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Effects of REM sleep deprivation on aggression and exploration in rats

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EFFECTS OF REM SLEEP DEPRIVATION ON AGGRESSION AND EXPLORATION IN RATS

A Thesis

Presented to

the Faculty of the Department of Psychology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Jose M. Bautista

May, 1994

APPROVED FOR THE DEPARTMENT OF PSYCHOLOGY

Robert A. Hicks PhD.

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APPROVED FOR THE UNIVERSITY

ABSTRACT

EFFECTS OF REM SLEEP DEPRIVATION ON
INTRASPECIES AGGRESSION AND EXPLORATION.

by Jose M. Bautista

This study attempted to clarify the relationship between REM sleep deprivation and the behaviors of intraspecies aggression and exploration. Thirty two Sprague-Dawley male rats were randomly assigned to a control group, and a REM sleep deprivation group (RSD). Exploration was measured as the percentage of time spent in the arm of a Y maze which held novel items. Video tapes were scored for three aggressive behaviors (chasing, mounting and pinning). No significant difference was found for the exploration variable. A significant effect was found for the aggression variable due to a decrease in aggression for the RSD group for the post-treatment session as compared to both the pre-treatment and post-recovery sessions. These findings suggest that when measured in an appropriate setting changes in intraspecies aggression can be found in REM sleep deprived animals; however, this type of aggression decreases as a consequence of RSD and not increases as has been hypothesized.

ACKNOWLEDGEMENTS

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Footnotes

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Effects of REM Sleep Deprivation on
Aggression and Exploration in Rats.

It has been argued that REM sleep serves to dissipate excess drive energy which is accumulated during wakefulness (Vogel, 1975). As a test of this hypothesis, a number of studies have been undertaken to measure the effects of deprivation of REM sleep on a variety of motivational behaviors. One of these motivational behaviors which should, hypothetically, be affected by loss of REM sleep is aggression. However, the studies which have considered the effects of REM sleep deprivation on the various forms of aggression have failed to reach a consensus. To elaborate, in the first study of its type, Morden, Conner, Mitchell, Dement, and Levine (1968) investigated shock-induced fighting (i.e., sham fighting in response to a train of electric shocks in two animals placed in a small enclosure) and reported that shock-induced fighting increased following REM sleep deprivation. However, these data can be challenged as an adequate test of this hypothesis because as others have pointed out (e.g., Blanchard, Fukunaga, Blanchard, and Kelly, 1977) shock-induced fighting is best described as a defensive behavior and is not related to true aggression. In another early test of this hypothesis, Sloan (1972) reported observing increased intraspecies aggression (i.e., aggression by one animal against another of the same species) in pairs of male rats following REM sleep deprivation. However, the methodology used in this study has been criticized in that the weights of his REM sleep deprived animals declined sharply during the course of the REM sleep deprivation treatment, and were substantially lower than controls. This suggests the possibility that the increases in aggression which he reported were not due to REM sleep deprivation per se, but rather may have been potentiated by a factor, such as stress, which contributed to the differential weight loss between his REM sleep deprived and control groups. In addition, Sloan noted that most of the aggressive behaviors were recorded and scored by one person who was not blind to the animals' treatment condition.

For another form of aggression, and consistent with the hypothesis that REM sleep deprivation increases motivational behaviors, Kuroda, Gonzales, Gomez, Reyes, and Hicks (1982) found an increase in nonspecific aggression (i.e., animals aggressing against a non-specific object) in young male and female rats, by measuring latency to push through a cotton barrier blocking a runway which the rats had been previously trained to cross to reach a food reward. These data were comparable to an earlier study in which Hicks, Hirshfield, Humphrey, Lauber, Giampaoli, and Hawkins (1981) found that REM sleep deprived animals were more likely to win using a food competition paradigm against non REM sleep deprived controls. Thus these studies tend to support the hypothesis that REM sleep deprivation increases a form of aggression, i.e., nonspecific aggression. In addition, in their investigation of the effects of REM sleep deprivation on interspecies aggression (i.e., rats attacking mice), Hicks, Moore, Hayes, Phillips and Hawkins (1979) demonstrated that REM sleep deprivation substantially increased the number of rats that attacked and/or killed mice both during an immediate post treatment test and after a 24-day recovery period.

