AN ANALYSIS OF BEHAVIORAL AND SEROTONERGIC MECHANISMS IN MALE RAT COPULATORY BEHAVIOR

by

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First, I would like to thank Freya Weizenbaum for her optimism and support, without those I would never have finished.

I would like to dedicate this thing to my wife who put up so well with the anger and the frustration.

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Organization of Copulatory Behavior in the Male Rat

Male copulatory behavior is usually described as being controlled by a minimum of two discrete mechanisms (i.e., Beach, 1956). The analysis presented in this paper was designed to further explicate the notion of multiple mechanisms, and the behavioral parameters subsumed under each of those mechanisms.

The copulatory behavior of the male rat is composed of several discrete behaviors; mounts, intromissions, and ejaculations. A mount consists of the male approaching the female from behind, clasping her around the midsection of her body, and displaying shallow pelvic thrusts. An intromission is a mount with the addition of penetration of the vagina by the male's penis. Thus, following completion of an intromission the male dismounts with a rapid or explosive motion, typically followed by genital grooming on the part of the male. An ejaculation consists of an intromission with the addition of deposition of sperm into the female's vagina. Ejaculations can be differentiated behaviorally from both mounts and intromissions. The ejaculatory pattern is characterized by a single deep thrust which is held for about a second, and followed by a spreading of the forepaws and a slow dismount.

A typical copulatory sequence in a sexually experienced male is as follows. The sequence of copulatory behaviors begins with the introduction of a behaviorally receptive female and a male to a mating arena. The male approaches the female and immediately investigates her anogenital region. Following this investigation the male mounts the

female and then rapidly proceeds to make his first intromission. He intromits approximately ten more times over the course of the next ten to fifteen minutes. Interspersed among these intromissions are several mounts. In addition to sexual behaviors, the male emits other behaviors which are not specific to a copulatory situation. These behaviors include grooming, rearing, and nasocontact with the female. The final component of an ejaculatory series is the ejaculation. Following an ejaculation the male grooms his genital area, then the rest of his body, and then lays down for approximately five minutes. This period of inactivity has been refered to as the post-ejaculatory interval (PEI). Some researchers divide this period of inactivity into an absolute and a relative phase. It is thought that in the absolute phase no amount of stimulation will induce the male to begin a new copulatory sequence, and that in the relative phase only rather intense stimuli will induce the male to begin a new copulatory sequence (Karen & Barfield, 1975). The end of the PEI is marked by the occurence of an intromission which also marks the beginning of the next copulatory sequence. The absolute and relative refractory periods have been operationally defined. The absolute refractory period is the time from ejaculation until the end of the male's 22 kHz vocalizations (VT). This period constitutes approximately three quarters of the total PEI. The remaining or nonvocalizing portion of the PEI is the relative refractory period (PEI-VT) (Barfield & Geyer, 1975).

The copulatory pattern of the male rat has been parametrically defined. The standard measures of copulatory behavior are the following: latency to the first intromission (IL), latency to the first

mount (ML), latency to ejaculation (EL), the average interval between successive intromissions (III), mount frequency (MF), intromission frequency (IF), and hit rate which is defined as the number of intromissions divided by the number of intromissions plus the number of mounts (HR).

The copulatory sequence of inexperienced male rats follows the same pattern as that described above for vigorous copulators except that the timing of the behaviors is slower, and there are more mounts included in the sequence. Briefly, the latency to the first mount generally will be much longer in inexperienced males, and a number of mounts will usually preceed the first intromission. While the experienced male will have about twice as many intromission as mounts throughout the copulatory sequence, the inexperienced male will have about an equal number of these two behaviors. The time between each intromission will be much longer in the inexperienced male, and the latency to its ejaculation will greatly exceed ten minutes. Finally, the PEI in these animals will be longer than five minutes.

The oldest view of this sequence of behaviors assumed that there was a single motivational force directing it, and that once the sequence was initiated it continued reflexively until it was completed (Miller & Dollard, 1941). Then Beach (1956) proposed a theory suggesting that the sequence of masculine copulatory behavior was composed of two distinct components. These two components are a sexual arousal mechanism (SAM) and an intromission and ejaculation mechansim (IEM). When Beach proposed these behavioral mechanisms he suggested that they represent the organization of masculine copulatory behaviors, and that they be

used to generate hypotheses about distinct anatomical referents or separate motivational components involved in control of specific behaviors. The sexual arousal mechanism channels the available behavioral arousal in order to produce the sexual arousal required for the initiation of the copulatory sequence. Therefore, the SAM is operationally reflected by the time required for the male to begin the copulatory sequence, which is usually measured as the latency to the first intromission. It is not clear from Beach's discussion of this mechanism how sexual arousal differs from a more general behavioral arousal, nor how the mechanism completes its process of channeling the behavioral arousal. It is clear that the sexual arousal is hypothesized to build up until it reaches some critical level, and then the second mechanism is triggered.

The second of the two mechanisms controls or organizes the execution of the copulatory sequence. This IEM regulates the copulatory behaviors which culminate in an ejaculation. This second mechanism is operationally reflected by the time required to achieve an ejaculation, and is usually measured by the latency to ejaculation. Beach also proposed that this mechanism controls the timing of the intromissions which lead to the ejaculation. The IEM then controls the entire sequence of copulatory behaviors up to and including the ejaculation.

The SAM and IEM are proposed to be consecutive rather than concurrent or overlapping, because the completion of the first mechanism leads to the activation of the second. The consecutive nature of these two mechanisms suggests that they may be independent. The exception to this independence is that the completion of the SAM may activate

the IEM. The speculation that these two mechanisms have independent control over the timing and occurrence of copulatory behaviors also suggests that there may be independent neural mechanisms controlling these two mechanisms.

The two component theory is widely accepted and often invoked to explain the results observed in physiologically oriented studies of masculine copulatory behavior (i.e., Ryan & Frankel, 1978). However, despite this wide acceptance for the two mechanism theory there does not seem to be many studies which directly test the independence of these two mechanisms. A recent study by Karen and Barfield (1975) provided support for a unitary IEM. In that study male rats were allowed to copulate to exhaustion. The curves of the rate of change for various copulatory parameters over successive ejaculations were compared. It was hypothesized that similar curves would indicate that the parameters of those curves are controlled by the same mechanism. From the comparison of these exhaustion curves it was determined that the latency to ejaculation, the average interval between intromission, and the frequency of intromissions all followed similar exhaustion patterns. According to the stated hypothesis, the similarity of these patterns was interpreted as indicating that all of these parameters are controlled by a single mechanism. In contrast, the exhaustion curves for the post-ejaculatory interval differed from those described by the latency to ejaculation. Furthermore, the PEI was divided by these researchers into a relative and an absolute phase, and the exhaustion curves for these two phases differed both from each other and from the other exhaustion curves. Again, according to the stated hypothesis, these

patterns were interpreted as indicating that the PEI is controlled by two relatively independent mechansims. Karen and Barfield interpreted their results as support first for a unitary IEM, and second for two separate recovery mechanisms within the post-ejaculatory interval which are both independent from each other and from the IEM. Because the usual measure of the SAM, the latency to the first intromission, does not lend itself to the above analysis, a specific examination of the SAM was not included in that study.

Despite such support Beach himself and others (Beach, Westbrook & Clemens, 1966; and Sachs & Barfield, 1976) have questioned the validity of the two mechanism hypothesis while still affirming the importance of such theoretical models. Conceptualizing the mechanisms which control male copulatory behavior is necessary for organizing the physiological mechanisms which control masculine copulatory behavior (Sachs, 1978). Therefore, there have been several recent attempts to organize the parameters of masculine copulatory behavior into larger units (Sachs, 1978; and Dewsbury, 1979). These studies have employed factor analytic techniques. The factor analysis technique organizes correlations among several variables in such a way that the common variance among variables is assessed; and the resulting correlational matrix is organized into common sources of variance of factors. This technique overcomes a major disadvantage of the correlation matrix, which is that a lack of significant correlation may obscure a common underlying variance. In addition, factor analysis accounts for redundant correlations, whereas a simple correlation analysis does not. As a result, factor analysis is a more rigorous statistical method for grouping variables into categories. These

categories, or factors, are interpreted as unitary constructs, or as underlying mechanisms.

Sachs, in 1978, statistically analyzed a single ejaculatory series of sexually experienced male rats. The copulatory data was collected in three independent laboratories (by Sachs at the University of Connecticut, by Barfield at Rutgers University, and by Bermant at the Battelle Seattle Research Center). Like Beach's original statistical analysis, Sachs presented the intercorrelations among copulatory parameters (ML, IL, MF, IF, HR, III, EL, VT, PEI-VT, and PEI). The intercorrelations were based primarily on Sach's and Barfield's data because Bermant's data did not include several of these measures. Statistically reliable intercorrelations were ML with IL, MF with HR, III with EL, III with VT, III with PEI, EL with VT, EL with PEI, VT with PEI, and PEI-VT with PEI. Sachs did not emphasize these results because in the factor analysis technique these correlations are only an intermediate step.

In Sachs' factor analyses four factors emerged: Copulatory Rate (III, EL, PEI, VT), initiation (ML, IL, PEI-VT), Hit Rate (MF, HR), and Intromission Count (IF, PEI-VT). He found the copulatory rate factor to account for the most variance, approximately 40%. Each of the other factors accounted for about 15% of the variance. In light of these results, Sachs hypothesized that there are at least four mechanisms underlying male copulatory behavior. These mechanisms are represented by the four factors but not necessarily equivalent to them.

Sach's four factors can be compared to Beach's two mechanisms. Sachs' Initiation Factor is obviously equivalent to Beach's Arousal Mechanism in that they both contain latency to the first mount and to

the first intromission. However, Sachs does not present a single factor which is comparable to the IEM. Rather Beach's IEM is represented in the three remaining factors. Of particular importance are the Copulatory Rate Factor and the Hit Rate Factor. The Copulatory Rate factor contains the two parameters in IEM that Beach considered most important i.e., the III and the EL. Therefore, the Copulatory Rate Factor also would appear to be a particularly significant factor. This factor denotes the two parameters which are direct indices of the timing of the execution of the copulatory sequence. In contrast, the remaining two factors, the Hit Rate Factor and the Intromission Count Factor, both contain parameters which reflect frequency of measures. Thus, the three factors related to the IEM divide that mechanism into three separate processes one of which refers to the duration of the IEM, another is concerned with the timing of events within the IEM and the third which relates to the frequency of the IEM events.

Dewsbury (1979) subsequently confirmed the general outline of Sachs' findings. In that study sexually vigorous rats were mated on three tests, 5 ejaculations per test. Dewsbury employed the same measures as Sachs with the exception of the vocalization parameters, as a result the VT and PEI-VT measures were not included in his factor loading patterns. Factor analyses on the first ejaculatory series of each test resulted in three factors. The loading patterns of the three factors with eigen values greater than one were similar to three of Sachs' factors.

Dewsbury considered these three factors similar enough to those of Sachs that he also named them Copulatory Rate Factor, Initiation

Factor, and Hit Rate Factor. On each of the three tests the Copulatory Rate Factor contained III, EL, and PEI, the Initiation Factor contained IL and ML, and the Hit Rate Factor contained HR and MF. The only factor which did not replicate was Intromission Count, and IF, the major component of that factor, was variably associated with each of the three factors in Dewsbury's analyses. Dewsbury interpreted the inconsistency in the intromission frequency variable as meaning that it was not strongly tied to any of the three factors, and therefore, the overall factor pattern was in agreement with Sachs' analyses. Supporting his interpretation were the results of the factor analysis of all five ejaculations, this analysis did retain a separate Intromission Count Factor. Intromission frequency strongly and consistently loaded on this factor. In addition, EL loaded on the Intromission Count Factor; however, this variable was inconsistent, and had low factor loading scores, approximately .40.

The above analyses all resulted from data collected on sexually vigorous rats. Such groups constitute a selected subset of the distribution of sexual vigor among laboratory rats. For example, in our lab (Goff & Weizenbaum, unpublished) approximately 30% of our animals will not have ejaculated at the end of five one hour tests. Analyses of the behavior of sexually vigorous male rats would be likely to reduce the variance because the range of individual differences was attenuated by the selection process. Therefore, it is not clear that the results of Sachs' and Dewsbury's Factor Analyses are similar because they represent a generalizable finding which is applicable to the whole population, or because they have both analyzed similar subsets

of that population. In order to reduce that bias in the present study ejaculatory experience was explicitly varied by selecting male rats that represented a broad range of sexual vigor.

METHODS

Subjects: Seventy-two, sexually naive male rats, approximately 90 days of age, were the subjects. These Spraque-Dawley rats were bred by Charles River Breeders and born in the colony at VPI & SU. The rats were individually housed in a reversed 14L/10D cycle, with lights out at 1200 EST. Lab chow and tap water were continuously available.

Stimulus animals: Fifteen females of the same strain and age as the experimental subjects were used as stimulus animals in the copulation tests. These animals were housed five in a cage with all other housing conditions similar to those of the experimental animals. These females were housed in a separate room from the males. Behavioral estrous was induced in these animals with subcutaneous injections of estradiol benzoate (.01 mg in .1 cc peanut oil) 78 and 30 hours prior to testing, and a subcutnaeous injection of progesterone (.02 mg in .1 cc peanut oil) 5 hours prior to testing.

Behavioral methods: Sexual behavior of the males was observed while they were paired with an estrous female. The mating arena (40 cm X 24 cm X 18 cm) was clear plastic, and its floor was covered with pine shavings. Observations of the sexual behavior of these animals was made for 30 minutes, or until the first intromission following an ejaculation, whichever occurred first. A thirty minute limitation on the observation period was chosen in order to reduce the number of animals which would gain complete copulatory experience on each trial.

The occurrence of mounts (approach from behind with palpation of the female's flanks and pelvic thrusting), intromissions (mounts with insertion of the penis, characterized by a quick dismount), and

ejaculations (intromissions with deposition of sperm, characterized by a longer duration mount, a spreading of the forepaws, a slow dismount, often vocalization, and genital grooming) were recorded on an Esterline Angus event recorder. From these records the latency to the first mount (time from the introduction of the female to the occurence of the first mount), the latency to the first intromission (time from the introduction of the female to the occurence of the first intromission), the latency to ejaculation (time from the occurence of the first mount to the occurence of the first ejaculation), the post-ejaculatory-interval (time from the occurence of an ejaculation to the next intromission), the frequency of mounts, the frequency of intromissions, the inter-intromission interval (the average time between successive intromissions), and the hit rate (the frequency of intromissions divided by the frequency of intromissions plus the frequency of mounts) were calculated.

