three or four in standard wire mesh cages and were maintained on a reversed 12-h dark/12-h light cycle with the lights off at 0900 hours. They were provided access to receptive females on four separate occasions and were then screened for copulatory proficiency. Ten males were excluded from the study due to failure to exhibit ejaculatory behavior; thus, 35 rats were used in this study. All males were gonadally intact and were given ad lib. access to Purina Rat Chow and tap water in a housing environment maintained at 21°C. At the time of behavioral testing, males were approximately 7 months of age and their body weights ranged between 500 and 600 g.

Stimulus animals consisted of 14 sexually experienced female Wistar rats which were used for sexual behavior testing of the males. Stimulus females were housed in groups of three or four in similar conditions. In addition, all stimulus animals had previously been bilaterally ovariectomized. At the time of testing, stimulus females were 8 months of age.

Apparatus. Standard triple-wire mesh cages were used for group housing of rats, and single-wire mesh cages for individual housing of rats. Sexual behavior and WDS testing were conducted in either clear Plexiglas chambers (30 × 30 × 30 cm) or in clear glass cylindrical chambers (30 cm in diameter × 46 cm in height) with San-i-cel bedding covering the floors. A stopwatch was used to record the length of testing intervals, and all observations were recorded manually on standard score sheets by a trained observer who was blind to the housing conditions of the rats being tested.

Estradiol benzoate and progesterone were obtained from Sigma Chemical Co. (St. Louis, MO) and were used to induce receptivity in stimulus females. (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was obtained from Research Biochemicals International (Natick, MA) and was used in Experiment 2. Twenty-six-gauge 0.5-in. stainless steel needles were used for all injections.

Procedure. When males were approximately 27 weeks of age, 18 of them were randomly assigned to the experimental group and were housed individually in single-wire mesh cages. The remaining 17 males formed the control group and were housed in groups of three or four in triple-wire mesh cages. All subjects were housed in the same colony room. After 34 days of differential housing, males were tested for sexual behavior and spontaneous WDS. Each experimental or control male was placed in a testing chamber and allowed 5 min to habituate before presentation of a receptive stimulus female. Females were made receptive by subcutaneous injection of 10 μg of estradiol benzoate (dissolved in 0.1 cm³ of peanut oil) 48 h prior to testing and 500 μg of progesterone (dissolved in 0.1 cm³ of peanut oil) injected subcutaneously 3 h before testing. Receptive females were rotated from one testing chamber to another every 10 min to maintain the sexual interest of males. Tests were terminated at 30 min or after ejaculation. The frequency of mounts (with pelvic thrusting) and intromissions prior to ejaculation were recorded. The time from the introduction of the stimulus female to the first mount (mount latency) and the first intromission (intromission latency) were also recorded. The ejaculation latency was calculated as the time interval between the first intromission and ejaculation. Copulatory efficiency was calculated by dividing the number of intromissions by the sum of the number of intromissions and mounts. A high score for copulatory efficiency indicates that more copulatory attempts result in intromissions than mounts; this measure is relatively independent of motivational changes (24).

Results and Discussion

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Group Housed</th>
<th>Individually Housed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copulatory efficiency</td>
<td>0.46 ± 0.03</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>Mounts</td>
<td>12.2 ± 1.4</td>
<td>23.5 ± 3.4</td>
</tr>
<tr>
<td>Intromissions</td>
<td>9.6 ± 0.8</td>
<td>9.8 ± 1.5</td>
</tr>
<tr>
<td>Mount latency (s)</td>
<td>60.5 ± 28.7</td>
<td>347.1 ± 132.8</td>
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<tr>
<td>Intromission latency (s)</td>
<td>340.7 ± 127.5</td>
<td>563.4 ± 152.8</td>
</tr>
<tr>
<td>Ejaculation latency (s)</td>
<td>875.5 ± 150.1</td>
<td>1122.7 ± 155.6</td>
</tr>
<tr>
<td>IntromIs/h</td>
<td>0.11 ± 0.01</td>
<td>0.30 ± 0.17</td>
</tr>
<tr>
<td>% ejaculating</td>
<td>76</td>
<td>61</td>
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Results were analyzed using an independent samples t-test. A Bonferroni adjustment of α level was performed to control power and decrease the probability of obtaining Type I errors. This was done by dividing the familywise error rate by the number of variables to be considered. (In this case, α level was set at 0.10/7 = 0.014). A familywise error rate of 0.10 was accepted in Experiments 1 and 2 in order that not all statistical power was lost to Bonferroni adjustment. Therefore, a probability level below 0.014 was necessary to achieve statistical significance in these measures of sexual behavior and WDS. Levene’s Test for Equality of Variance was performed on each variable prior to t-test analyses.