In general, the preceding studies all provide support for the hypothesis that REM sleep deprivation increases aggression, regardless of the type of aggression under consideration. However, with the exception of the Sloan (1972) study, these studies did not measure intraspecies aggression, and as was mentioned, this study is hard to interpret on methodological grounds. Further, others who have measured the relationship between REM deprivation and intraspecies aggression in rats have not confirmed Sloan's results.

For example, Kuroda (1984) failed to find an increase in intraspecies aggression in male rats, using the intruder paradigm. This paradigm makes use of the territoriality of the animal against an intruder. In another study, Peder, Elomaa, and Johansson (1986) did not find an increase in intraspecies aggression in REM sleep deprived male and female rats following reductions in enclosure dimensions. More recently, Shaw, Puentes, Reis and Hicks (1990) using groups of three rats consisting of a REM sleep deprived animal and two controls (i.e., a dry control and a wet control) in an open field apparatus, reported that REM sleep deprivation had no activating effect on intraspecies aggression. In explaining their results, Shaw and his colleagues suggested that this lack of effect of REM sleep deprivation on aggressive behavior might be due to the activation of another behavior which is incompatible with the expression of this form of aggression. To elaborate, it was suggested that if REM sleep deprivation serves to potentiate some behaviors, it may do so in a differential way depending on the behavior in question. In order to explore this idea, Bautista, Grunow, Lawrence, Lucero, Nelson, McCullough, Pass and Hicks (1993) in an attempt to investigate the possible relationship between REM sleep deprivation and stress (in the form of food deprivation) as a justification for the contradictory literature, reported no increase in aggression for REM sleep deprived animals whether food deprived or not. Thus, if exploration is affected preferentially to intraspecies aggression, the effect of REM sleep deprivation on this type of aggression would be masked by this preference and thus an effect on intraspecies aggression would not be detected if measured in a manner where both behaviors were possible. If exploration was increased by REM sleep deprivation, as has been reported by Hicks and Moore (1979) for animals of the age used in Bautista's study, this increase could account for the lack of effect of the REM sleep deprivation procedure on intraspecies aggression since aggressive involvement and exploration appear to be incompatible behaviors.

Measuring Exploration

Typically, the measurement of exploration in rats involves placing these animals into an open field apparatus with grid marking on the floor, and counting the number and type of grid crossings the animal makes in a predetermined amount of time. However, there is a problem with this kind of methodology if it is to be used concurrently with an effort to measure aggression in the same animals. This problem is that many aggressive behaviors such as chasing involves movement by the animal possibly involving many grid crossings, which should not be counted towards a measure of exploration, but which are hard to divorce from such under these conditions. Thus, this study looked at the relationship between REM sleep deprivation and exploration by borrowing from the study done by Moore, Hayes and Hicks (1979) which explored the relationship between REM sleep deprivation and preference for novelty in rats. This study used a modified Y-maze consisting of a start box and two identical interchangeable arms, one of which contained novel items. By calculating the proportion of time spent by each animal in the novel arm of the Y-maze, a measure of exploration was obtained.

This study was designed to investigate the effects of REM sleep deprivation on both intraspecies aggression and exploration by measuring these dependent variables simultaneously in an apparatus that minimizes the confounding of these variables. Based on the literature reviewed, it was hypothesized that REM sleep deprived animals would not exhibit an increase in aggressive behavior, but would show an increase in exploratory behavior.

Method

Animals

The animals were 32 male Sprague-Dawley rats, 60 days old when they arrived from the vendor. They were housed in 19.7 liter buckets specially equipped with feeders and water bottles, allowing the animals ad lib access to food and water. Lighting was on a 12:12 hour light:dark cycle, with lights on at 8:00 AM.