Procedure: Subjects were selected on the basis of an initial 30 minute screen for copulatory behavior. This screening procedure consisted of placing a male individually in a mating arena with a stimulus female. Occurence of ejaculations and frequency of intromissions were recorded during the 30 minute test. Experimental subjects were selected based on these two measures. This selection was made in such a way as to provide the widest possible range of individual differences in copulatory behavior. Specifically, the range of copulatory behavior represented in the subject sample included animals which ejaculated in a very short time, animals which did not mount in the 30 minutes, and animals representing the range between these two extremes. Thus

the sample included in subsequent analyses represented a broad distribution of sexual vigor.

Copulatory behavior of the 72 rats was observed on four separate days, within a thirteen day period. On these tests the males were placed in the observation arena, allowed one minute acclimation to that arena, and then an estrous female was introduced into the arena. Observations of copulatory behavior were made for 30 minutes or until the occurence of the first intromission following an ejaculation, which ever occured first. All testing was carried out between 0100 and 0500 PM EST. The test room was illuminated with 60 watt red lights.

RESULTS

The first statistical approach to these data was to determine when each of the eight parameters became stable. Determination of stability would permit collapsing the data for those trials, and would in turn reduce variance due to extraneous factors. The multivariate analysis of variance (MANOVA) was used to test for differences in the 8 copulatory parameters across the four trials. Only animals which completed a copulatory sequence (ejaculated) on all four trials were included in the MANOVA, because this statistical technique requires complete data. Twenty-seven animals met this criterion. Following a significant overall statistic (Wilk's L = .259, F approximation - $F(24,116) = 2.89$, $p < .001$) each of the eight parameters were analyzed separately in a one way analysis of variance (n = 27). The purpose of these statistics was to determine which parameters contributed to the significant overall statistic.

The MANOVA described above did not include animals which had not ejaculated in all four tests but did exhibit some copulatory behavior on each trial. For example, an animal could have mounted and intromitted in the first two trials and ejaculated only on trials three and four. In order to confirm the pattern described by the MANOVA, the individual ANOVA's were repeated with the addition of those subjects which contributed a score to that particular parameter on all four trials. This resulted in an n of 49 for ML, 61 for IL, 72 for MF and IF, 59 for III, and 66 for HR. The results of the individual ANOVA's are presented in Table 1. These analyses showed that the only set of trials for which all eight parameters were stable in both statistical

Table 1

Means and Standard Errors for Eight Parameters of Male Rat Copulatory Behavior.

parameter	n	Trials 1	2	3	4	F	p
ML	27	266±54[a]	191±42[b]	228±52[a,b]	138±29[b]	2.80	.05
	49	375±55[a]	211±40[b]	249±43[b]	174±31[b]	4.86	.01
IL	27	288±54[a]	119±35[b]	114±33[b,c]	56±9[c]	15.44	.001
	61	452±63[a]	212±35[b]	121±20[c]	82±15[c]	25.06	.001
MF	27	4.1±.7	2.0±.3	3.2±.6	3.5±.8	1.70	NS
	72	3.8±.6	2.4±.4	2.9±.5	3.8±.6	1.29	NS
IF	27	19.3±1.9[a]	12.7±1.9[b]	15.6±1.0[a]	15.9±1.4[a]	3.90	.05
	72	9.6±1.3[c]	11.1±1.0[b,c]	13.2±1.0[a,b]	15.5±1.0[a]	6.99	.001
III	27	38.7±2.6	61.0±5.9	42.0±4.9	43.4±4.4	1.20	NS
	59	77.9±19.4	85.8±9.0	57.5±13.3	57.7±5.6	2.01	NS
HR	27	78.7±2.6	81.7±1.9	79.4±2.3	79.7±2.5	.12	NS
	66	57.3±4.9[b]	79.1±2.6[a]	74.2±3.4[a]	80.5±2.0[a]	12.98	.001
PEI	27	300±11.8	299±14.2	305±12.1	320±12.9	.50	NS
EL	27	630±58.9[a]	582±54.0[a,b]	569±57.0[b,c]	554±54.1[c]	4.16	.01

Different superscripts indicate means which are significantly different by a Duncan's Multiple Range test, $\alpha < .05$.

tests were trials three and four.

Since the behavior of the animals was stable over the last two trials, the mean of those trials was obtained for all the rats that had ejaculated on both tests three and four. These values were used in the factor analysis because they provided three advantages. First, they allowed for elimination of some of the variation that may have been due to the separate tests, error variance. Second, they provided a larger sample than would have been possible if all four trials had been used because factor analysis requires complete data; many fewer animals (about 30%) achieved ejaculations in the first two trials. Third, they allowed inclusion in the analysis of animals which achieved their first ejaculation on the third trial, as well as animals which achieved their third ejaculation on that same trial, and thus the sample retained a range of individual differences, as had been sought in the original selection of animals. Fifty-three animals were included in the factor analysis.

The intercorrelations among the eight measures of copulatory behavior are presented in Table 2. There were significant correlations between ML and IL; PEI and MF; III and IL, EL, MF; and HR and EL, MF. These significant correlations are similar to those reported by Sachs (1978) and Dewsbury (1979).

The factor analysis retained the three factors which had eigen values greater than one. A varimax rotation was performed on these factors. The rotated factor pattern is presented in Table 3. In keeping with convention, and in an attempt to increase replicability, a variable was considered to load on a factor if it had a loading score

Table 2

Intercorrelations of Eight Parameters of Male Rat Copulatory Behavior

	ML	IL	EL	MF	IF	PEI	III	HR
ML	1.00	.47**	.14	-.17	.02	.04	.21	.02
IL		1.00	.26	.03	-.11	.12	.32*	-.01
EL			1.00	.39**	.11	.06	.61**	-.60**
MF				1.00	.13	.28*	.37**	-.60**
IF					1.00	-.09	-.12	.08
PEI						1.00	.25	-.08
III							1.00	-.27

*p < .05, **p < .01

Table 3

Rotated Factor Pattern for Male Rat Copulatory Behaviors

Measure	Factor 1 Copulatory-Efficiency	Factor 2 Initiation	Factor 3 Intromission Count
EL	.783	.328	.118
III	.602	.438	-.275
HR	-.829	.116	.117
MF	.832	-.102	-.011
ML	-.041	.821	.029
IL	.077	.823	-.130
PEI	.233	.018	-.585
IF	.171	-.087	.824
% Variance	30.57	21.02	14.28
Cumulative Variance	30.57	51.59	65.88

greater than .50 (or accounted for more than 25% of the variance in that variable). Factor 1 includes variables from both the Copulatory Rate and Hit Rate Factors and is named Copulatory Efficiency. The latency to ejaculation, inter-intromission interval, and the number of mounts all had large positive factor loading scores, while the hit rate ratio had a large negative factor loading score on this first factor. Factor 2 closely resembles the factor which Sachs had labled Initiation, and thus is entitled initiation here as well. Both latency to the first mount and the latency to the first intromission had large positive factor loading scores on factor 2. Factor 3 conforms to the factor which Sachs has labled Intromission Count, and is likewise labled in Table 3. The only two variables which loaded on this factor were frequency of intromissions and post-ejaculatory interval; PEI had a negative factor loading score. Together the three factors accounted for about 65% of the total variance.

DISCUSSION

The present findings further support the hypothesis that male rat copulatory behavior is organized into discrete mechanisms. The confirmation of separable behavioral mechanisms was obtained by factor analysis of eight standard parameters of male copulatory behavior. The application of the factor analytic technique to the data resulted in three factors with eigen values greater than 1.0. These three factors, Copulatory Efficiency, Initiation, and Intromission Count each contained a unique set of variables. The Copulatory Efficiency Factor was composed of EL, III, HR, and MF. The Initiation Factor was composed of ML and IL. The Intromission Count Factor was composed of IF and PEI. The meaningfulness of the factors can be appreciated by comparing these variable clusters with the classic formulations of Beach and with the results of behavioral and physiological studies of male rat copulatory behavior.

The Initiation Factor in the present analysis was loaded with the same variables as those which loaded consistently on the Initiation factor in both Sach's (1978) and Dewsbury's (1979) analyses. The present Copulatory Efficiency factor seems to be an amalgam of the previous Copulatory and Hit Rate factors because it preserved the pattern of signs of the factor loading scores previously reported for the Copulatory Rate and Hit Rate factors. The combining of these two factors into a single factor, may be due to idiosyncratic sample differences. Thus, the present results are consistent with the general pattern reported by both Sachs (1978) and Dewsbury (1979). Confirmation of these findings using a behaviorally heterogeneous sample lends additional support to

the hypothesis that the relationships among the variables, as defined by factor analysis, are valid representations of underlying controlling mechanisms. In addition the results of the present and previous factor analysis are similar to Beach's conceptual model.

When Beach proposed his two factor theory in 1956 he described the process by which he generated the theory. Prior to 1956 models based on a notion of a unitary drive had predominated in most behavioral models including those concerned with sexual behavior. However, around 1955 Beach, along with Jordan, generated data which caused Beach to review the unitary formulation and eventually to suggest an alternative, the two factor theory. Beach and Jordan (1956) investigated the effects of sexual exhaustion on mating parameters. There were several interesting findings; three of these suggested that a reformulation was necessary. Specifically, it was found that both EL and IF decreased as the animal approached sexual exhaustion, whereas PEI increased over successive ejaculatory series. This data suggested that the copulatory sequence was not based on a single mechanism, because the pattern of change in these parameters was not unidirectional. It was on the basis of these findings that Beach proposed that PEI was controlled by a mechanism which was independent of the one controlling EL and IF. They also found that sexual rest was associated with a decrease in the ML, IL and PEI, while the EL showed little change. Thus the data further suggested an elaboration on the variables controlled in the two postulated mechanisms. This pattern suggested that the PEI, the ML, and the IL might be controlled by the same mechanism which would differ in turn from the mechanism controlling the EL. The

mechanism regulating ML, IL, and PEI was called the Arousal Mechanism. The remaining parameters (EL, III, and IF) were conceptualized as being controlled by the mechanism for ejaculation, the Copulatory-Ejaculatory Mechanism. In his subsequent theoretical paper in 1956, Beach expanded the ideas formulated earlier by Beach and Jordan, and also made some revisions of those ideas.

Beach still proposed two mechanisms; one primarily relating to pre-copulatory events and the other to copulatory behaviors. The pre-copulatory mechanism was called the Sexual Arousal Mechanism (SAM) and contained only two measures, the latencies to the first mount and to the first intromission. In the earlier paper the Arousal Mechanism had contained a third measure, the PEI. However, in the later paper Beach was no longer willing to include the PEI in the SAM because he thought the PEI might not be a unitary parameter. The second mechanism was the Intromission and Ejaculation Mechanism (IEM) and it was hypothesized to govern the III and the EL. In this paper then Beach was specifically excluding the IF from the IEM. Unfortunately since Beach does not present his data base the reasons for his exclusion of this parameter are unclear. However, he posits that it is the simple occurence of intromissions rather than the number of intromissions which are important for maintaining the excitation necessary for achieving an ejaculation.

The results of the present statistical analysis conform well with Beach's conceptualization of male rat copulatory behavior. The current Copulatory Efficiency Factor relates well to Beach's IEM, as major components of each are the III and the EL. The current factor also includes

two other variables, HR and MF, which Beach did not include in his formulation because they were not commonly used until the early 1970's. The Initiation Factor in the present analysis conforms well to Beach's SAM as both depend on IL and ML for their definition. And it is interesting to note that the Intromission Count Factor contains the two parameters which Beach himself excluded from his two mechanisms.

The results of the present analysis indicates that intromission frequency is associated with PEI, and that these variables are subsumed within one factor, the Intromission Count Factor. An additional feature of the Intromission Count Factor merits attention. The variables it contained, IF and PEI, were not themselves correlated. This result is similar to those of previous behavioral and physiological analyses of male rat copulatory behavior in which IF and PEI have been found to be related to the same variable or factor, but not to be correlated with each other (Szechtman, Lambrou, Caggiula & Redgate, 1974; Sachs, 1978). The consistent finding of an association between IF and PEI and some third variables in the absence of a correlation between the two copulatory parameters indicate that, in the present analysis, a suppressor variable may be affecting the Intromission Count Factor.

One possible candidate for the suppressor variable is the female partner (Sachs, 1978), because she has been shown to participate in both the control of intromission frequency and PEI duration (Adler, 1969; Barfield & Geyer, 1975; and McClintock & Adler, 1978). Additionally, recent behavioral analyses by McClintock, Anisko and Adler (in press) suggest that our hypothetical suppressor variable might be related to other social behaviors, specifically the relative dominance

position of the male. In an indepth analysis of rat sociosexual behavior McClintock et al. (in press) demonstrated that the ejaculatory series of subordinate males contained fewer intromissions and a longer PEI, than did those of dominant males. These results suggest that the Intromission Count Factor may include social variables other than masculine copulatory behavior. In this context it is also of interest to reconsider the present procedure of selecting for heterogeniety of ejaculatory experience. This subject manipulation was dependent upon individual differences in the rats' mating activity, or sexual vigor. Others have also demonstrated that sexual vigor, IF, and PEI are related. Szechtman et al. (1974) have shown that differences in sexual vigor are correlated with differences in corticosterone response to mating. They found that "sluggish" copulators increased serum corticosterone concentrations in response to mating, i.e., there was a positive correlation between PEI and corticosterone. Only one other parameter of the ejaculatory series correlated with corticosterone, IF, and that variable correlated negatively: as noted above PEI and IF were not themselves significantly correlated. The concordance between the findings of Szechtman et al. (1974), and the present analysis support our hypothesis that the Intromission Count Factor is particularly sensitive to behavioral variance in the subject population. In addition, these results further support the suggestion that the Intromission Count Factor is related to several aspects of psychoneuroendocrine function, rather than being linked solely to copulatory performance.

Ideally at this point it would be possible to cite findings which indicate that specific neuroanatomical or neurophysiological correlates

affected exclusively the variable clusters from each of the three factors. This is a significant issue because most of the physiological investigations of male copulatory behavior do not include the full complement of variables that were included in the above factor analysis. Therefore, a thorough comparison between the above variable clusters and the results of these studies is difficult.

