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RAPID COMMUNICATION

Relation of Spontaneous Wet Dog Shakes and Copulatory Behavior in Male Rats[1]

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WATSON, N. V. AND B. B. GORZALKA. *Relation of spontaneous wet dog shakes and copulatory behavior in male rats.* PHARMACOL BIOCHEM BEHAV **37**(4) 825–829, 1990.—Pharmacological manipulation of 5-HT_2 activity has yielded equivocal effects on male rat sexual behavior. Both facilitation and inhibition of copulation have been reported following treatment with 5-HT_2 antagonists. "Wet dog shake" (WDS), a component of the Serotonin Behavioral Syndrome, is largely mediated by 5-HT_2 receptors. The present series of experiments were aimed at determining whether WDS might yield a spontaneous behavioral measure of 5-HT_2 activity that converges on the pharmacological data. In Experiment 1, spontaneous WDS was recorded in 58 male rats paired with receptive females. Normal copulators (Studs) exhibited significantly less WDS than did noncopulators (Duds). In Experiment 2, the males were additionally paired with males and nonreceptive females. In these situations, WDS did not discriminate Duds from Studs, but Studs scores differed from each other across the three different partner conditions. Lastly, in Experiment 3, treatment with the selective 5-HT_2 agonist DOI potently inhibited copulatory responses and increased WDS. Overall, the data from the three experiments suggest that 5-HT_2 activity mediates an inhibition of male rat sexual behavior.

Wet dog shakes Sexual behavior DOI Serotonin

COMMENCING with the initial report of 2 distinct types of receptors for serotonin (5-HT) in brain tissue (25), much work in the last decade has focussed on the pharmacological and physiological description of multiple 5-HT receptors in brain. To date, the evidence suggests that there are as many as six or more pharmacologically distinct central 5-HT binding sites that have been characterized as functional receptors (3, 8, 26). With regard to overt behavior, however, the influence of activation of each of these receptor subtypes remains incompletely characterized. Although 5-HT has been implicated in a variety of behaviors, including anxiety, emesis, copulation, aggression and others, the respective contributions of the various receptor subtypes to the expression of these behaviors is still largely unknown.

Recent work suggests that the individual 5-HT receptor subtypes have differing roles in the sexual behavior of rats. In addition, these functions appear to differ between the sexes (1, 10, 17, 23). Pharmacological manipulation of 5-HT_2 activity has yielded mixed effects on the sexual behavior of male rats. The 5-HT_2 antagonists pirenperone and ketanserin have both been reported to inhibit male rat sexual behavior (22). This suggests that 5-HT_2 receptors mediate a facilitation of sexual behavior. However, the newer and more selective 5-HT_2 antagonists LY53857 and LY281067 have been reported to facilitate copulation in male rats (7). These compounds were also effective in reversing the inhibition induced by treatment with the selective 5-HT_2 agonist DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane]. This second line of evidence thus suggests an inhibitory influence of 5-HT_2 receptor activity on male rat sexual behavior. Overall, therefore, evidence on the role of 5-HT_2 receptors in male rat copulatory responses remains equivocal.

Wet dog shaking (WDS), an element of the "Serotonin Behavioral Syndrome," appears to be primarily mediated by 5-HT_2 activity (12, 14, 30). A wet dog shake is a paroxysmal rotational shudder of the head and shoulders; other behaviors in the syndrome include reciprocal forepaw treading, Straub tail, hindlimb abduction and others (2, 4, 11, 16). The syndrome is induced by treatment with 5-hydroxytryptophan (5-HTP) plus carbidopa, or tryptophan plus a monoamine oxidase inhibitor, presumably re-

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sulting in supranormal or "spill-over" activation of serotonergic mechanisms (13,14). Generally, it has been found that WDS behavior is attenuated by administration of 5-HT antagonists, especially those that are more selective for 5-HT_2 receptors, such as ritanserin, ketanserin, pirenperone, cinanserin, cyproheptadine and pizotifen. It is elicited by 5-HT_2 agonists such as LSD and quipazine [(19, 21, 29); for review: (14)].