REM sleep deprivation and controls

REM sleep deprivation was achieved using the inverted flower-pot technique described in detail by Hicks and Moore (1979). Basically, this technique makes use of the muscular atonia characteristic of REM sleep by placing the animal on a small platform (6.5 cm diameter) surrounded by water. When the animal enters into REM sleep, it looses muscle tone and comes in contact with the water thus awakening. As a control for the wet environment a "Wet control" is usually used as a part of this REM sleep deprivation procedure. This control places the animal atop a larger platform (10.5 cm) than that used for REM sleep deprivation, allowing the animal to attain fairly normal sleep while being in a wet environment. However, previous experience with this control treatment has consistently yielded results that are intermediate between those from the REM sleep deprived animals, and those from a dry control which consists of the animal being placed into a treatment which is identical to that in which the animals were adapted (i.e., no water, with a platform in place). The reason for these intermediate results stem most likely from the reported partial loss of REM sleep for animals placed into a wet control condition, especially during the first few days of treatment (Vogel, 1975). Since this study used a treatment period of four days, and previous research of this type has shown no real benefits from using a wet control group, this study only compared REM sleep deprived animals with dry controls.

Apparatus

The animals were tested for aggression and exploration in a modified Y-maze consisting of a start box (38 x 38 cm) with two identical interchangeable arms attached, each 70 cm in length and 25 cm in width. The arms were positioned so that the animals had a clear view of both from the start box. The interior of the Y-maze was painted a medium gray color. One of the arms was equipped with Velcro strips to which novel items consisting of children's toy blocks were attached.

The floor of the Y-maze was covered by a removable Plexiglas surface for easy cleaning between trials to eliminate any scent signals left by the previous group of animals.

Intraspecies aggression

In order to record the target behaviors which made up the dependent variables in this study, a video camera was suspended above a Y-maze for taping of each group of animals. Each group of animals was video taped for 20 minutes before entering into a four day treatment period (REM sleep deprivation or dry control), immediately after the four days of treatment, and four days after the end of the treatment period. Trained judges who were blind to the animals' condition scored the tapes for the aggressive behaviors of chasing, mounting and pinning. These behaviors were defined as follows: chasing, the pursuit of one animal by another including direct nose to tail contact; mounting, the climbing of one rat upon another directly from behind including grasping with both forelimbs; pinning, the full body turning of one rat by another placing it on its back with the perpetrator laid atop.

Exploration

Exploration was scored by measuring the amount of time spent in the novel arm of the Y maze by each rat and converting it into a percentage by dividing by the total time spent in the maze (20 minutes) and multiplying by 100. We anticipated that the REM sleep deprived animals would tend to leave the others to explore their novel environment.

Procedure

Animals were placed into their modified buckets upon arrival from the vendor. For the first 10 days they were handled for seven minutes each day, and placed into the apparatus alone and without any novel items for 3 minutes to adapt them to the test conditions. On the eleventh day, the animals were divided into tetrads (i.e., groups of four) according to their weight (i.e., the four heaviest together, then the next four heaviest, and so on). The decision to use groups consisting of four animals was made to allow the opportunity for aggressive behaviors among the animals. To clarify this point, if, as was hypothesized here, the deprivation of REM sleep would activate an animal's exploratory behavior, then we must provide this animal the opportunity to express that behavior so that we could record it. If we had tested only two animals at a time, a REM sleep deprived animal and a dry control animal, then any aggression on the part of one animal would have impacted the other's ability to express some other behavior, i.e., exploration. Within each tetrad, each animal was randomly assigned to one of two conditions; i.e., the dry control condition which was identical to their adaptation period (i.e., dry bedding in their respective buckets), or the REM sleep deprived condition which is described above. Each tetrad consisted of two animals in each of these two conditions. Each animal within each tetrad was also randomly assigned one of four markings (using an odorless, waterproof, non-toxic ink marker) for identification on the video tapes; a circle, an "X", a filled circle, or two horizontal left to right stripes. These were chosen because of the ease of identification of animals when on videotape. Animals remained in their respective tetrads for the remainder of the study.