Consideration of the factors individually also does not simplify this problem. For example, much of the neuroanatomical work has been centered on making small lesions in portions of the limbic system. While that strategy has been productive, there may be drawbacks in that strategy for uncovering an area which influences every parameter of the Copulatory Efficiency Factor. The drawback results from the possibility that any portion of the limbic system may not be sufficient for the integration of the several parameters subsumed within the Copulatory Efficiency Factor. Second, most of that research has been centered on discovering manipulations which eliminate copulator behavior. This approach makes it difficult to discern any effects which may be evident on the parameters of the Copulatory Efficiency Factor, i.e., if the animal does not copulate at all, how can its execution of the copulatory sequence be evaluated?

Similar issues arise in consideration of the physiology of the Intromission Count Factor. Notice that in the above discussion of the Intromission Count Factor that results which corresponded with the ICF variable clusters were related to diffuse interactive systems, i.e., endocrine and social processes. While it is possible that such a system is primarily controlled from a single neurological system it is more likely that the

pattern of results is a function of a convergence of several different systems. Thus, a search for a single location within the brain may be frustrating in that many of the components of the overall system may be easier to locate than the process which is crucial for coordinating all of those components. Furthermore, since the hormonal influence is likely wide spread in the brain, that influence may represent the variable which is the neurological correlate of the Intromission Count Factor, and thus this factor may not have a more specific neuroanatomical correlate. In other words, looking for the neuroanatomical locus of a correlate of a single factor may be futile because that correlate may lie in a relatively diffuse system within the brain, or it may be represented outside of the central nervous system in the endocrine system or even be a function of the behavioral interaction with the estrous female.

Furthermore, each of the factors contain several different parameters, and thus they represent an organization of behaviors, and not specific structures. In other words, it is likely that each of the behaviors included in a factor is represented by a specific neurological structure which controls that specific behavior, but there is also a larger system, the factor, which influences each of these smaller systems. Thus in searching the central nervous system for the neurological correlate of a factor one must be careful to consider the role of larger systems/processes which serve an integrative behavioral function.

Despite the above admonitions, there is evidence to indicate that at least the Initiation Factor has some control arising from the hypothalamus. A number of studies have indicated that lesions of the medial preoptic area (mPOA) results in a complete loss of copulatory behavior

(Larsson, 1979; and Sachs, 1978). The medial forebrain bundle (MFB) is a fiber system which innervates the mPOA. Lesions of the MFB also disrupt copulatory behavior in male rats. However, a study by Caggiula, Antelman, and Zigmond (1973) indicates that this disruption of copulatory behavior is exclusively a result of a lack of initiation rather than a disruption of the entire copulatory sequence. In that study rats which received lesions of the MFB either had longer latencies to the first mount and intromission, or did not copulate without additional behavioral manipulations, i.e., tail pinch. Aside from that disruption the copulatory sequence proceeded in a normal manner. The authors interpreted these results as indicating that the nerve tract which was disrupted by their lesions was responsible for innervating an area which involved the initiation, but not the execution of the copulatory sequence.

Thus, there is evidence that the MFB and the mPOA may be involved in the control of the initiation of copulatory behavior exclusive of any control over the execution of that sequence. Given that finding one would expect to uncover a similar neurological system involved in the control of the Copulatory Efficiency Factor. At present there is not any such evidence. There are a number of studies on the limbic system which indicates that some of those structures may be involved in controlling the execution of copulatory behavior, but those studies do not indicate an exclusive control. For example, Valcourt and Sachs (1979) found that male rats with lesions of the bed nucleus of the stria terminalis had increased frequency of mounts, inter-intromission interval, and latency to ejaculation, and a decreased hit rate ratio. These four variables were related in an identical pattern to the Copulatory Efficiency

Factor in the present analysis. However, the other parameters of the copulatory sequence were also affected by the lesions. Thus, it is not clear if the structure is important only for the control of the Copulatory Efficiency Factor and that the other findings were secondary to this effect, or if the structure is important for such a wide range of behavior that the copulatory sequence in its entirety falls within its influence.

Sachs (1978) has suggested that the lack of a clear conceptual organization in the search for the neuroanatomical centers involved with control of copulatory behavior has led to a proliferation of disjointed research which is more interested in uncovering where these centers are than in uncovering how those centers exert their control. I share that view. In order to alleviate this problem Sachs (1978) suggests several specific adjustments in the strategy for research in this area. First, the focus on an unknown neuroanatomical center should be reduced. Rather than a "let's take it out and see what happens" attitude, the decision to lesion an area should be guided by knowledge of which other areas that one is related to, and what behaviors are influenced by those other areas. Second, it may be that more fruitful results will be obtained if concentration is first aimed at broad systems such as the neurotransmitter systems, rather than concentrating on a specific neuroanatomical locus, as those systems have a broader influence and would be more likely to influence a number of parameters. Third, the experimental design of neurological studies of copulation needs to be organized to directly determine if copulatory behavior is being affected primarily or secondarily by the lesion. For example, might a decline in copulatory behavior

following a lesion be due to a sharp decrease in olfactory sensitivity, or a general change in tactile sensitivity rather than being due to a specific disruption of copulatory behavior?

Experiment 2

The preceeding factor analysis suggests that there are three separable mechanism which organize the components of male rat copulatory behavior. One interpretation of this analysis is that there are neural correlates of these mechanisms. In a recent review of the literature concerning central control of male rat copulatory behavior Sachs (1978) suggested several possible candidates for those correlates. Of those candidates Sachs (1978) chose to focus his study on the hypothalamus as the likely site of the neurological correlates of the behavioral mechanisms. While this is a reasonable possibility, the fact that each of the neuroanatomically defined divisions of the hypothalamus serves as a junction for several neuroanatomical and neurochemical systems makes analyses at this level somewhat imprecise. This imprecision makes the recent body of work having to do with the serotonergic system particularly appealing. There are a number of studies (e.g., Del Fiacco et al., 1970; and Larsson et al., 1978) which suggest that the serotonergic system is involved in control of both the Initiation and Copulatory Efficiency factors. Furthermore, the evidence suggests that the influence on the two factors may arise from separable components of the serotonergic system.

The results of the serotonergic investigations are especially relevant to the current analyses, e.g. the hypotheses generated from the factor analysis, because the results are consistent with positing separable Initiation and Copulatory Efficiency factors. Moreover, the variable clusters posited by the present factor analysis appear to be validated in the serotonin studies. In contrast the findings from other neural systems do not relate consistently to the variable clusters generated by Beach

and by the recent factor analyses of Sachs, Dewsbury, and Goff and Weizenbaum. Therefore, the present study was undertaken to preliminarily investigate the hypothesis that the serotonergic influence on male rat copulatory behavior included both the initiation and execution components, and that these separate influences were located within different neuroanatomical structures.

The Serotonergic System

Serotonin or 5-Hydroxytryptamine (5-HT) is one of the major putative monoaminergic neurotransmitters. Using the histoflouresence technique developed by Falck and Hillarp (Falck, Hillarp, Thieme, & Torp, 1962) it was possible for Dahlstrom and Fuxe (1965) to localize the cells of origin for serotonin. Nine distinct groups of serotonin containing somas have been identified. All of these cell groups lie in the midsagital plane of the brain, and are located in the midbrain, pons, and medulla. The present review will focus on the midbrain raphe because that portion of the raphe system, as I shall document below, is the only part of the raphe system to be clearly implicated as influencing male copulatory behavior.

The two major midbrain cell groups which contain serotonin are the dorsal raphe nucleus and the median raphe nucleus. The efferent connections of these are primarily to the forebrain through the medial forebrain bundle, and into the cerebellum. More specifically, some of the efferent connections from the midbrain raphe nuclei which have been identified are described next. Conrad, Leonard, and Pfaff (1974) demonstrated efferent projections in the albino rat from the area of the midbrain raphe including both the dorsal and median nuclei, to the hypothalamus, the anterior amygdala, the olfactory tubercle, the preoptic areas, the hippocampus, and the habenular nuclei among others. Bobillier and his coworkers (1975) made an attempt to separately analyze the efferent projection from the two midbrain raphe nuclei in the cat. This analysis revealed that the dorsal raphe nuclei projects to the periaqueductal gray region, the median raphe, the thalamus, and the

median and lateral preoptic regions among other nuclei, while the median raphe nucleus projects to the pontine reticular formation, the mamillary bodies, the thalamus, the lateral geniculate body, and the hippocampus among other areas.

Nonetheless, it is not yet entirely clear what all of the efferent projections from the two midbrain nuclei are, or even which of the identified projections belong to which of the two nuclei (Lorens, 1978). This confusion is due in part to the fact that some of the efferent axons from the dorsal raphe nuclei course ventrally as they leave the dorsal raphe and pass close to the median raphe nucleus (Bobillier et al., 1975). Since the best studies of the efferent projections from these two nuclei used anterograde techniques (Conrad et al., 1974; and Bobillier et al., 1975) the results for the efferent projections from the median nucleus are probably confounded to some extent by the fibers en passage through that nucleus from the dorsal raphe nucleus. This confound occurs because when a radioactive dye is injected into a nuclear group, as is done in anterograde techniques, the dye is picked up by the axons passing through that area as well as by the cell bodies in that area. The dyes used are usually proteins, and are transported down the axons, away from the cell bodies, and thus later visualization indicates the terminals of those neurons which picked up the dye. For the current work, the fact that the two nuclei of the midbrain raphe have separable efferent projections to different parts of the forebrain is of primary importance.

Since the serotonergic system does not make up a unitary system, i.e., there are different areas of the brain which are inervated by

the serotonergic system, it is clear that independent manipulation of these separable systems would be likely to lead to different behavioral consequences. This issue is important for assessing the role of the midbrain raphe with the previous experiments concerned with 5-HT control of male copulatory behavior. Briefly, those studies typically employed systemic depletion of serotonin, and therefore, the locus of the effective depletion is unclear. Therefore, the bulk of the experimental evidence demonstrating serotonergic involvement in male copulatory behavior does not indicate the specific site within the raphe complex of that effect. Nonetheless there are a few studies which suggest that the critical serotonergic area is in the midbrain.

The Role of the Serotonergic System in Control of Male Copulatory Behavior

The raphe nuclei, and their neurotransmitter 5-HT have been implicated in the control of the copulatory behavior of the male rat. Specifically the reports to be reviewed here suggest that serotonin may in fact be acting on separate aspects of copulatory behavior in the male, the Initiation factor and the Copulatory Efficiency factor.

There are a number of studies which indicate that the 5-HT system is involved with control of the initiation of copulatory behavior, the Initiation factor. Although these studies do not present sophisticated behavioral analyses, they have produced interesting findings on the role of serotonin and the control of the initiation of copulatory behavior in the male rat. Castrated male rats not receiving testosterone replacement typically exhibit very little mating behavior. In a study by Gessa et al. (1970) sexually naive rats were castrated and then

administered either a dose of testosterone which was not sufficient to induce copulatory behavior, pCPA, or both compounds. pCPA is a specific depletor of serotonin (Koe and Weissman, 1966). The animals which received only testosterone did not display any copulatory behavior, nor did the animals which only received pCPA. However, administration of the two compounds together produced mounting behavior in these rats. No data was reported for any other copulatory parameters, and there was no information presented to interpret the absence of these data. These results indicate that testosterone and serotonin may act synergistically to activate male copulatory behavior.

Del Fiacco, Fratta, Gessa, and Tagliamonte (1970) have provided some data on the possible independence of the effects of serotonin depletion on the separate stages of copulatory behavior. In this experiment sexually naive male rats were either castrated or sham operated, then half of each of these two groups was treated with pCPA while the other half was treated with saline. The proportion of animals which displayed mounts, intromissions, or ejaculations in the presence of an estrous female were the only data reported. Neither of the castrated groups (saline or pCPA treated) displayed any of the copulatory behaviors. However, pCPA treatment did increase the number of intact males displaying each of the three behaviors, indicating that the effect of 5-HT on copulatory behavior are not independent of testosterone. The data that are particularly relevant to the present hypotheses were that if an animal mounted, it always continued through the copulatory sequence until it ejaculated. It was as if the effective treatment unblocked some mechanism which was inhibiting the initiation

of copulatory behavior, but not affecting the execution of that behavioral sequence once it was initiated. Once again this may not be a complete evaluation of the effects of 5-HT because intact saline rats also copulated to ejaculation. Moreover, latency and frequency data were not reported, so there is no way to determine if pCPA treatment affected any of the execution components. Therefore, the absence of an effect of serotonin depletion on the Copulatory Efficiency factor is only a tentative conclusion.

Ginton (1976) presented data which indicates that non-copulating males will copulate following treatment with pCPA. The animals used in this study were drawn from the normal population of male laboratory rats, but they were selected for their persistent lack of copulatory behavior despite repeated exposures to estrous females. Ginton found that following treatment with pCPA more than 85% of the former non-copulators mated to ejaculation. However, like del Fiacco et al. (1974) he did not report any of the traditional measures of copulatory behavior and so a precise evaluation of the possible selective effects of depleting 5-HT on the Initiation and Copulatory Efficiency mechanisms is not possible. Nonetheless, these data are consistent with the findings of del Fiacco et al. (1974), again it appears as if the depletion of serotonin plays an important role in permitting the initiation of the copulatory sequence. Moreover, once initiated, the ejaculatory sequence followed a species typical pattern.

The above data clearly indicates that depleting serotonin in a rat which is not likely to copulate (castrated or non-copulator) will increase the probability that it will copulate. Complementary to this

hypothesis are a number of studies which demonstrate that an accumulation of 5-HT in the central nervous system will depress copulatory behavior. In the following two studies 5-HT accumulation was accomplished by administration of pargyline, an MAO inhibitor. Pargyline produces an accumulation of serotonin in the central nervous system by disrupting the mechanism which breaks down the monoamines after they have served their transmitter function. This disruption is the result of the pargyline binding to the monoamine oxidase which is the enzyme responsible for the initial oxidization of the monoamines. Because pargyline disrupts all of the monoamine oxidases (MAO's) and increases the concentration of all of the monoamines in the central nervous system this drug must be administered alone to one group and in combination with pCPA to a second group in order to determine if a behavioral effect is due to a specific build up of serotonin or a more general build up of all the monoamines (Cooper et al., 1978). If the effect of the pargyline is due to the accumulation of 5-HT rather than the other monoamines, then the effect should be reversed in the pargyline plus pCPA group because the pCPA will reduce the amount of serotonin available, but not affect the other monoaminergic systems. In fact when pargyline alone was administered to a group of intact male rats there was a significant decrease in the number of males copulating when compared to a non-treated control group. This decrease was reversed by the administration of pCPA (Ahlenius et al., 1971; and Gessa & Tagliamante, 1974). These data indicate that if serotonin accumulates in the central nervous system that initiation of copulatory behavior will be decreased.