Data collected following intrathecal injection of serotonergic drugs and the 5-HT selective neurotoxin 5,7-dihydroxytryptamine suggest that WDS is mediated by brain 5-HT_2 receptors rostral to bulbospinal serotonergic neurons (6). Since WDS is also not affected by total ablation of frontal cortex, which is rich in 5-HT_2 binding sites (20), it appears that the cells mediating the response are located in the mid- or hindbrain, although no precise localization has yet been made.

It seems reasonable to suppose that the frequency of WDS occurring spontaneously during other behaviors might provide a type of "bioassay" of concurrent serotonergic activity. More specifically, combining measures of WDS with measures of male rat sexual behavior might yield a behavioral index of 5-HT_2 activity that converges on the pharmacological data, with regard to the effects of 5-HT_2 receptor activity on male rat copulatory responses.

In any group of male rats, a certain proportion reliably fails to initiate normal copulation when confronted with a receptive female, even after numerous trials (5,28). This presents the opportunity to perform a natural experiment, in which the responses of noncopulators are compared with those of normal copulators. In the present experiments, the incidence of spontaneous WDS was compared between copulators and noncopulators under various conditions.

GENERAL METHOD

Animals

Male and female Sprague-Dawley rats were derived from stock originally obtained from Charles River Canada Inc., Montreal. Rats were housed separately by sex, 6 per cage in standard wire mesh cages, with free access to food and water. All animals were maintained on a reversed 12/12 hr light cycle.

Approximately 70-day-old female rats were bilaterally ovariectomized while under sodium pentobarbital anesthesia (65 mg/kg IP). Males were approximately 100 days old when tested.

Behavioral Testing

All testing was performed during the middle 1/3 of the dark cycle. The experimental males were placed in clear Plexiglas chambers (30 × 30 × 45 cm), and allowed five minutes to habituate to their surroundings. At the end of five minutes, a stimulus animal was introduced, and behavioral observations commenced. In each experiment, the incidence of WDS produced by the male rats was recorded over a period of 60 minutes. Additionally, the occurrence of mounts, intromissions and ejaculations was scored in Experiments 1 and 2, and ejaculations were scored in Experiment 3.

EXPERIMENT 1

In the first experiment, the relationship of spontaneously occurring WDS to varying copulatory proficiency was examined in 58 sexually inexperienced male rats.

Procedure

After habituation to the observation chambers, males were presented with a receptive female. Receptivity was induced in the females by subcutaneous injection of 10 μg estradiol benzoate (Steraloids) 48 hours prior to testing, and 500 μg progesterone (Steraloids) 4 hours prior to testing. Steroids were dissolved in 0.1 ml peanut oil.

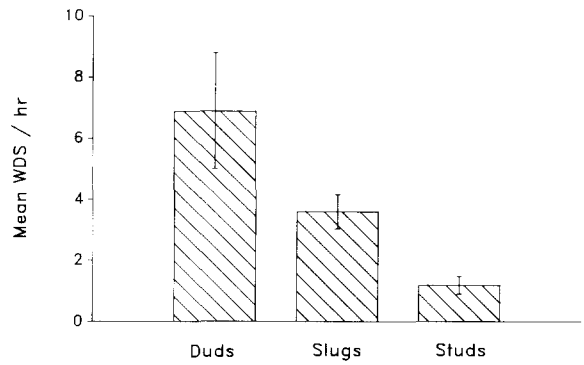

FIG. 1. Spontaneous wet dog shakes (WDS) during exposure to sexually-receptive females (given as mean ± S.E.M.). "Duds" (n = 8): males that showed no sexual behavior. "Slugs" (n = 7): males that showed mounts, or mounts and intromissions, but failed to ejaculate during the test session. "Studs" (n = 43): males that ejaculated at least once during the test session.