After all the animals had been assigned their respective conditions and markings, each tetrad was in turn placed into the start box of the Y-maze and then video taped for 20 minutes. At the end of the 20 minute test session the animals were removed from the Y-maze and replaced into their original buckets, except that the animals in the REM sleep deprivation condition were now in buckets with small platforms and water in place.

After four days of treatment, the animals were again marked and the testing procedure repeated. Following this testing session, the animals were returned to their buckets, all now with dry bedding, for four days of recovery. After the four day recovery period, the animals were placed into the Y-maze and the testing procedure was repeated.

During each of the 18 days over which this study took place, weights were taken for each animal, their buckets cleaned and food and water replaced as needed. All activities took place between the hours of 9:00 AM and 11:00 AM. In order to accomplish these tasks (including the videotaping) within this time period, the study was done in two stages, each consisting of sixteen animals, and each taking 19 days to complete (18 days for the study, plus one day in which they were received from the vendor).

At the end of the data collection the video tapes were scored. This was done by judges who had been previously trained for a period of one month, to score the behaviors outlined in this study using video tapes from a prior study which looked at the same behaviors. Each judge was randomly assigned an equal number of animals to score in each condition (REM sleep deprived or Dry control), so as to distribute any variability in their scoring evenly among the conditions. Also, each judge scored the same animal for each taping session (pre-treatment, post-treatment and post-recovery) so as to minimize the variability in scores for each animal.

Results and Discussion

Table 1 shows the intraspecies aggression score means and standard deviations for dry control and REM sleep deprived groups for the three testing sessions. A three (testing session) by two (sleep deprivation) MANOVA with repeated measures was done using these scores (see Appendix B). This analysis revealed a significant within subjects effect

Table 1.

Means and Standard Deviations for aggression scores for Dry control (DC) and REM sleep deprived (RSD) groups for each of the three testing sessions.

	Pre-treatment		Post-treatment		Post-recovery	
Group	Mean	S.D.	Mean	S.D.	Mean	S.D.
DC	18.9	9.2	16.9	10.0	15.9	8.7
RSD	20.1	12.5	12.4	8.7	18.5	11.1

for the testing session ($F_{2,60}$ = 3.35, p = .042), as well as no significant interaction ($F_{2,60}$ = 2.03, p = .141).

Planned comparisons by means of t tests for related measures were performed for the REM sleep deprived group between pre-treatment and post-treatment, and between post-treatment and post-recovery testing sessions for the aggression scores. As was expected from the results of the MANOVA above, comparisons of the aggression scores did yield significant differences between the pre-treatment/post-treatment scores (t_{15} = 2.60, p = .020), and the post-treatment/post-recovery scores (t_{15} = -2.94, p = .010).

Prior studies have shown either an increase in intraspecies aggression following REM sleep deprivation, or no significant effect. However, on closer inspection, two prior studies (Bautista et al., 1993 and Shaw et al., 1990) have both shown a decrease in aggression following REM sleep deprivation, although, the overall effects for treatments did not reach significance. Most studies which have used the inverted flowerpot method to induce REM sleep deprivation in rats have made use of a wet control which, as described earlier, utilizes a platform larger than that used to achieve REM sleep deprivation, but which controls for the wet environment present in the procedure. It is possible that the use of a wet control group, which has been shown to deprive the animals of some REM sleep, may dilute the variance in aggression scores sufficiently to make any significant effect undetectable using an overall test such as an analysis of variance. Table 2 shows the intraspecies aggression data from Bautista et al. (1993). Reexamination of these data revealed that when taken alone, the aggression scores for the non food-deprived REM sleep deprived group do show a significant effect between pre-treatment and post-treatment testing sessions (t_{13} = 5.45, p <.001), as well as between the post-treatment and post-recovery testing sessions (t_{13} = -2.67, p = .019). Using the data reported by Shaw et al. (1990) which are presented in Table 3, t tests were computed for both the

Table 2.