Another method for studying the effects of the accumulation of 5-HT is by administering the immediate precursor of serotonin, 5-Hydroxytryptophan (5-HTP). Malmnas (1973) administered 5-HTP to a group of castrated male rats which were maintained on subthreshold doses of testosterone to see if the 5-HT accumulation antagonized the effects of testosterone. The number of 5-HTP treated males mounting an estrous female declined compared to non 5-HTP animals. Contrary to these findings Soulairac and Soularaic (1975) present data which indicate that administration of 5-HTP may have a slight facilitatory effect on copulatory behavior in the male rat. Since none of the effects were stable over time, and this is the only report of a facilitatory effect of 5-HTP, the significance of their findings is difficult to assess. Overall, the investigations described above indicate that serotonin has an inhibitory effect on the initiation of male copulatory behavior, but it does not address the possibility that 5-HT also affects the execution of the copulatory sequence.

The ability of the serotonergic system to modulate the execution components of copulatory behavior in sexually experienced male rats was demonstrated in a study by Salis and Dewsbury (1971). In this study depletion of 5-HT was accomplished by systemic administration of pCPA. Salis and Dewsbury administered pCPA to a group of male rats and observed their sexual behavior. The pCPA treated animals demonstrated a general enhancement of copulatory behaviors when compared to a saline treated control group. The frequency of mounts, the inter-intromission interval, the latency to ejaculation, the frequency of intromissions, and the PEI were all significantly reduced in the pCPA group. The reduction in

the latency to ejaculation, number of mounts, and the inter-intromission interval indicates that the serotonergic system influences the execution of the copulatory sequence. The measures of the Initiation factor were not changed by the administration of pCPA in this study. In summary, these results indicate that when 5-HT is systemically depleted in sexually experienced rats that the execution components of their copulatory behavior will be enhanced, while initiation is unaffected. However, an affect on the execution is not consistently reported. Whalen and Luttge (1970) showed that pCPA treatment did not enhance the copulatory behavior of male cats which were selected for sexual vigor. Gessa et al. (1971) have suggested that these negative results were due to a ceiling effect as a result of selecting "sexually vigorous" animals. In other words, an animal probably has a maximum level of copulatory performance which it can not exceed, and thus depleting serotonin would not further enhance copulatory behavior in these high performance animals. A similar hypothesis can be advanced to explain the occurence of facilitation of initiation components in sexually naive but not in sexually experienced male rats. In fact, experienced copulators show a near zero latency to the first intromission. Thus these animals probably have a minimum latency to the first intromission which they can not physically exceed.

These pharmacological studies suggest that serotonin may be acting to control two seperate components/mechanism of copulatory behavior in the male rat. These two components are an initiation mechansim and an execution or copulatory efficiency mechanism. Basically the above results indicate that if an animal is not likely to copulate and sero-

tonin is depleted that animal will be more likely to initiate a copulatory sequence. If the animal is already likely to copulate, and it is not yet at peak copulatory efficiency, and 5-HT is systemically depleted then the execution of its copulatory behavior will likely be enhanced. Given that these two seperable effects result from systemic depletion of serotonin, it is interesting to consider how these two effects might be mediated in the central nervous system.

The raphe extends from the medulla to the pons and midbrain; my hypothesis is that the two behavioral effects may be mediated by the nuclei of the midbrain raphe complex. This hypothesis is based on the findings of studies that have attempted to specifically evaluate the role of individual nuclear groups of the raphe system in the control of male rat copulatory behavior. Two of these studies induced cell distruction with localized injections of 5,7-Dihydroxytryptamine (5,7-DHT). 5,7-DHT is taken up into the cell as if it were 5-HT and then disrupts the metabolism of the cell to such an extent that the cell eventually dies (Cooper et al., 1978). Larsson and his coworkers (1978) injected 5,7-DHT into the midbrain raphe nuclei of sexually naive male rats which had been castrated and were receiving subthreshold testosterone replacement therapy. The proportion of these treated males mounting and ejaculating was greater than a saline injected control group. No mention was included of the latency to ejaculation or any other more specific measures of copulatory performance. Once again this study demonstrates that depleting serotonin enhances the probability that an animal will initiate a copulatory sequence, and that once initiated the animal will complete that sequence. Gessa and Tagliamonte (1975)

also reported an enhancement of sexual behavior in the male rat following localized injections of Dihydroxytryptamine into the raphe nuclei of sexually naive male rats. Although they failed to report which nuclei were affected by the injections they did report results similar to those of Larsson et al. (1978). Finally Sheard (1973) made separate electrolytic lesions in the two midbrain raphe nuclei and then studied the occurence of a number of behaviors, including sexual behavior. While his neuroanatomical technique was elegant, his behavioral techniques were inadequate for an evaluation of copulatory behavior because he did not use behaviorally receptive females in his observations of copulatory behavior. Given that inadequacy it is not surprising that no copulatory behavior occured in his animals. Thus no firm conclusions about the specific role of these two major nuclear groups in the control of copulatory behavior are available from this study. Nonetheless at least one of these three studies directly suggests that the midbrain raphe nuclei may be involved in the control of the initiation of male copulatory behavior.

Other Behavioral Systems Affected by the Serotonergic System

The classic work of Jouvet (1973) has demonstrated that the serotonergic system in general and the midbrain raphe nuclei in particular have a role in the control of sleep. The medullary and dorsal raphe nuclei have been implicated in the inhibition of pain (Mayer & Price, 1976). There is also data which indicates that the midbrain raphe inhibit behavioral arousal (Fibiger & Campbell, 1971; Marby & Campbell, 1974; and Jacobs et al., 1974). Some other behaviors which are generally enhanced following forebrain depletion of serotonin include aggres-

sion (Sheard, 1973), food intake (Waldbig, Bartness & Stanley, 1981), open-field activity (Jacobs et al., 1974; and Srebro & Lorens, 1975), wheel running behavior (Shahid Salles et al., 1979), and startle reflex (Davis, Astrachan & Krass, 1980). Furthermore, acquisition of an active avoidance response is disrupted by depletion of forebrain serotonin (specifically median raphe lesions) while acquisition of a passive avoidance response is unaffected by those same manipulations (Heybach et al., 1978).

The broad range of behavioral systems affected by the serotonergic system which arises primarily from the midbrain raphe nuclei suggests that this system may be involved in a general inhibition of motor behaviors. Thus it is possible that any changes in copulatory behavior following manipulation of that system may be the result of this more general control of motor behaviors, rather than an effect which is specific to copulatory behavior.

Hypotheses

The primary hypothesis suggested by the above findings was that the 5-HT system influences two separate mechanisms of male copulatory behavior (initiation and execution), and that the neuroanatomical locus of these controls may be different. More specifically, it is proposed that the dorsal and median raphe nuclei of the midbrain raphe could be independently affecting different aspects of male copulatory behavior which are the SAM and IEM described by Beach (1956) or the Initiation and Copulatory Efficiency factors described earlier. In order to evaluate these possibilities the dorsal and median raphe nuclei were lesioned in two separate groups, and the copulatory behavior of these two groups

was compared to appropriate control groups. In addition a group with both nuclei lesioned was also included to see if any effects from one of the two separate lesions would override the other, or if those effects would augment each other.

In addition, the method was designed to address another issue related to the expectation that the lesions would alter the initiation phase of the copulatory sequence. In order to assess the specificity of any changes in the initiation phase of the copulatory sequence measures of other social behaviors which are not specific to copulation were taken during the mating tests, and a separate measure of activity was obtained in an open-field test. I have suggested that changes in the initiation phase might reflect a general change in arousal rather than a specific alteration in sexual arousal.

METHODS

Subjects: Seventy-two, sexually naive male rats were the subjects. These Spraque-Dawley rats were bred by Charles River Breeders and born in the colony at VPI&SU. The rats were individually housed in a reversed 14L/10D cycle, with lights out at 1200 EST. Purina Lab Chow and tap water were continuously available.

Surgery: Three groups (n=18) received electrolytic coagulation of specific midline cell groups. The electrolytic lesions were made by passing 1.0mA anodal current between an electrode and the sterotactic frame. The electrode was a .3mm platinum-iridium wire insulated with teflon except for a .05mm tip. In order to facilitate accurate placement of the electrode a 23 gauge needle was used as a placement cannula. The lesioning electrode extended 4mm beyond the ventral extent of the guide cannula.

All surgery was performed while the animal was under sodium pentobarbital anesthesia. The coordinates for the lesion placements were derived from Pellegrino, Pellegrino, and Cushman (1970) as well as pilot work. The bite bar of the sterotaxic frame was set at 5.0mm above the interaural plane in accordance with the atlas by Pellegrino, Pelligrino, and Cushman (1970). Furthermore, all AP coordinates are with reference to Bregma, and the DV coordinates are with reference to the dural surface of the brain.

One group received lesions of the median raphe nuclei (MED); the coordinates used for these lesions were: -6.0 AP, 0.0 L, and -8.0 DV. Current was passed for 20 seconds. A second group received lesions of the dorsal raphe nuclei (DOR); the coordinates for these lesions were:

-6.0 AP, 0.0 L, and -5.4 DV. Current was passed for 15 seconds. The shorter lesion was made in the dorsal nucleus because it is smaller than the median nucleus and this shorter lesion would not do as much extra nuclear damage. A group of animals received lesions in both of these areas (COM). These lesions were accomplished by passing current at both of the sites described above in a single surgical session.

The sham operated control group (n=9) received surgery similar to that described for the lesioned groups except that the electrode was lowered to just above the DV coordinate for the dorsal nucleus, and no current was passed. A fifth group (n=9) was used as a control for the effects of surgery, this group was an unoperated control group.

Stimulus animals: Fifteen females of the same strain and age as the experimental subjects were used as stimulus animals in the copulation tests. These animals were housed five in a cage with all other housing conditions similar to those of the experimental subjects. These females were housed in a seperate room from the males. Behavioral estrous was induced in these animals with subcutaneous injections of estradiol benzoate (.01mg in .1cc peanut oil) 78 and 30 hours prior to testing, and a subcutaneous injection of progesterone (.02mg in .1cc peanut oil) 5 hours prior to testing.

Behavioral methods: Sexual behavior of the lesioned males and their controls was observed while they were paired with an estrous female. The mating arena (40cm X 24cm X 18cm) was clear plastic, and its floor was covered with pine shavings. Observations of the sexual behavior of these animals was made for 30 minutes, or until the first intromission following an ejaculation, whichever occurred first. A

thirty minute limitation on the observation period was chosen in order to reduce the number of animals which would gain complete copulatory experience on each trial. Pilot work had indicated that by the second trial about 50% of a sample of rats would be completing the copulatory sequence within 30 minutes.

The same parameters of copulatory behavior were quantified in this experiment as those in the first. Those were in IL, EL, PEI, III, IF, MF, and HR.

In order to evaluate the general activity level and non-copulatory responses to the stimulus females some non-copulatory behaviors were also recorded on the Esterline Agnus. These behaviors were chosen on the basis of earlier observations and include: locomoting about the observation arena, rearing (removal of both forepaws from the floor), general grooming, ano-genital grooming, investigation of the ano-genital region of the female, any non-copulatory contact with the female, rooting in the bedding, and laying down. Furthermore, those behaviors not involving the female were recorded for five minutes before the introduction of the female to the observation arena. From these records the frequency and total time of each behavior was calculated.

In order to evaluate the level of general motor activity observation of locomotion in the open-field was made. These observations were made in a plywood arena (91cm X 91cm X 23cm) finished in polyurethane and marked off into sixteen 23cm squares. The number of inner squares entered, the number of outer squares entered, and the number of rearings were recorded. The number of inner and outer squares entered were recorded separately because it had been suggested that this separation

provides a more sensitive measure of effects of manipulations of the central nervous system on activity.

Procedure: The experiment was executed in three identical replications. Twenty-four subjects were evaluated in each replication. A single pre-surgical observation of copulatory behavior was made along with observations of the non-copulatory behaviors in the mating arena and an observation of open-field activity was made two days later. Following this observation of copulatory behavior the animals were matched according to their copulatory efficiency. This matching was made according to three criteria: first the latency to ejaculation, then the number of intromissions, and then the latency to the first intromission. Following this matching procedure animals were assigned to each of the five groups according to a block randomization procedure. A total of 18 animals was assigned to each of the lesion groups, and nine animals were assigned to each of the control groups. All surgery was performed ten to fourteen days following the initial observation of copulatory behavior. Following a ten to fifteen day recovery from surgery the behavioral observations were repeated.

A malfunction in the recorders used to quantify the non-copulatory behaviors in the five minutes prior to the introduction of the female, and during the copulation tests resulted in much of the baseline data and some of the post-surgical data being uninterpretable. For that reason only the post-surgical measures were analyzed. Furthermore, the COM group was not included in any of those analyses because the above technical difficulty reduced the sample of available observations for this group to two. A separate analysis of the pre-surgical scores did

indicate that there were no differences between the groups, therefore any differences between the groups in their post-surgical observations would not be due to differences in the baseline of those behaviors.

Histology and Neurochemistry: Subjects were sacrificed by rapid decapitation. The brain was removed immediately following the decapitation, and the forebrain was disected away from the rest of the brain and weighed. This tissue was placed in 6ml of .1M $HCLO_4$ in an homogenizing tube, the tube was placed in a beaker of ice, and the brain tissue was mechanically homogenized. This homogenate was centrifuged at 8000g for 20 minutes. One milliliter of the supernatant of the centrifuged homogenate was removed, and 100ml of Acetate buffer was added. This super aliquot was vortexed, sealed with parafilm, and frozen for later analysis to determine monoamine, and amine metabolite contents by High Pressure Liquid Chromotography (see appendix 1 for details of this procedure). Specifically forebrain levels of serotonin, its metabolite 5-HIAA, dopamine (DA), its two metabolites dopac, and homovanilic acid (HVA), and norepinephrine (NE) were measured.