Test results are presented as means and standard errors in Table 1. In addition, the percentage of animals which achieved ejaculation are reported in Table 1. Four of 17 group-housed animals and 7 of 18 individually housed animals did not ejaculate. Rats that failed to reach ejaculation during the test interval were dropped from the data analyses of mount frequency, intromission frequency, and copulatory efficiency, and missing latency scores were set to the maximum (1800 s). Levene’s Test for Equality of Variance was significant (p < 0.05) for mounts, intromissions, mount latency, and WDS. For these variables, a Welch’s correction to the degrees of freedom was performed prior to t-test analyses. An independent samples t-test indicated a significant increase in the number of mounts prior to ejaculation for the individually housed group, t(13.31) = 3.09, p < 0.01. Copulatory efficiency was significantly lower in individually housed rats, t(22) = 3.92, p < 0.01. No other significant differences were found.
although mount latency was close to reaching significance, \( r(18.58) = 2.11, 0.05 > p > 0.014. \) However, this measure failed to reach significance after Bonferroni adjustment to \( \alpha \) levels.

The present results replicate previous findings (7) that specific components of the sexual response pattern, such as frequency of mounts, copulatory efficiency, and mount latency, rather than all sexual responses, are affected by individual housing.

Casual observation of individually housed rats revealed that relative to group-housed rats, they exhibited the following symptoms: i) aggressiveness and tendency to bite when handled, ii) caudal dermatitis near the base of the tail, and iii) the loss of hair from the back of the head. This is consistent with the observations of Hatch et al. (15) that individual housing of rats is stressful.

The lack of significant differences for WDS is not surprising since only four rats displayed any WDS. Spontaneous WDS occurs relatively infrequently and the period of behavioral testing may have been too brief to provide sufficient data for statistical analysis. Because of the low frequency of WDS, it is not possible to attribute differences in sexual behavior to altered 5-HT2A activity.

**EXPERIMENT 2**

It was shown in Experiment 1 that individually housed male rats were impaired in their sexual performance. The lack of significant differences in spontaneous WDS behavior may be attributed to the overall infrequent display of this behavior. It is possible to increase the frequency of WDS either by testing for a significantly longer period of time or by administering a 5-HT2A agonist (28). In Experiment 2, both sexual behavior and WDS were observed following the administration of the 5-HT2A agonist DOI.

**Method**

Animals from Experiment 1 were maintained under their previously assigned housing condition for an additional 4 days. Thirty minutes before testing, each male was injected intraperitoneally with 1 mg/kg DOI dissolved in 0.9% saline (1 mg/cm³) which was prepared fresh just prior to the test session. Stimulus females were injected 48 h prior with 10 \( \mu \)g of estradiol benzoate and 3 h earlier with 500 \( \mu \)g of progesterone. Sexual behavior and WDS were scored, as previously described in Experiment 1.

As in Experiment 1, a Bonferroni adjustment of the familywise error rate was performed to control for Type I errors. Therefore, on measures of sexual behavior and WDS, each independent samples \( t \)-test must have yielded a probability level below 0.014 to be deemed as significant. In addition, Levene’s Test for Equality of Variance was performed on each variable prior to \( t \)-test analyses.

**Results and Discussion**

All results are presented as means and standard errors in Table 2. In addition, the percentage of animals which achieved ejaculation are reported in Table 2. Nine of 17 group-housed animals and 12 of 18 individually housed animals did not ejaculate. As in Experiment 1, rats that failed to achieve an ejaculation during the test session were dropped from the analyses of mount frequency, intromission frequency, and copulatory efficiency. Levene’s Test for Equality of Variance was significant (\( p < 0.05 \)) for mount frequency and intromission latency. For these variables, a Welch’s correction to the degrees of freedom was performed prior to \( t \)-test analyses. Table 2 suggests a general trend in the direction of increased mounts, reduced copulatory efficiency, and longer latencies in the individually housed group. These effects were not significant although statistical significance was close for intromission latency, \( r(28.6) = 2.21, 0.05 > p < 0.02. \) However, the frequency of WDS was significantly higher in the individually housed rats, \( r(33) = 2.68, p < 0.014. \)

Results from this experiment indicate that although a trend was present, there was no significant decrease in the sexual behavior of individually housed rats relative to group-housed rats. This is probably because DOI administration significantly reduced the number of animals ejaculating from 24 of 35 in Experiment 1 to 14 of 35 in Experiment 2. A McNemar change test confirmed that this reduction was significant (\( p < 0.01 \)). It is unlikely that prior testing 4 days earlier in Experiment 1 affected sexual performance in Experiment 2. It has been shown that male rats completely recover from the effects of sexual satiation within 96 h (17). In the current study, Experiments 1 and 2 were separated by a 96-h interval even though animals were not tested to sexual satiation in Experiment 1. Furthermore, a \( t \)-test comparing overall copulatory efficiency of group-housed animals before and after DOI treatment revealed that DOI administration induced a significant decrease, \( t(20) = 2.69, p < 0.02. \) This suggests that potential differences between individually housed and group-housed rats on measures of sexual behavior after DOI treatment may not be apparent due to the dampening effects of DOI on sexual behavior. This is consistent with reports of the inhibiting effects of DOI on male rat sexual behavior (11,28,29). Despite the inhibiting effect of DOI on ejaculatory behavior and copulatory efficiency, differences in WDS frequency between individually and group-housed animals were significant. This may reflect increased 5-HT2A activity in the individually housed rat.