The incidence of each of the following 4 behaviors was recorded for each male rat: WDS, mounts with pelvic thrusting, intromissions, and ejaculations. During each 60-minute session 4 males were evaluated simultaneously, in separate chambers. Stimulus females were rotated between males every 15 minutes.

Results and Discussion

The experimental males were classified into groups on the basis of their sexual behavior in the testing sessions. Inspection of these data suggested that, in fact, three groups could be discriminated on the basis of their sexual performance (see Fig. 1 for details). The incidence of spontaneously generated wet dog shaking for these three groups is presented in Fig. 1. Mean WDS scores differed markedly between the copulators ["Studs": (5)] and noncopulators ["Duds": (5)], with Duds expressing about four-fold more shaking than Studs. The Slugs group, consisting of males that mounted or mounted and intromitted during the test session, but did not ejaculate, appears to be a subset of Duds, and showed an intermediate level of WDS.

Overall analyses of the three groups were significant by both nonparametric (Kruskal-Wallis ANOVA: $\chi^2 = 20.1$, $p < 0.0001$) and parametric [ANOVA: $F(2,55) = 14.46$, $p < 0.00005$] tests. Subsequent pairwise comparisons using Mann-Whitney U-tests were significant ($p < 0.05$) for all comparisons except Duds vs. Slugs, and using the Newman-Keuls procedure all pairwise differences were significant ($p < 0.05$). Thus, the mean WDS scores for the three experimental groups were all significantly different from one another (although the difference for Duds and Slugs was marginal). Both sexual performance and WDS thereby appeared to discriminate between the Duds, Slugs and Studs groups.

EXPERIMENT 2

The results of Experiment 1 suggested that male rat sexual behavior is incompatible with the expression of WDS. However, the observed effect might be an artifact of some other mediating

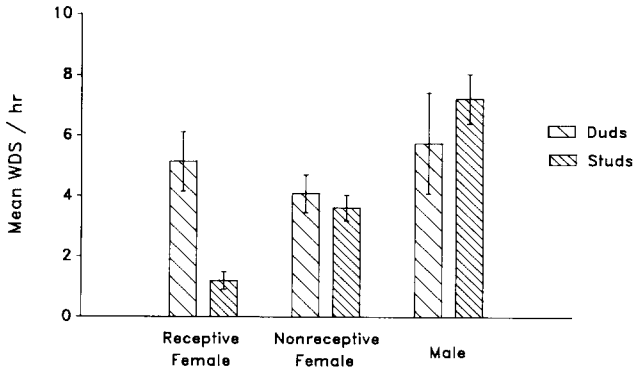

FIG. 2. Spontaneous wet dog shakes (WDS) performed by male rats with various types of partners in the testing chamber (given as mean ± S.E.M.). "Duds" (n = 15): males that failed to ejaculate during tests with receptive females; "Studs" (n = 43): males that ejaculated at least once.

variable. For instance, a difference in autonomic activity or general arousal levels might predispose certain males to both shake excessively and fail to copulate, without the two behaviors being directly related. If this were true, one would expect to find the difference in WDS between Duds and Studs to occur regardless of the type of stimulus animals presented. It was therefore of interest to determine what pattern of results would emerge when the male rats were paired with other types of stimulus animals.

Procedure

The same 58 male rats were tested for the incidence of spontaneous WDS following the procedure used in Experiment 1. However, in addition to the receptive females, the experimental males were also presented with nonreceptive female and male stimulus animals, in counterbalanced fashion. Testing continued until each experimental animal had been tested with each type of partner. As previously, the test duration was one hour, and stimulus animals were rotated between the experimental males every 15 minutes.