The rest of the brain was placed in 40% formalin until it hardened, then it was cut frozen into 50μ horizontal sections. These sections were stained with cresylecht violet for evaluation of the lesion sites.

RESULTS

Histology and Neurochemistry

All histological materials were examined in order to select those cases which demonstrated complete destruction of the target nuclear groups. This examination revealed that six animals had complete destruction of the dorsal raphe nucleus; a sample case is presented in Figure 1. Two animals had complete destruction of the median raphe nucleus; a sample case is presented schematically in Figure 2. Two animals had complete destruction of both nuclear groups, a sample case is presented schematically in Figure 3. In addition to the animals which had anatomically complete lesions there were 8 rats which demonstrated more than 50% destruction of the target nuclear group, and 13 cases for which histological materials were unavailable for microscopic investigation.

The main hypothesis being examined here was concerned with depletion of serotonin in the forebrain as a result of specific lesions. Animals from the partial lesion groups and the group of animals for which histological materials were not available were matched with those animals which had identified and complete lesions of the target nuclear groups. This matching was made specifically on the basis of the results of the neurochemical analyses of forebrain monoamine content.

Matching was completed on the basis of several criteria. First, the animal under consideration for inclusion in an experimental group had to have been drawn from an appropriate surgical group; for example, an animal being considered for the COM group must have received surgery intended to make a combined lesion. Second, the forebrain measure of

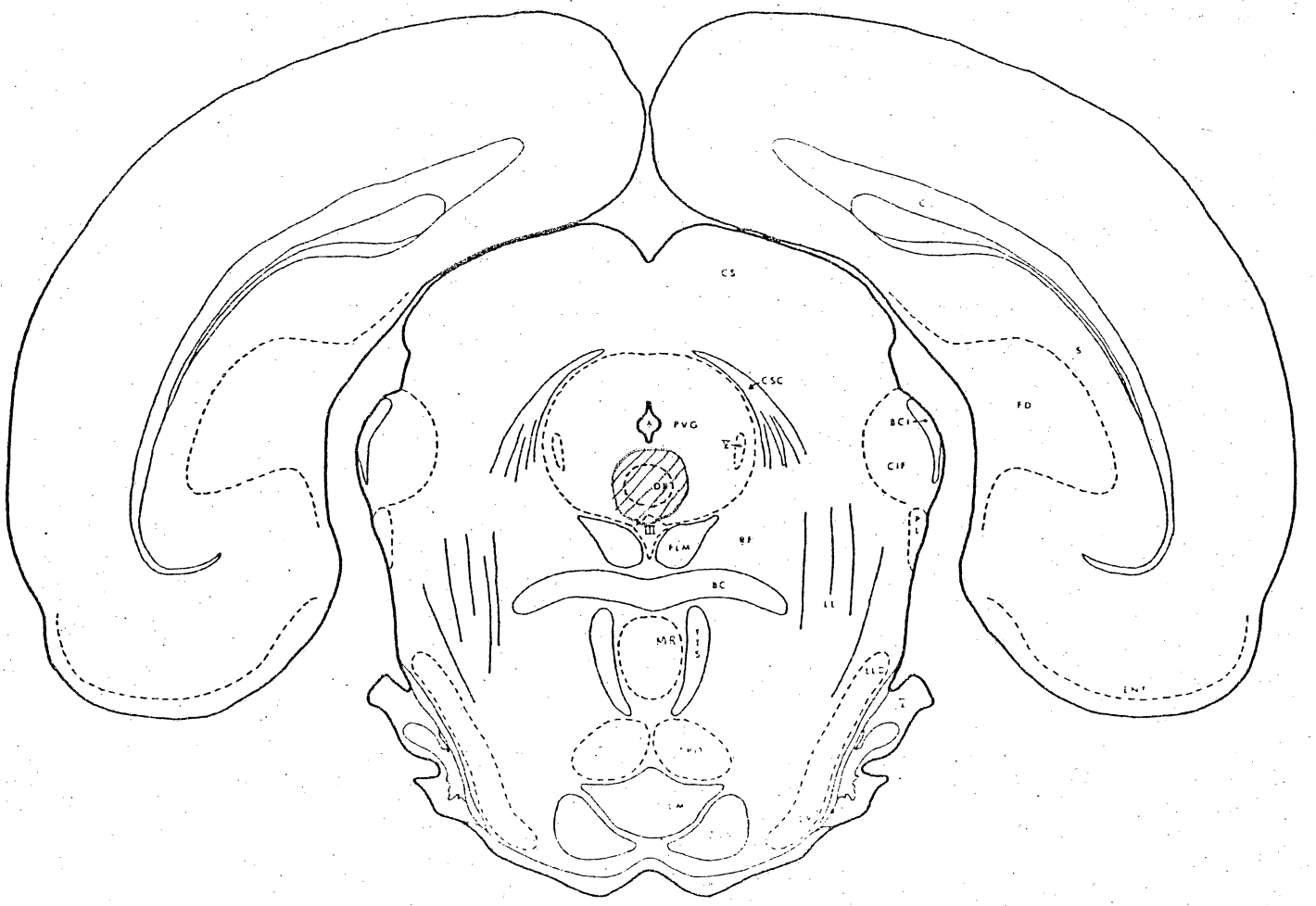

Figure 1. Schematic representation of a horizontal section of the rat brain through AP-6.0 illustrating a dorsal raphe lesion, after Pellegrino, Pellegrino, & Cushman (1979). Abbreviations A-third ventricle, BC-Brachium Conjunctiuum, DR-Dorsal Raphe, FLM-medial longitudinal fasiculus, MR-Median Raphe, PVG-peri-ventricular grey, TPO-Tegmental nucleus of Pons, TTS-Tectospinal tract, III-Nucleus of the oculomotor nerve, V-nucleus of the Trigeminal Nerve.

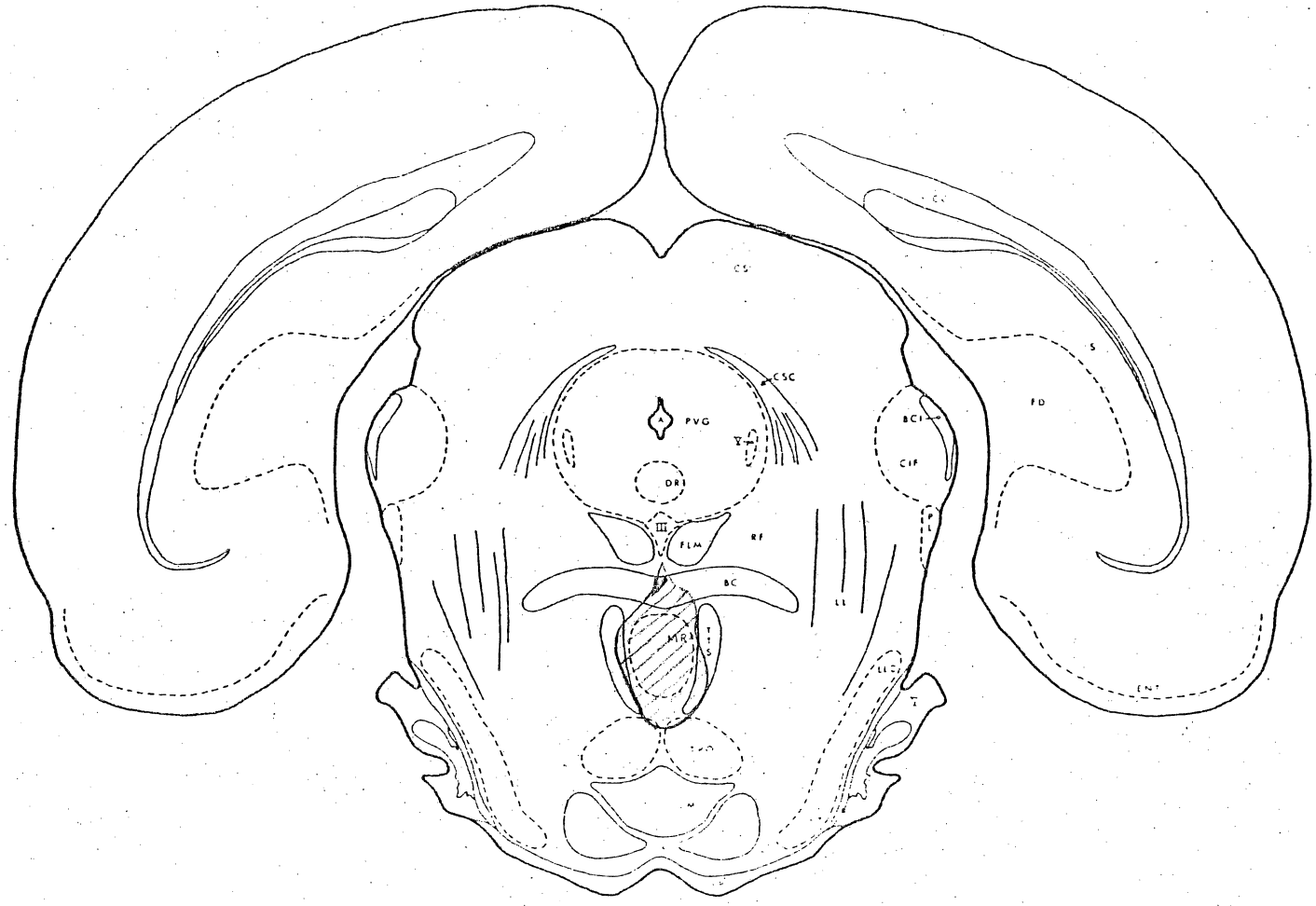

Figure 2. Schematic representation of a horizontal section of the rat brain through AP-6.0 illustrating a median raphe lesion, after Pellegrino, Pelligrino, & Cushman (1979). See Figure 1 for abbreviations.

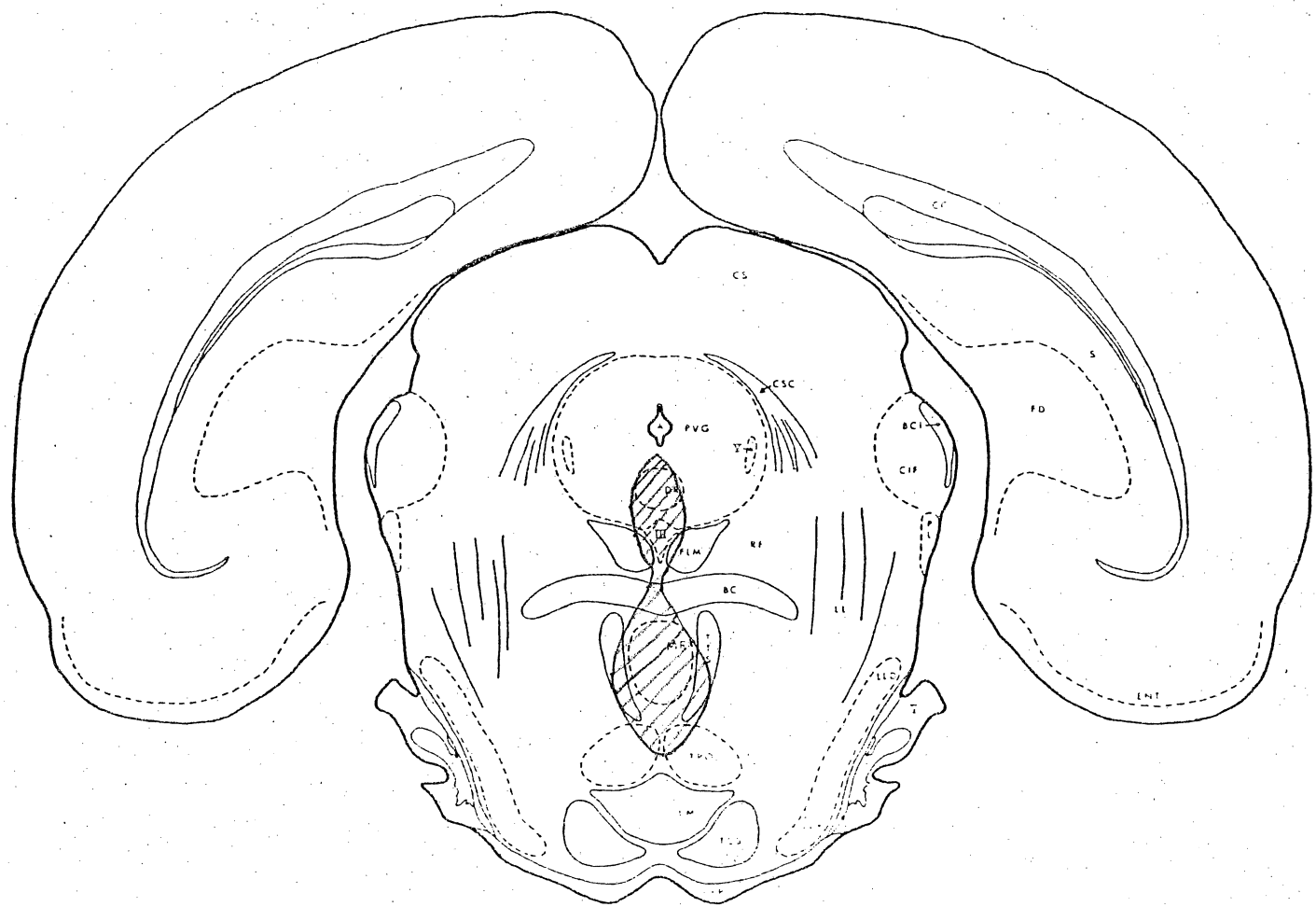

Figure 3. Schematic representation of a horizontal section of the rat brain through AP-6.0 illustrating a combined raphe lesion, after Pellegrino, Pellegrino, & Cashman (1979). See Figure 1 for abbreviations.

serotonin of the animal under consideration must have fallen within the range of the serotonin content of the identified lesion cases appropriate to that group. In addition the forebrain measures of norepinephrine, dopamine, 5-HIAA, HVA, and dopac must have fallen within the range of the values of the identified lesion cases. Following this matching procedure three animals were added into the dorsal lesioned group, five were added into the median lesioned group, and two were added into the combined lesioned group. Thus the total number of subjects in each of these groups were: nine in the dorsal group, seven in the median group, and four in the combined group.