**GENERAL DISCUSSION**

The present series of experiments indicate that short-term individual housing of male rats has inhibitory effects on the spontaneous expression of sexual behavior and facilitatory effects on the DOI-induced expression of WDS. Previous work has shown that central activation of 5-HT2A receptors concurrently induces WDS and inhibits male sexual behavior (29). Therefore it is possible that individual housing acts, at least in part, via a 5-HT2A receptor mechanism and this may involve an increase in the density of postsynaptic 5-HT2A receptors.

In addition to impairing male sexual performance, individual housing in the rat activates the HPA axis and causes an elevation in corticosterone levels (2,19). Studies in which male rats were stressed via other methods have demonstrated an inhibition of sexual behavior and elevation of corticosterone levels (12). Therefore, the increase in corticosterone levels following chronic individual housing may contribute to the observed reduction in sexual behavior.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Group Housed</th>
<th>Individually Housed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copulatory efficiency</td>
<td>0.32 ± 0.04</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Mounts</td>
<td>26.2 ± 5.9</td>
<td>32.0 ± 3.2</td>
</tr>
<tr>
<td>Intromissions</td>
<td>10.4 ± 1.9</td>
<td>10.0 ± 1.8</td>
</tr>
<tr>
<td>Mount latency (s)</td>
<td>758.0 ± 210.1</td>
<td>981.1 ± 202.3</td>
</tr>
<tr>
<td>Intromission latency (s)</td>
<td>942.4 ± 205.5</td>
<td>1493.6 ± 140.5</td>
</tr>
<tr>
<td>Ejaculation latency (s)</td>
<td>1245.5 ± 156.9</td>
<td>1399.3 ± 159.1</td>
</tr>
<tr>
<td>WDS/h</td>
<td>7.2 ± 2.3</td>
<td>18.6 ± 3.4</td>
</tr>
<tr>
<td>% ejaculating</td>
<td>47</td>
<td>33</td>
</tr>
</tbody>
</table>
The increase in DOI-induced WDS evident in Experiment 2 suggests activation of 5-HT_{2A} receptors. This is consistent with findings that rearing rats in isolation produces a significantly greater number of 5-HT_{2A}-mediated back muscle contractions (30). Evidence indicates that social isolation significantly elevates plasma corticosterone levels (2, 19). It has been reported that chronic administration of corticosterone results in an upregulation of 5-HT_{2A} receptors (16). Similarly, others have demonstrated an increase in 5-HT_{2A} binding in the parietal cortex of chronically stressed male rats (22). Also, corticosterone treatment increases the frequency of WDS in the male rat (16). The suggestion that corticosterone may be implicated in the upregulation of 5-HT_{2A} receptors is supported by results from the present study in that individually housed rats displayed more WDS behavior. The present data are consistent with the possibility that the detrimental effects of individual housing on male sexual performance are mediated by corticosterone. Studies in progress in our laboratory now reveal that corticosterone treatment inhibits male sexual performance (13).

Evidence indicates that short-term individual housing alters central serotonergic function. Three months of isolation has been shown to result in diminished neuronal sensitivity to serotonin in the striatum and nucleus raphis in the male rat (23). Additionally, isolation increases synaptosomal serotonin uptake in the striatum of alcohol-prefering rats (6). It is possible that increases in WDS in the present study reflect increases in 5-HT_{2A} receptor density. However, it is unlikely that decreased serotonergic function is responsible for an increase in 5-HT_{2A} receptor density. As noted earlier, the regulation of 5-HT_{2A} receptors does not follow the classical rules of receptor regulation and 5-HT_{2A} receptors do not upregulate in response to most pre- and post-synaptic manipulations (25). Therefore, it is possible that the upregulation of 5-HT_{2A} receptors occurs independently of presynaptic events. To date, no studies have directly investigated the effects of social isolation on 5-HT_{2A} receptor density. However, studies have demonstrated that other serotonergic receptors are altered in response to isolation. Individual housing of rats for 3 months significantly decreased the affinity of 5-HT_{1A} receptors (27). In addition, evidence connotes that another receptor of the 5-HT_{1A} family, the 5-HT_{1C} receptor, is more responsive in rats that were reared in isolation (9). Further studies need to be done to clarify directly what effect individual housing has on 5-HT_{2A} receptor density or sensitivity.

To summarize, the present data demonstrate that the stress of short-term individual housing of the adult male rat impairs sexual performance and increases WDS behavior. Both decreased sexual behavior and increased WDS are correlated with an increase in 5-HT_{2A} receptor activity. These results suggest a functional interaction between the HPA axis, 5-HT_{2A} receptor activity, and sexual behavior.

REFERENCES


25. Sanders-Bush, E. Adaptive regulation of central serotonin receptors