Means and Standard Deviations for aggression scores for non food deprived REM sleep deprived (RSD) animals for each of the three testing sessions. Bautista et al. (1993).

Pre-treatment		Post-treatment		Post-recovery	
Mean	S.D.	Mean	S.D.	Mean	S.D.
45.9	16.5	26.9	14.6	43.9	16.4

Table 3.

Means and Standard Deviations as reported by Shaw et al. (1990) for intraspecies aggression scores and dominance scores for REM sleep deprived (RSD) animals for each of three testing sessions (i.e., pre-treatment, post-treatment, and 10 day post-recovery).

Behavior	Pre-treatment		Post-treatment		10 Day Post-recovery	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Aggression	39	29	28	23	39	33
Dominance	151	31	118	35	151	23

aggression and dominance scores that they reported for their REM sleep deprived group, with the result that the differences between the pre-treatment/post-treatment means and the post-treatment/post-recovery means were not significant, i.e., $t_9 = .94$ and $t_9 = -.86$ respectively. However, the differences between these sets of means for the dominance scores for the REM sleep deprived animals were both significant, i.e., for the pre- and post-treatment means ($t_9 = 2.23$, $p < .05$) and for the post-treatment/post-recovery means ($t_9 = 2.49$, $p < .05$). Both of these studies measured intraspecies aggression in an open field apparatus (81.3 X 81.3 cm) which was smaller than the apparatus used in this study, which may account for the discrepancy between the larger intraspecies aggression scores reported in those studies as compared to this study. However, the three intraspecies aggressive behaviors measured in all three studies were the same, the duration of the REM sleep deprivation treatment was the same, and among the results from Bautista et al. (1993), and Shaw et al. (1990) the aggression scores reported are comparable. Thus in all of these related studies the pattern of the means reported for the REM sleep deprived animals is consistent with the data from this study. That is, a decrease in aggression scores for the post-treatment testing session followed by a return to pre-treatment levels after recovery from REM sleep deprivation.

Table 4 shows the exploration score means and standard deviations for dry control and REM sleep deprived groups for the three testing sessions. In order to test the hypothesis that REM sleep deprivation would increase exploration, a three (testing session) by two (sleep deprivation condition) MANOVA with repeated measures for the testing variable was performed comparing pre-test, post-test and post-recovery exploration scores (see Appendix C). The results of this test revealed no significant effect of treatment condition ($F_{1,30} = .09$, $p = .765$), or testing session ($F_{2,60} = .57$, $p = .568$), as well as no interaction between the treatment variable and the testing session variable

Table 4.

Means and Standard Deviations for exploration scores for Dry control (DC) and REM sleep deprived (RSD) groups for each of the three testing sessions.

	Pre-treatment		Post-treatment		Post-recovery	
Group	Mean	S.D.	Mean	S.D.	Mean	S.D.
DC	43.7	6.3	40.4	7.0	42.8	4.6
RSD	43.2	5.8	42.7	9.6	42.1	4.7

($F_{2,60}$ = .45, p = .638). Similar planned comparisons were performed on this data by means of t tests which yielded no significant difference for the exploration variable for either the pre-treatment/post-treatment scores (t_{15} = .14), or the post-treatment/post-recovery scores (t_{15} = .20). This is not the first study to fail to show such an effect (Boyaner, 1970; Hicks, Thomsen, Pettey & Okuda, 1976). Although the current study differs from prior studies in that it attempted to measure exploration in more than one subject at a time, it seems that this alone is not enough to make the results of this study ineligible for comparison to prior studies. It also seems clear that the methods used in these studies are not the source of the disparate results; two of these studies (Albert, Cicala, & Siegel, 1970; Hicks, et al., 1976) used an activity box (i.e., an open field equipped with items for the animals to interact with), and two (Boyaner, 1970; Moore et al., 1979) used a Y maze similar to the one in the current study. Since only one study using each method showed an effect, the method by itself seems to not be a factor in the results.