Student's t tests of the six neurochemical measures from the unoperated and sham operated control groups indicated that there were no significant differences on any of the measures between these two groups. Therefore, they were combined and treated as a single control group for the remainder of the neurochemical analyses. Seperate one-way analyses of variance were performed on each of the neurochemical measures with groups as the independent variable in that analysis. Duncan's Multiple Range Tests were used to test for differences between the means of specific groups (α=.05). Significant differences were found between the groups for measures of forebrain content of 5-HT ($F(3,33)=7.67$, $p < .001$), 5-HIAA ($F(3,33)=6.56$, $p < .002$), DA ($F(3,33)=3.26$, $p < .05$), and Dopac ($F(3,33)=3.12$, $p < .05$). There were no significant differences in forebrain content of HVA ($F(3,33)=2.24$, $p > .05$) or NE ($F(3,33)-2.05$, $p > .05$). Figure 4 presents histograms of the means and standard errors of forebrain content of 5-HT and 5-HIAA (expressed in nanograms of compound per grams of brain tissue analyzed) for each of the four groups.

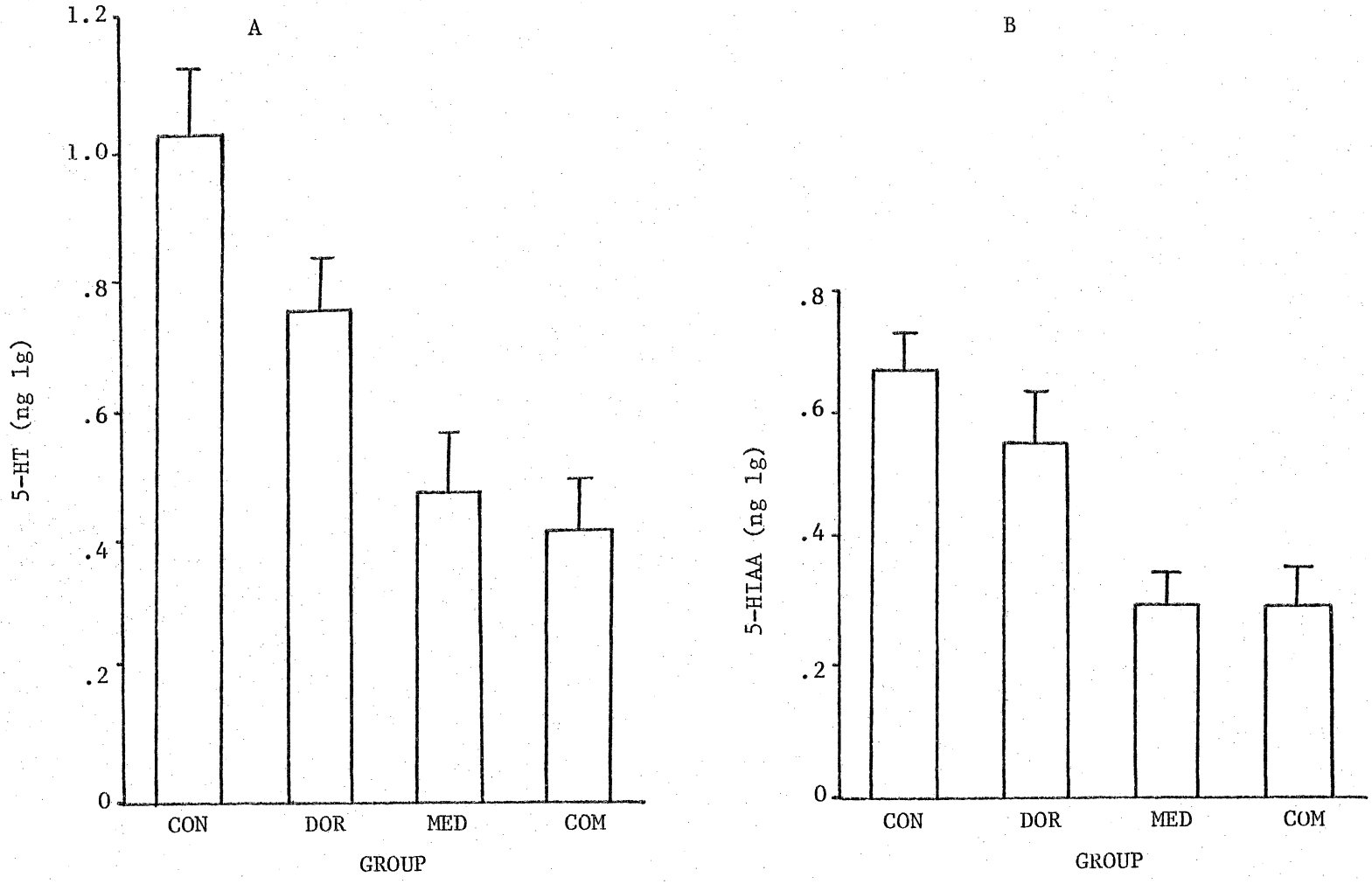

Figure 4. Mean forebrain concentrations of 5-HT and 5-HIAA in control and lesion groups.

Figure 5 presents histograms of the means and standard errors of forebrain content of dopamine and dopac for each of the four groups. Serotonin was significantly lower than the control group in all three of the lesioned groups. 5-HIAA was significantly reduced from the control level in the MED and COM groups. Dopamine and dopac were reduced from the levels of the control group only in the combined lesion group. Thus the four groups differed on the neurochemical measures for which they were selected.

Compairson of Control Groups

Separate two way analyses of variance were performed on each of the behavioral measures with pre- or post-surgery and unoperated or sham operated as the two independent variables in the analyses. No significant differences were observed either in the comparisons of the two control groups, or in the comparison of the interaction of group with pre- post-surgery. Therefore, the two control groups were treated as a single group in all of the analyses with the lesion groups.

Where possible (group n's greater than 2) data was evaluated with a similar two-way ANOVA. This analysis and the type IV correction for the sums of squares derived from a regression approach to the ANOVA allowed for a reduction in the bias toward type I errors which can result from unequal cell n's. The type four correction depends on the regression approach to calculation of the sums of squares for each of the independent variables included in the analysis (Kerlinger & Pebhazar, 1973). Then rather than calculating the sums of squares as the traditional approach does, the sums of squares for each of the independent variables is calculated with the variance common to all of the other independent variables partialed out. Thus, because the variance which is due to the unequal cells is also a portion of the

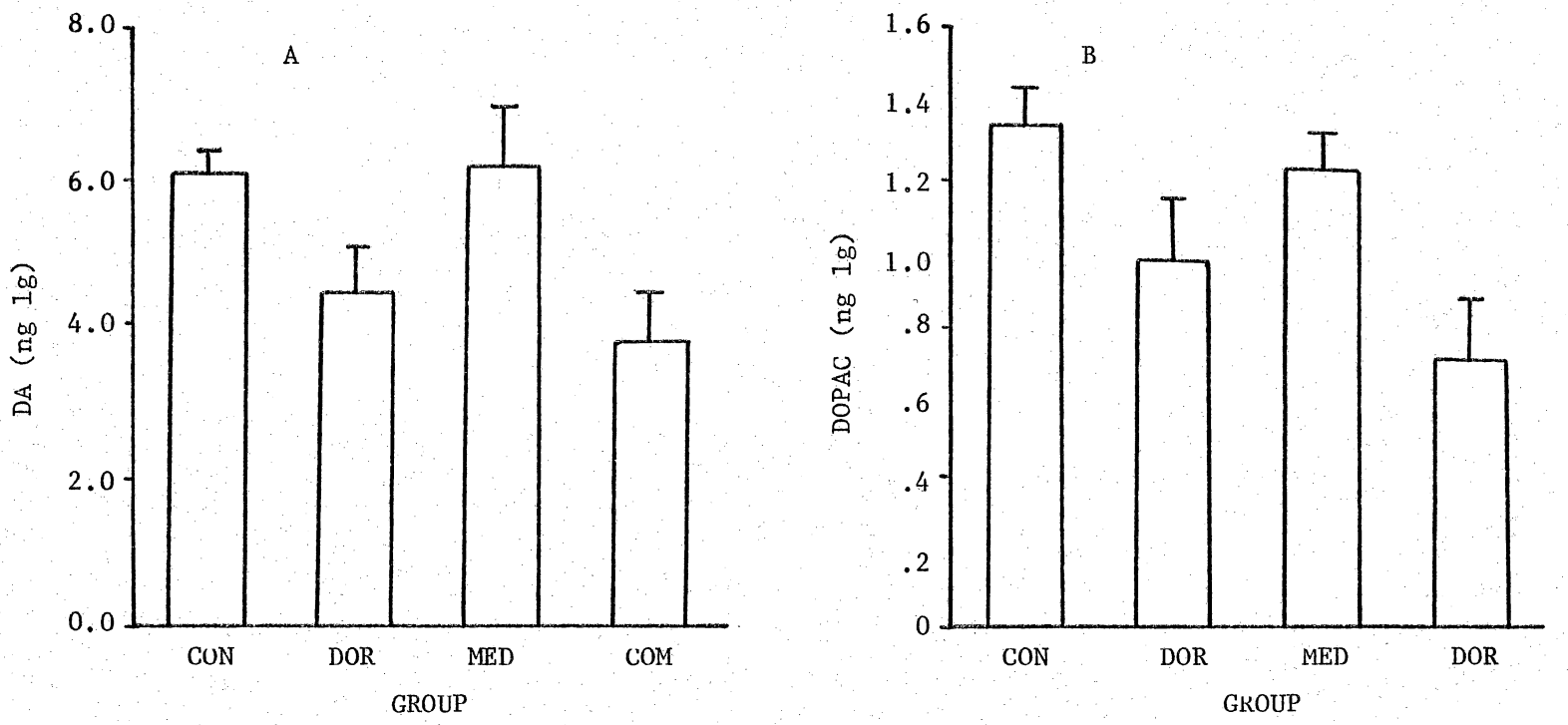

Figure 5. Mean forebrain concentrations of DA and Dopac in control and lesion groups.

variance observed for each independent variable that bias is partialed out of the tested sums of squares. For the sake of consistency when the two-way analysis of variance was not possible, a one-way ANOVA is presented with groups as the independent variable, and post-surgical measures as the dependent variable. In all cases these data were also analyzed with a Kruskal-Wallis one-way analysis of variance and the same results were obtained.

Open-Field Activity

The analyses revealed significant interactions between time of measurement and group for the total number of squares entered ($F(3,32) = 8.39$, $p < .001$), the number of outer squares entered ($F(3,32) = 8.27$, $p < .001$), and the number of inner squares entered ($F(3,32) = 3.65$, $p < .05$). The interaction of time of measurement and group for the frequency of rears in the open field approached significance ($F(3,32) = 2.66$, $p = .06$). The main effect for groups was not significant for any of the measures.

The post-surgical measures of the number of inner squares entered is presented in Figure 6A, the number of outer squares entered is presented in Figure 6B, and the total number of squares entered is presented in Figure 6C. The Duncan's Multiple Range Test indicated that the MED group entered more inner squares in the post-operative measure than in the pre-operative measure, and this was also true for the COM group, furthermore, the COM group entered more inner squares in the post-operative test than the congrol group did. There were no differences in the number of inner squares entered by the DOR group and the CON group.

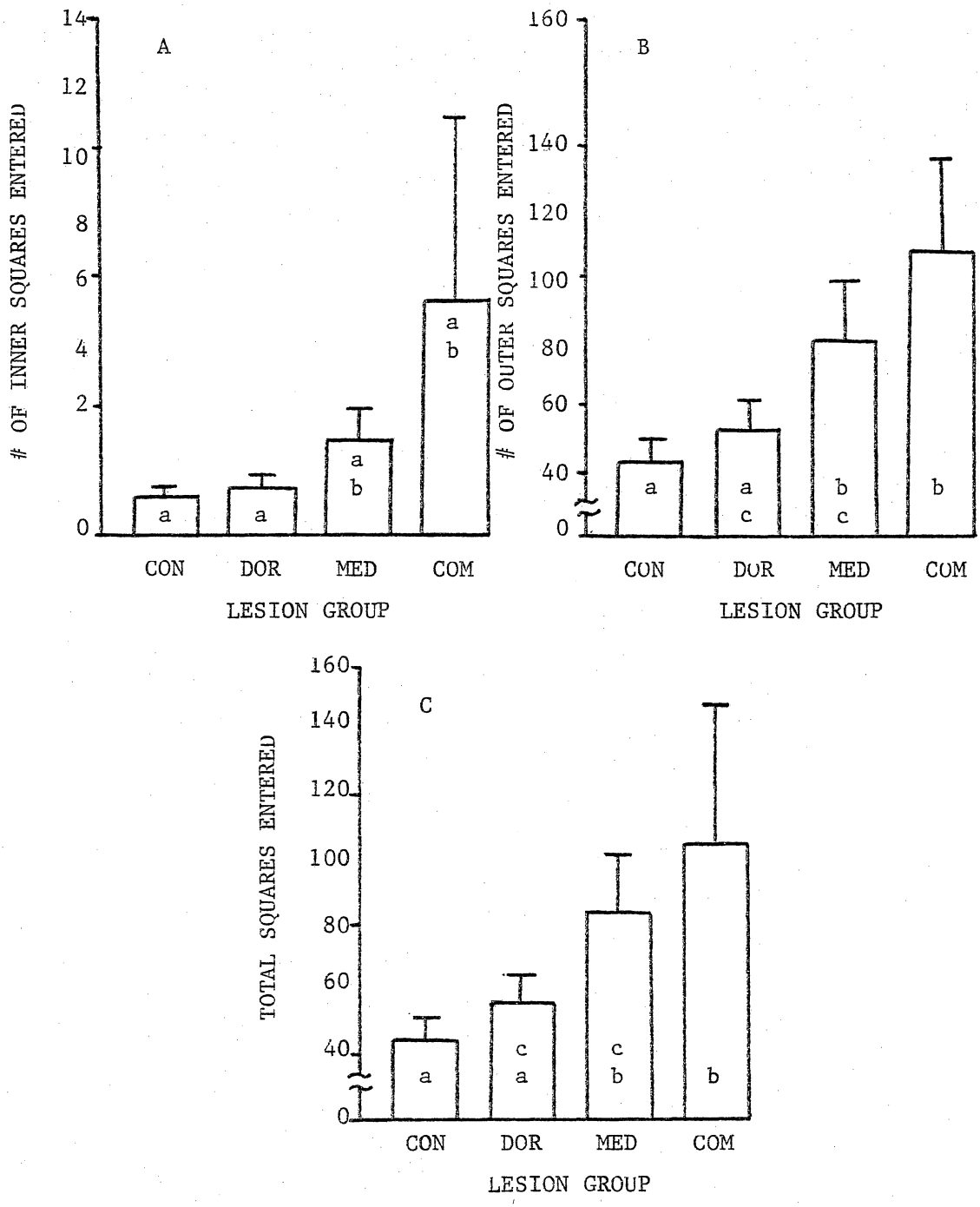

Figure 6. Mean number of squares entered in the open field by lesioned and control groups. Panel A is number inner squares entered, panel B is number outer squares entered, and panel C is total number of squares entered.