Results and Discussion

Results of Experiment 2 are presented in Fig. 2. Since Slugs appear to be a special case of the Dud category, Slugs are combined with Duds in the figure. Spontaneous WDS was significantly greater in Duds than in Studs when paired with a receptive female. However, Duds and Studs displayed equivalent levels of WDS when paired with either nonreceptive females or males. Interestingly, while the mean WDS scores for Duds did not differ across the 3 test conditions (Friedman ANOVA, $\chi^2 = 0.433$, NS), mean WDS scores for Studs were significantly different ($\chi^2 = 43.41$, $p < 0.0001$). Wilcoxon paired comparisons showed that the Studs scores differed from each other under each of the three conditions ($p < 0.0005$ for each comparison). Thus, Studs showed maximally suppressed WDS in the receptive female condition, moderate levels of WDS when exposed to a nonreceptive female, and high levels of WDS when exposed to another male. Subjectively, these effects did not appear to be related to overall activity levels, as Duds explored their cagemates quite actively throughout the test session. Differences in the incidence of grooming did not appear to explain the effects, either. In fact, copulating males appear to engage in far more grooming than do noncopulators. However, these measures were not quantitatively evaluated.

The results of Experiment 2 thus support and extend the suggestion that spontaneous WDS and male rat copulation are mutually exclusive. Moreover, Stud males show significantly less WDS in the presence of nonreceptive females than in the presence of males. Since the Stud animals were not copulating in either of these situations (although a few Stud males were observed to attempt to mount the nonreceptive females), the marked suppression of WDS seen in tests with receptive females is not readily explained in terms of differential activity levels or as an epiphenomenon of the copulatory behavior itself.

EXPERIMENT 3

The results of Experiments 1 and 2, coupled with the pharmacological evidence of 5-HT_2 mediation of WDS, support the idea that the spontaneous WDS expressed during other behaviors might reflect concurrent serotonergic activity. This notion would be strengthened by evidence from pharmacological manipulation. In Experiment 3, a preliminary drug study was conducted in which male rat copulatory behavior and simultaneously occurring WDS were examined in rats treated with the selective 5-HT_2 agonist DOI (9).

Procedure

Three groups of 20 sexually experienced males received either 0.1 mg/kg DOI (Research Biochemicals), 1.0 mg/kg DOI, or the saline vehicle in an equivalent volume (approximately 0.4 ml). DOI HCl was dissolved in physiological saline immediately before administration, and all injections were given subcutaneously 30 minutes prior to behavioral testing, which consisted of scoring for the incidence of WDS and ejaculations by the males while paired for 60 minutes with a receptive female.

Results and Discussion

The effect of the DOI treatment on sexual behavior and simultaneous WDS is depicted in Fig. 3. Overall tests of these measures were performed using the Kruskal-Wallis nonparametric ANOVA: these revealed that DOI had a significant effect on both the number of ejaculations ($\chi^2 = 21.02$, $p < 0.0001$), and on WDS ($\chi^2 = 32.24$, $p < 0.0001$). Subsequent paired comparisons (Mann-Whitney U-test) revealed that all three observed levels of WDS were significantly different from one another ($p < 0.0005$), with the greatest incidence of WDS occurring at the highest dose of DOI (1.0 mg/kg). Concurrently, significantly fewer ejaculations occurred in animals that received 1.0 mg/kg DOI than in either of the other two groups.

GENERAL DISCUSSION

Wet dog shaking appears to discriminate male rats of varying copulatory proficiency. The first experiment showed that Studs produced very few spontaneous shakes, and Experiment 2 confirmed that this effect was limited to situations in which a receptive female was present. In a third experiment, the selective 5-HT_2 agonist DOI (1.0 mg/kg) was found to exert reciprocal effects on WDS and sexual behavior, totally suppressing sexual behavior while maximally increasing WDS.