Taken together, the lack of effect of REM sleep deprivation on exploratory behavior and the decrease in aggressive behavior found in this study fail to support the general motivational hypothesis of REM sleep advanced by Vogel (1975). In fact, the data on aggressive behavior seems to argue against such a hypothesis. Further research into this relationship should take into account the confounding elements elucidated by this study; for example, the measurement of exploration would perhaps be better achieved separately from the measurement of aggression, by testing one animal at a time rather than concurrently as this study did.

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SAN JOSE STATE UNIVERSITY

Appendix A

A campus of The California State University

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April 26, 1993

TO: Jose Bautista, MA in Psychology candidate

FROM: Kevin Jordan, MA Coordinator

RE: Design and Analysis review of thesis proposal

Drs. Feist and Huntsman have read your thesis proposal for the Design and Analysis Committee. Both reviewers found the design and analysis to be quite sound. Dr. Huntsman had some specific comments for you to consider for the final version of the thesis, so I have attached the manuscript for you. Given the reviewers' favorable comments, the proposal is of course approved.

Based on this committee's approval, the collection of data for your thesis is approved contingent on documentation of compliance with university policy regarding the use of animal subjects in research. University policy requires the approval of your project by the Institutional Animal Care and Use Committee. Since the research you will undertake has an approved protocol as part of Dr. Hicks' grant, please provide me with a file copy documenting such approval. After that copy is part of your file, you may begin collecting data.

Congratulations on your progress to date! We look forward to the continuation of your fine performance in the program.

cc: Feist
Hicks
Huntsman
Phillips (via Hicks)
Veregge (Biological Sciences)
file

San Jose State University
Institutional Animal Care and Use Committee

LETTER OF OFFICIAL PROTOCOL REVIEW

Date: January 30, 1991

Dear Dr. Robert A. Hicks:

The animal care and use portion of your research proposal indicated below was reviewed by the Institutional Animal Care and Use Committee (IACUC). The IACUC Protocol Number indicated below should be used when ordering animals for this study, and on grant and contract proposals to fund this study. This protocol number may be used ONLY by the principal investigator and other participants included in the protocol. **The IACUC must be notified in writing of any proposed changes to this approved protocol, and approval must be granted in writing before any change is instituted.**

If you have any questions, please contact Dr. Miriam Saltmarch at **924-3118.**

APPROVAL FOR THE IACUC COMMITTEE

Miriam Saltmarch, Ph.D., Chair, IACUC

Project Title: Sleep and Health Related Behaviors

Protocol No.: 534

Approval Date: 1-30-91 **Expiration Date:** 7-31-95

Species To Be Used: Rats **Total No. of Animals:** 370

Principal Investigator: Dr. Robert A. Hicks

Department: Psychology **Phone:** (408) 924-5659/7233

Co-Investigator: Nathan Phillips

X This application was approved without modification.

____ This application was approved with the following mandatory changes:

____ This application was **not** approved for the following reasons:

cc: University Animal Care Office
Chair: Investigator's Department, or Departmental Animal Committee

SJSU/UAC/1989
jdm

Appendix B

MANOVA tables for the intraspecies aggression data.

Between-Subjects Effects

	SS	DF	MS	F	sig of F
Within Cells	5873.31	30	195.78		
Constant	28187.76	1	28187.76	143.98	.000
Condition	1.26	1	1.26	.01	.937

Within-Subject Effects.

	SS	DF	MS	F	sig of F
Within Cells	3362.00	60	56.03		
Test	375.52	2	187.76	3.35	.042
Condition by Test	227.15	2	113.57	2.03	.141

Appendix C

MANOVA tables for the exploration data.

Between-Subjects Effects

	SS	DF	MS	F	sig of F
Within Cells	987.15	30	32.90		
Constant	173264.03	1	173264.03	5265.59	.000
Condition	2.99	1	2.99	.09	.765

Within-Subjects Effects

	SS	DF	MS	F	sig of F
Within Cells	2889.15	60	48.15		
Test	54.98	2	27.49	.57	.568
Condition by Test	43.61	2	21.81	.45	.638