The pattern of differences was identical for the number of outer squares entered and the total number of squares entered.

The COM group entered fewer squares than the CON group in the pre-surgical observation, all other groups entered the same number of squares in the pre-surgical observation. In the post-surgical measurement the MED group and the COM group entered more squares than the CON or DOR groups which entered the same number of squares.

Copulation Measures

The proportion of animals from each group which initiated copulatory behavior, and those which completed that behavioral sequence were compared. In order to make these comparisons the proportion of each group performing an appropriate behavior (i.e., ejaculation) was calculated. Because of the small sample sizes no statistical test is appropriate for these data. However, an interesting pattern of differences emerged and those data are presented here.

Comparisons were made for the number of animals from each group achieving at least one intromission because this would indicate the number of animals initiating copulatory behavior. Likewise, number of animals from each group achieving more than one intromission were compared because this would indicate the number of animals proceeding with the copulatory sequence but not necessarily completing that sequence. And finally the number of animals from each group achieving an ejaculation were compared because this would indicate the number of animals completing the copulatory sequence. The pre-surgical and post-surgical proportions of animals reaching each of the above criteria, and the difference between those two proportions are presented in Table 4.

Table 4

Proportion of animals from each group attaining three behavioral stages within the copulatory sequence.

		CON n=14	DOR n=9	MED n=7	COM n=4
%≥1 Intro.	Pre	41	44	57	25
	Post	41	56	57	50
Change		0	+12	0	+25
%>1 Intro.	Pre	35	44	43	25
	Post	35	56	43	50
Change		0	+12	0	+25
% 1 Ejac.	Pre	12	0	0	25
	Post	6	22	14	25
Change		- 6	+22	+14	0

Notice that the pre-surgical values are approximately comparable, and that the post-surgical values are generally greater than these baseline values. A greater proportion of animals in the lesioned groups achieved at least one intromission. However, since the MED and control groups showed no change from the pre-surgical baseline it is likely that the lesion was not the cause of that difference. The lesions were effective in increasing the proportion of animals in the DOR and COM groups which initiated a copulatory sequence compared to the control animals. A similar pattern of results is observed in the proportion of animals continuing with a copulatory sequence. A greater proportion of the lesioned animals achieved an ejaculation. Again one of the groups did not show a change from its baseline performance and thus the post-surgical percentage of animals achieving an ejaculation in the COM group may not indicate an effect of the surgery. However, since the CON group did show a decline, the lack of change in the COM group may in effect represent an improvement. Clearly, both the DOR and MED groups were more likely to achieve an ejaculation in the post-surgical trials.

The means and standard errors for the traditional measures of male copulatory behavior obtained in the post-surgical trial are presented in Table 5. There were no significant differences between the groups on any of these measures.

Non-Copulatory Activity Measures

The proportions of animals engaging in each of the non-copulatory activity measures taken during the five minutes prior to the observation of copulatory behavior, and during the observation of copulatory behavior were compared. For most of those measures 100% of the animals

Table 5

Means and standard errors of the copulatory parameters taken during the post-surgical observation.

	CON	DOR	MED	COM
MF	11.1 ± 3.1(7)*	11.2 ± 4.5(9)	12.7 ± 7.5(7)	13.3 ± 8.8(4)
IF	5.4 ± 2.1(17)	5.8 ± 2.5(9)	8.0 ± 4.9(7)	5.3 ± 3.5(4)
ML	388.8 ± 139.9(14)	268.8 ± 154.9(7)	485.7 ± 171.0(5)	105.1 ± 92.4(2)
IL	442.4 ± 237.5(7)	526.1 ± 188.8(5)	442.0 ± 165.6(4)	262.3 ± 248.1(2)
EL	1290.1 ± - (2)	910.9 ± - (2)	1771.2 ± - (1)	1011.2 ± - (1)
PEI	331.8 ± - (1)	248.1 ± - (2)	282.8 ± - (1)	429.8 ± - (1)
III	114.3 ± 22.9(6)	95.6 ± 11.1(5)	153.3 ± 90.1(3)	116.5 ± 31.8(2)
HR	25.9 ± 8.4(14)	47.7 ± 15.4(7)	41.9 ± 14.1(5)	39.0 ± 15.2(2)

*The number in parentheses indicates the number of observations.

from each group displayed the behavior at least once, however, for some of the less probable behaviors differences between the groups were obvious. The proportions of animals from each group grooming and rooting in the bedding of the observation arena prior to the introduction of the stimulus female are presented in Table 6. There was a smaller proportion of animals in the MED group as compared to the CON group which groomed at least once in the post-surgical trial. And there was a smaller proportion of animals in the DOR group which rooted in the bedding at least once in the post-surgical trial. The pattern of change from the pre- to the post-surgical observation is interesting for this behavior. While there was little change in the CON group there was a large increase in the proportions of animals from the MED and COM groups which rooted in the bedding, and a slight decline in the DOR group. It could be argued that the change observed in the MED and COM groups represents a regression toward the mean of the population which is best represented by the CON group in this sample. On the other hand the DOR group showed a change away from the value predicted by this assumption.

The proportion of animals grooming their genital region, rooting in the bedding, and making investigative contact with the females genital region are presented in Table 7. The COM group had a higher proportion of animals grooming their genital region in the post-surgical observation period as compared to the CON group. However, since none of the groups changed from their baseline performance (with the exception of the DOR group) this difference is probably due to a difference in the initial levels of performance in the groups, and not to the surgical

Table 6

Proportions of animals from each group displaying grooming and rooting behaviors in the five minutes preceeding the mating tests.

		CON	DOR	MED	COM
%≥1	Pre	92	100	100	100
Groom	Post	91	88	60	100
Change		1	-12	-40	0
%≥1	Pre	89	60	50	50
Root	Post	82	50	80	100
Change		- 7	-10	+30	+50

Table 7

Proportion of animals from each group displaying genital grooming, rooting, investigation of the female partners genital region during the observation of copulatory behaviors.

		CON	DOR	MED	COM
%≥1	Pre	67	50	67	100
G. Groom	Post	64	63	60	100
Change		- 3	+13	- 7	0
%≥1	Pre	78	80	67	100
Root	Post	86	50	80	100
Change		+ 8	-30	+13	0
%≥1	Pre	83	75	100	100
Contact	Post	71	75	20	0
Change		-12	0	-80	-100

manipulation. A greater proportion of the animals in the DOR group displayed at least one instance of rooting in the bedding during the observation of copulatory behavior. The same pattern is evident here as was observed in the analysis of behaviors in the five minute adaptation period. The DOR group declined sharply from the baseline observation while the other groups gained or stayed at the same level. Finally, the MED and COM groups had a smaller proportion of animals making investigative contact with the female.

There were no significant differences among the four groups in the means of any of the measures of activity taken during the five minutes prior to the introduction of the estrous female. The means and standard errors for these measures are presented in Table 8. Furthermore, there were not significant differences among the four groups in the means of any of the measures of non-copulatory activity taken during the observation of mating behaviors. The means and standard errors of those measures are presented in Table 9.

Table 8

Means and standard errors of the post-surgical activity measures taken prior to the introduction of an estrous female.

	CON n=14	DOR n=8	MED n=5	COM n=2
# Rear	19.6 ± 1.7	18.6 ± 1.0	17.2 ± 1.5	10.5 ± 6.5
# Groom	3.0 ± .7	3.9 ± 1.0	3.2 ± 1.3	2.5 ± 1.5
# Root	3.2 ± 1.1	1.6 ± 1.0	4.4 ± 2.5	7.0 ± 6.0
Time Moving	120.2 ± 14.9	110.0 ± 13.3	109.7 ± 18.1	112.2 ± 19.0
Time Grooming	15.9 ± 3.9	16.2 ± 4.6	17.7 ± 8.5	7.9 ± 6.3
Time Rooting	12.1 ± 5.5	4.7 ± 3.1	18.0 ± 13.0	24.5 ± 22.4

Table 9

Means and standard errors of social behavior measures taken during the post-surgical observation of copulatory behavior.

	CON n=14	DOR n=8	MED n=5	COM n=2
# Rear	47.9 ± 6.8	40.5 ± 6.1	57.4 ± 12.1	26.5 ± 16.5
# Groom	34.0 ± 4.8	37.5 ± 8.9	36.2 ± 6.9	30.0 ± 24.0
# Genital Groom	5.6 ± 2.3	10.9 ± 6.0	3.6 ± 3.1	17.5 ± 16.5
# Roots	10.5 ± 3.7	2.8 ± 1.6	11.0 ± 6.5	5.0 ± 2.0
# Contacts	41.4 ± 8.5	33.1 ± 7.3	39.0 ± 16.1	29.9 ± 13.5
# Genital Contacts	4.2 ± 1.3	5.3 ± 1.7	2.8 ± 2.8	0.0 ± 0.0
Time Rearing	339.5 ± 87.6	294.1 ± 85.2	363.4 ± 94.7	97.0 ± 25.3
Time Grooming	314.9 ± 54.8	274.5 ± 92.1	224.4 ± 55.1	207.8 ± 99.9
Time Rooting	54.1 ± 18.2	12.2 ± 8.8	55.6 ± 37.2	12.6 ± 4.7
Time Moving	318.7 ± 44.6	222.2 ± 64.8	383.0 ± 98.5	145.4 ± 9.5

DISCUSSION

The change in copulatory behavior following lesions of the dorsal raphe nucleus reported here replicate the results of earlier work on the effects of 5-HT depletion on copulatory behavior. Specifically, the current results indicate that when compared to the control group the dorsal lesioned group had 1. a greater proportion of animals which displayed at least one intromission, 2. a greater proportion of animals which continued to intromit after that first intromission, and 3. a greater proportion of animals which ejaculated. There were no significant changes in other traditional measures of masculine copulatory behavior as a function of the lesions of the dorsal raphe. However, the above differences indicate that the animals in the dorsal lesioned group were more likely to initiate and continue with a copulatory sequence.

The above results are consistent with the findings of del Fiacco et al. (1975) in which systemic depletion of 5-HT in sexually naive rats resulted in an increase in the proportion of animals which intromitted or ejaculated. The above results also are consistent with the findings of Larsson et al. (1978) in which destruction of both midbrain raphe nuclei with 5.6 DHT in a similar improvement in male copulatory behavior. The current results then further confirm that the serotonergic system in general, and the midbrain raphe in particular have an inhibitory role in controlling male copulatory behavior. Furthermore, the present results extend the earlier findings by indicating that it is specifically the dorsal raphe nucleus which is the origin of this inhibitory influence.

The current results, and the other work discussed tend to indicate that the dorsal raphe has an inhibitory influence on the initiation

phase of copulatory behavior. However, that is not to infer that the dorsal raphe has no effect on the execution of the copulatory sequence. There is in fact some evidence indicating that this nuclear group is also involved in the execution components of masculine copulatory behavior. In the current study every animal with a dorsal raphe lesion which initiated a copulatory sequence did sustain that sequence, but did not necessarily achieve an ejaculation, whereas, in previous studies it was always the case that an animal which initiated a copulatory sequence completed that sequence. The infrequency of ejaculations in the current study was probably due to the constraint of the thirty minute observation period. Specifically these sexually inexperienced animals may not have had sufficient time to complete a copulatory sequence in the thirty minute test. Support for this explanation is provided by the results of a recent study by McIntosh (1980) using sexually experienced copulators.

McIntosh (1980) has demonstrated that the dorsal raphe nucleus does have an inhibitory influence over the execution mechanism. In that study lesions of the dorsal raphe of sexually experienced rats resulted in significant decreases in the frequency of intromissions preceeding an ejaculation, the latency to ejaculation, and the PEI. The decrease in the latency to ejaculation indicates that the execution phase of these lesioned animals' copulatory behavior was enhanced.

When the data presented by McIntosh are compared to the earlier findings an interesting empirical question is raised. That is, what is the precise relationship between the level of previous copulatory experience and disruption of the serotonergic system in determining the behav-

ioral outcome? Based on the present results and those of McIntosh (1980) and Larsson et al. (1978) I have generated the following hypothesis. The effects of 5-HT depletion in sexually inexperienced animals is to increase the proportion of animals initiating a copulatory sequence, and in experienced copulators is to enhance the execution of the normally initiated sequence (McIntosh, 1980) and in males which are at the peak of their performance depletion of 5-HT will have no further effect (Larsson et al., 1978). The interesting part of this hypothesis is that it stipulates that 5-HT depletion does not facilitate copulation behavior beyond its natural limits, which suggests that there may be an overriding physical constraint on this behavioral sequence.

It is interesting to note that the behavioral effects in the current study were not accompanied by a large decrease in the level of forebrain 5-HT. The current results showed that there was about a 25% depletion in forebrain 5-HT following the lesions of the dorsal raphe. Earlier studies which employed systemic depletion techniques and demonstrated behavioral effects similar to those observed in the present study (i.e., del Fiacco et al., 1975) have reported 5-HT depletion of as much as 80%. Thus the current results may be interpreted to indicate that it is not the amount of systemic depletion that determines the behavioral outcome, but the anatomical locus of the effective depletion.