Taken together, the results of these experiments support the conclusion that copulatory behavior in the male rat is incompatible with the expression of WDS. Unlike the data obtained from Duds, who maintained consistently high levels of WDS regardless of partner in the testing chamber, WDS was virtually absent in Studs paired with receptive females. Moreover, Studs showed significantly increased WDS when paired with nonreceptive fe-

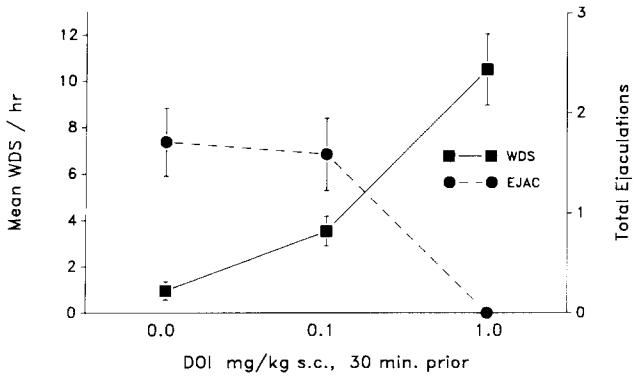

FIG. 3. Effect of DOI on wet dog shakes (WDS) and ejaculations (given as mean ± S.E.M.), in males paired with receptive females (n = 20 per treatment).

males, and even higher levels when paired with other males. Thus, in Studs, the characteristics of the partner in the testing chamber yielded an effect on the spontaneous expression of WDS that resembled a dose-response. The significant difference in WDS between the nonreceptive female and male partner conditions reflects either a net increase in Stud WDS scores with male partners, or a partial attenuation with nonreceptive females. One admittedly speculative interpretation of the difference is that it may reflect differential involvement of a mechanism that inhibits sexual behavior under inappropriate conditions. Such a mechanism would make ecological sense, since inappropriate sexual advances would be at best somewhat costly, in terms of energy expended in attempting to inseminate a nonreceptive female, or in provoking aggression if directed at a male. Despite the lack of receptivity, proceptive behavior and appropriate olfactory cues in the nonreceptive female condition, it seems reasonable to assume that the nonreceptive females still possess more sexually stimulating characteristics than do the male partners, and that such stimuli may partially reduce the inhibition. If the hypothesized mechanism relied on a tonic 5-HT_2-mediated influence, this differential inhibition might also be manifest in the differing levels of WDS. In any case, given that WDS is indicative of concurrent 5-HT_2 activity, the data from Studs suggest greater 5-HT_2 activity in the presence of male partners than nonreceptive females.

Other possible influences on the 5-HT syndrome that have been discussed in the literature include noradrenergic and dopaminergic activity, certain hormone actions and other mechanisms (6, 14, 15, 18). However, the evidence generally supports the conclusion that the elements of the syndrome are 5-HT mediated, with other transmitters or mechanisms serving ancillary or permissive functions (14). In addition, although morphologically similar to the pinna reflex exhibited by many species in response to mechanical stimulation of the ear canal, WDS and the pinna reflex appear to be physiologically unrelated (19). WDS is reliably and potently induced by 5-HTP loading or treatment with serotonin agonists (12,14). In fact, pharmacologically induced WDS in conjunction with the 5-HT behavioral syndrome has gained some acceptance as a model of neuronal 5-HT activity [e.g., (21,30)]. In the present study, stimulation of 5-HT_2 receptors with the 5-HT_2 agonist DOI produced, simultaneously, increased WDS and maximal inhibition of sexual behavior. Similarly, the selective 5-HT_2 antagonist ritanserin potently blocks phencyclidine-induced WDS in rats (24). Evidence that ritanserin is also effective in reversing DOI-induced inhibition of male rat copulatory behavior (27), further supports the notion of a relationship between 5-HT_2 activity, WDS and sexual behavior.

In summary, to the extent that WDS provides a valid index of concurrent 5-HT_2 activation, the present results extend the pharmacological data suggesting that 5-HT_2 receptors mediate an inhibition of male rat sexual behavior. Moreover, spontaneous WDS may provide a useful noninvasive approximate measure of 5-HT_2 activity during other types of behavior. Work is underway to determine the reliability and specificity of the spontaneous WDS measure in this context.

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