The effective locus of the inhibitory influence which arises from the dorsal raphe to regulate copulatory behavior remains an empirical question. However, there are a number of likely anatomical sites at which to begin looking for that locus. The axons from the cells

located in the dorsal raphe are among the fibers which compose the medial forebrain bundle (MFB) and these cells have many terminal fields including one in the medial preoptic are (mPOA) (Bobillier et al., 1975). Both the MFB and the mPOA have been implicated in the control of male copulatory behavior (Larsson, 1979). Lesions of the mPOA disrupt male copulatory behavior (van de Poll and Dis, 1979; and Hart, 1980). This disruption is typically described as a reduction in the animals ability to initiate a copulatory sequence, and it has been reported that animals with small mPOA lesions which are induced to initiate copulation continue until ejaculation (Larsson, 1979). It has also been demonstrated that a similar effect is found when the MFB is lesioned. That is the initiation, but not the execution of copulatory behavior is disrupted by lesions of the MFB (Caggiula et al., 1973). Thus it seems that at least two structures which are neuroanatomically related to the dorsal raphe are also implicated in control of the initiation of male copulatory behavior.

There is additional neuroanatomical and physiological data to indicate that the serotonergic system amy act through the mPOA to influence masculine copulatory behavior. For example, testosterone has been demonstrated to bind in the mPOA (Sar and Stumpf, 1973), and implants of testosterone in this area will restore copulatory behavior in castrated male rats (Lisk, 1967). There is also a clear demonstration of the dependence of the serotonergic system for regulation of the rate of synthesis of serotonin on the presence of testosterone (Engel et al., 1979). These four converging pieces of evidence: 1. the projection from the dorsal raphe to the mPOA, 2. the similar behavioral

effects of manipulations of the two structures, 3. the dependence of serotonin on the presence of testosterone for control of its rate of synthesis, and 4. the common location of 5-HT and testosterone receptors in mPOA, make this structure a likely choice to begin a search for the effective locus of the inhibitory influences on the control of masculine copulatory behavior which arise in the dorsal raphe.

There was only one other behavioral change evident following the dorsal raphe lesions, this was a decreased proportion of animals rooting in the bedding during the five minutes preceeding and concurrent with the observation of copulatory behavior. This behavior was quantified originally because it was observed in pilot observations and an attempt was made to quantify as many behaviors as was possible in addition to the copulatory behaviors. There is evidence to indicate that this behavioral change makes sense from the perspective of possible interactions of the 5-HT projection with other structures of the central nervous system.

In order to understand how the lesions may have produced this change in behavior it will be necessary to describe the neuroanatomy of the olfactory system, and make one assumption about the motivation for the rooting behavior. The first order projections of the primary olfactory system arise from the olfactory bulb, and these cells have dendritic branches which extend into the receptor area of the olfactory epithelium. These first order neurons have efferent projections to the olfactory tubercle, the periform area of the amygdala, and the corticomedial nucleus of the amygdala. From these areas this system sends its second order neurons centrally, and these inervate much of the limbic

system. In addition a separable system arises from the amygdala and has the cortex as its eventual target, the second order neurons of this cortical fugal system projects to the dorsomedial nucleus of the thalamus (Heimer, 1972). There is a remarkable overlap between the projections of the dorsal raphe nucleus and this primary olfactory system. Efferent projections from the dorsal raphe nucleus have been demonstrated to the olfactory bulb, the olfactory tubercle, and the periform nucleus in the amygdala (Bobillier et al., 1975). Since the 5-HT system usually acts as an inhibitory influence on sensory input (Cooper et al., 1978), it is reasonable to hypothesize that this system of common innervation normally acts to dampen or modulate the nervous system's response to olfactory stimuli. In this case that system was disrupted by the lesions of the dorsal raphe, and thus the lesioned animals were experiencing intensified CNS responses to the olfactory stimuli available as compared to the control animals. Thus if the motivation for rooting in the bedding was investigation of subtle olfactory cues, and the lesioned animals were experiencing an intensified central nervous system reaction to those stimuli, then they would likely decrease olfactory investigatory behaviors. This is in fact what was observed. The reason that there was not a comparable change in investigatory contacts with the ano-genital area of the female is not known.

This influence of the 5-HT system on olfactory sensitivity is an interesting empirical question which has ramifications for the serotonergic systems influence on other behaviors. For example Larsson (1979) tends to argue that the olfactory system is the key to understanding the limbic systems control of the initiation of copulatory behavior. Thus

he would likely aruge that the two behavioral effects observed here, the increased proportion of animals copulating, and the decrease in the investigation of the bedding material by the dorsal raphe lesioned animals could have been mediated by the same olfactory mechanism, and that this mechanism increases activation of a behavioral mechanism much like the SAM.

The lesions of the median raphe nucleus did not have any effect on copulatory behavior with the possible exception that the proportion of animals achieving an ejaculation may have been increased in this group. While this difference is a possible interpretation of the results, a more conservative approach would indicate that there was no difference between these lesioned animals and the control group on this measure. The statistical outcome was likely due to an unusual change in the proportion of animals in the control group achieving an ejaculation. The more usual pattern for this measure is that with each successive trial a greater proportion of the group achieves an ejaculation (Goff and Weizenbaum, 1981), with the current data a decline was noted between the pre-surgical and post-surgical observations of the control group. If the control group had not changed, there would have been no difference between the proportion of animals which exhibited an ejaculation in the two groups. (However, this basis for comparison would not have changed the difference observed between the DOR and CON groups.) The comparison between these two groups on this measure remains an open question. For the sake of taking the more conservative approach I will assume that there was no difference between these two groups on the proportion of animals achieving an ejaculation.

Before closing the discussion of this issue it may be of some value to present the opposing arguments on the basis of the neuroanatomical data avaiable. Like the dorsal nucleus, the cells of the median nucleus give rise to axons which travel in the MFB. There is evidence which indicates that the fibers of the MFB influence the execution stage of copulatory behavior. Stimulation of the MFB causes a decrease in the latency to ejaculation (Eibergen and Caggiula, 1973). As many different neurotransmitter systems both ascend and descend through the MFB, this stimulation may or may not have affected specifically a system which overlaps with the serotonergic system arising from the median raphe nucleus. However, there are clearly different projections from the two raphe nuclear groups which have fibers in the MFB. The projections from the median raphe include many structures within the limbic system such as the septal nuclei, the habenula, and the hippocampus (Bobillier et al., 1975). However, none of these structures have been implicated in the neural control of masculine copulatory behavior (Larsson, 1979), while the mPOA projection from the dorsal raphe nucleus has been implicated in the neural control of copulatory behavior. Until further evidence indicates some reason for concluding that the median raphe is involved in the inhibition of male copulatory behavior, it will be tentatively concluded that no such role exists, and that the dorsal raphe is the important nucleus for the control of masculine copulatory behavior.

There were other behavioral differences evident following the lesions of the median raphe nucleus. These effects included a marked increase in activity in the open field. These results replicate the

findings of Srebro and Lorens (1975) and Jacobs et al. (1975). The latter authors concluded that this behavioral effect was due to a functional depletion of 5-HT in the hippocampus as that structure had been implicated in open-field activity previously. It is interesting to note that this increase in activity did not correlate with a change in copulatory behavior. This lack of a correspondence indicates that the influences of the serotonergic system on different behaviors is not due to a single mechanism of action, but rather to specific effects on different behavioral systems.

A second behavior that was affected by these lesions was a decrease in the proportion of animals investigating the genital area of the female during the observation of copulatory behavior. This phenomena can be explained by reference to overlapping neuroanatomical data and a behavioral effect of lesions of the point of that intersection. As described above there is a portion of the olfactory system in which secondary efferents synapse in the dorsomedial nucleus of the thalamus (Heimer, 1972). The median raphe nucleus also has an efferent projection to this nucleus (Bobillier et al., 1975). Furthermore, Sapolsky and Eichman (1980) have demonstrated that lesions of this nucleus reduce the occurence of vaginal investigation by male hamsters, but does not affect their copulatory behavior. It is striking that the same results were obtained in both the current study and the study reported by Sapolsky and Eichman.

The decrease in the proportion of median raphe lesioned animals which exhibited grooming behavior observed in the five minutes before the introduction of the female is consistent with a phenomena observed

following systemic depletion of 5-HT. Kutsher and Yamamoto (1979) observed that there was a decrease in the occurrence of grooming behaviors following systemic depletion of 5-HT with para-Chloroamphetamine (pCA). This change was part of an overall behavioral syndrome which included the occurrence of some abnormal behaviors. The reduction in the occurrence of grooming behaviors in Kutscher and Yamamoto's observations was accompanied by a decrease in other usual behaviors (i.e., rearing) and an increase in the occurrence of unusual stereotyped behaviors. While the animals used in the present study were not specifically evaluated for the presence of any stereotypy, no abnormal or unusual behaviors were noted in any of the animals during any of the behavioral observations. Regardless, if the current observation of a decrease in the proportion of animals grooming is due to the same mechanism altered by the pCA, then the effective site of action for that syndrome is likely originating in the median raphe.

The results for the combined lesion group will only be interpreted in terms of their similarities with the effects of the individual lesions. The increased proportion of these animals displaying more than one intromission, and thus continuing with a copulatory sequence is probably due to damage to the dorsal raphe as they also received that lesion. The absence of a significant increase in the proportion of animals which initiated or completed a copulatory sequence in these animals may be due to either the small sample size, or to an interaction with some mechanism which was effected by the inclusion of damage to the median raphe.

The increase in activity observed in the open-field is interpreted

as inclusion of damage of the same structure as was disrupted by the lesions of the median raphe. Likewise, the decrease in the proportion of animals investigating the genital region of the female was probably due to some damage to the median raphe nucleus.

The results of this study suggest a number of interesting hypotheses, and indicate the beginning of the solution to the puzzle of the loci of the effect of systemic depletion of 5-HT on masculine copulatory behavior. First, the change in copulatory behavior was observed only following the lesions of the dorsal raphe, thus indicating that this cell group is the location of the cells of origin for the inhibitory influence on copulatory behavior by serotonin. Second, a theme that occurred at least twice in explaining the other behavioral result was that the serotonergic system and the olfactory system may be enmeshed. Some of the behavioral effects observed following depletion of 5-HT may be explained by the interaction of these two systems. And third, this interaction between the olfactory system and the serotonergic system, and the ability of this interaction to explain some of the results of this experiment raises the possibility that the behavioral effects seen in copulatory behavior following 5-HT depletion may be secondary to a change in the olfactory system.

While the above results are not overwhelming, they do deserve consideration because they suggest some new hypotheses about how the copulatory sequence of the male rat is affected by other variables. Specifically, it seems that there may be considerable interaction between the olfactory system and the serotonergic system influence on the copulatory behavior of the male rat. Moreover, the pattern of behavioral

differences may be consistent with positing two separable influences from the olfactory system.

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Appendix I

HPLC procedures: Two injections from each sample were made into the High Pressure Liquid Chromatographic column in order to fully seperate all of the monoamines for quantification. The first injection was a 60ml sample of the super aliquot. For these injections the buffer (pH=4.36) was designed seperate peaks for Dopac, 5-HT, HVA, and 5-HIAA (see Table 10 for buffer recipe).

Before the second set of injections were made the catecholamines were seperated from the acids in the super aliquot with an alumina extraction procedure. In this procedure catecholamines are bound to alumina HCl and the acids are rinsed out of the remaining solution, then the catecholamines are rinsed off of the alumina and placed back in suspension. The eight step procedure for this extraction is described below. 1) A 200ml of super aliquot was added to 20mg of sized and charged alumina HCl. 2) 0.5ml of 1M tris-acetate buffer (pH=8.6) was added to the above solution and shaken for 10 minutes. 3) A centrifugal filter was constructed from a pipette tip filled with glass wool and suspended in a test tube. The liquid and the solid alumina (with the catecholamines now bound to it) were both placed in these filters. 4) The filters were centrifuged at 100g for 1 minute, the liquid which had been drawn through the filters was then discarded. 5) The solid alumina was rinsed with 0.5ml HOH and centrifuged, agin the liquid was discarded. This step was repeated. 6) The filter containing the solid alumina was transfered to a clean test tube and 100ml of .8M $HCLO_4$ was added to the alumina in order to rinse off the catecholamines which were bound to the alumina. This solution was then centrifuged at 100g

for one minute. 7) The alumina was rinsed one last time by adding 300ml of HOH to the filters and centrifuging. 8) The filters were removed, the liquid was vortexed to assure mixing of the added water, acid, and catecholamines; the liquid was centrifuged again to reduce any remaining alumina. 80ml of this solution was injected into the HPLC a buffer (pH=2.97) designed to seperate peaks for DA, NE, and Epinephrine was used (see Table 10 for buffer recipe).

Table 10

Buffer Recipes

Quantities for Unextracted Samples	Chemical	Quantities for Extracted Samples
34g	Citric Acid	23g
9g	Sodium Acetate	3g
100mg	Sodium Octyl Sulfonate	900mg
1800ml	HOH	1800ml
125ml	MEOH	100ml

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AN ANALYSIS OF BEHAVIORAL AND SEROTONERGIC MECHANISMS IN MALE RAT COPULATORY BEHAVIOR

by

Dennis McKevitt Goff

(ABSTRACT)

The copulatory performance of male rats (Sprague-Dawley) was quantified, and the factor analytic technique applied to the data. Since factor analysis assesses common variance, subject selection was organized so as to maximize behavioral heterogeniety. Three factors were retained in the statistical analysis. The variables in two factors, Copulatory Efficiency and Initiation, were similar to those contained in the two factors posited by Beach (1956). The third factor was Intromission Count; it contained 2 variables, intromission frequency (IF) and post-ejaculatory interval (PEI). Unlike the variables in the other two factors, however, IF and PEI were not significantly correlated in a simple correlation analysis. The absence of a correlation suggested that the Intromission Count factor contained a suppressor variable. Although the identity of the hypothesized suppressor variable is not known, others have shown that IF and PEI are systematically related to adrenal hormones, the female's behavior and to dominance position. Therefore, the present results suggest that the Intromission Count factor may bear a significant relationship to a broad range of social behaviors, in addition to copulation. In a second experiment an attempt was made to independently manipulate the Initiation and Copulatory Efficiency factors by making elecrolytic lesions of either the median or dorsal raphe nuclei. While there were no significant differences among the groups on measures of copulatory or non-copulatory social behav-

iors, a pattern of differences in those behaviors emerged which suggested that the serotonergic system may interact with the olfactory system to influence the two copulation factors.