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14. ABSTRACT Obesity and metabolic diseases are known to be tightly linked to arousal-sleep cycles. Further, both metabolic disease and arousal are known to have significant impacts on cognitive function in humans and animals. Importantly, the armed forces represent a population at significant risk for increased stress and disrupted arousal-sleep cycles. Because the incidence of metabolic disease and obesity is increasing, even in these physically fit individuals, understanding the interactions between these systems is highly significant. Further, some anti-fatigue pharmacologies (e.g., modafinil) are already used in military settings, though their long-term effects on metabolism or central nervous system function are not well-understood. We have completed Year 1 of the proposed funding period to assess the physiological and behavioral effects of this pharmacology on rat subjects. Our first year data demonstrate that chronic administration of intraperitoneal modafinil decreases food intake and body weight in rats. Additionally, we observed that acute central modafinil has deleterious effects on some hippocampal-dependent forms of learning. These findings support our overall hypothesis that pharmacological activation of the central orexin system may modulate energy balance. Ongoing studies are assessing the effects of treatment on insulin sensitivity and also the effects of drug withdrawal on body weight regulation and cognitive function.					
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Introduction:

The incidence of obesity is escalating to epidemic proportions in all segments of society. Even among the military, with much higher levels of fitness than civilian populations, are experiencing a rapid increase in obesity and metabolic disease. Recent research has suggested an important connection between arousal/stress physiology and metabolism. One important basis for this connection may be the brain orexin-A system, which is also the principal target of the anti-fatigue drug modafinil. However, the specific molecular machinery remains unidentified, as do the behavioral effects of manipulating this system. **Objective/Hypotheses:** We have hypothesized that modafinil and other anti-fatigue drugs may act by modulating metabolic pathways in the central nervous system. We also hypothesize that chronic stress and disruption of arousal-sleep system leads to impaired metabolic function and increased susceptibility to obesity. Finally, we hypothesized that central metabolic pathways can be activated by foods and nutritional interventions, in lieu of pharmacological manipulation, with less risk of long-term metabolic complications. To assess these hypotheses, we are conducting several studies in rat subjects. The primary study designs include administration of modafinil and assessment of the molecular and genetic effects (Project 1) as well as behavioral consequences (Project 2).

Body:

During the first year of funding, we have made significant progress toward the stated aims and objectives. All proposed studies for Year 1 have been completed and we detail the results and conclusions here. For each study, we list the statement of work task, specific objectives, methods employed, and results obtained. Figures referenced are presented in an appendix at the end of the report.

Project 1 Year 1 Tasks:

Task 1: Assess the metabolic effects of chronic orexin-A activation by modafinil (1-18 months)

- Assess chronic effects on food intake, energy expenditure, metabolic rate, and body adiposity.
- Measure insulin sensitivity and glucose homeostasis.
- Assess the effect of modafinil to exacerbate the metabolic effects of stress and circadian disruption.

Experiment Series 1.1 Methods

Adult, male, Long-Evans rats were used in all of these experiments. The choice of diets for these experiments was critical. In this case, we use a diet that is 40% fat (by calories) with the predominant source of fat coming from saturated fat (1). We have a great deal of experience with this particular diet and it is much closer to a typical human diet than is standard rodent chow which is inconsistent in its ingredients and only 8% fat. Rats were given modafinil at a dose of 4.0 mg/kg once per day by oral gavage. These doses are approximately equivalent to doses used to increase performance in sleep-deprived humans. The key end points to be measured were food intake, body weight and body fat. .

Experiment Series 1.1 Results

We observed that chronic modafinil administration significantly reduced body weight and body adiposity in rats. Figure 1 depicts total body weight across days of treatment with either modafinil or vehicle. Figure 2 depicts that daily gavage with 4.0 mg/kg modafinil transiently decreased food intake. However, as noted in that figure experimental rats resumed food intake similar to that observed in control animals after 7-8 days of treatment. Figure 3 depicts body adiposity as measured by NMR. As can be seen in that figure, and consistent with patterns of food intake, modafinil treatment reduced (albeit slightly) the total level of body fat after 1 day of treatment, but there were no differences after 2 weeks dosing. Statistical significance was assessed by repeated ANOVA using Tukey's HSD post-hoc tests. Asterisks depict significant differences between modafinil and vehicle-treated rats.

The next set of experiments will determine the effect of modafinil on propensity to gain weight when exposed to a high-fat diet after the modafinil exposure. Measures will include body weight, body fat and also measures of glucose tolerance as well. This is more analogous to the situations where modafinil is used in warfighters where they take the drug for some period of time in order to remain alert and then cycle off of it. Thus a key question becomes whether modafinil exposure increases the risk for metabolic disease.

Project 2 Year 1 Tasks:

Task 1: Identify and measure acute effects of modafinil on cognition and behavior (1-12 months)

- Measure effects of modafinil and orexin-A activation on key cognitive parameters: spatial memory, stress performance, motoric effects, and perception (months 1-6).
- Identify specific beneficial effects of acute delivery. Identify specific negative consequences of acute delivery (months 7-12).

Experiment Series 1.2 Methods

In the first series of experiments, rats received acute administration of modafinil (0.1 and 1.0 nmole/1 μ l determined by pilot testing) or vehicle 1-hr prior to training in standard memory tasks for rodents: novel object recognition (NOR) and the passive avoidance task (details described below). In the second series of studies, we investigated the

NOR test: Rats will be injected intraperitoneally (i.p.) with modafinil or vehicle 1-hr prior to training session with two identical objects. For and 24-hr later, they are returned to the training/testing context and one of the objects is replaced with a novel object (e.g., paper weight or coffee cup). We use a digital-video based computer analysis to calculate percent time investigating the objects. Percent time at the novel object is used as an index of memory. This test requires 3 days habituation, 1 day for training and 1 day for test.

Passive avoidance test: Rats received ICV 0.1, 1.0 nmoles modafinil or vehicle 1-hr prior to training in the passive avoidance task. In this task, rats are presented with a moderate tone on one side of a conditioning chamber. When rats "escape" the presence of this tone by moving into the opposite chamber, the doorway is closed and rats receive a mild foot-shock. They are returned to the apparatus after 24 hours for testing. IN both instance, we measure the latency to enter the opposite side of the chamber to avoid the moderate tone stimulus. An increase in latency indicates memory of the footshock.

Elevated plus maze (EPM) and open-field tests: We have also administered icv (0.1 nmoles and 1.0 nmoles) modafinil or vehicle 1-hr prior to acute testing in critical measures of stress and anxiety. The EPM and open field tests are standard measures of stress and anxiety in rats and mice. Here, we assessed the effects of modafinil to increase time spent in the closed arm of the EPM, and also decrease general activity in the open field (both are indicators of stress). An elevated plus maze constructed of 1/8" polypropylene plastic was used. Each of four arms (10 x 50 cm) is adjoined by a 10 x 10 cm intersection. The base of the maze is constructed such that the arms are elevated 50 cm above the ground. Animals will be placed in the center of the apparatus facing an open arm and allowed to freely explore the apparatus for 5 min for behavioral observation. Briefly, rats were treated ICV 1-hr prior to testing. Separate cohorts of rats were used for EPM and open-field. In both instance, tests were conducted at lights-out (the time of greatest activity for rodents) and both tests were 20 minutes in duration.

Intraventricular Cannulation. Animals were shaved and surgically prepped. A 2-cm midsagittal skin incision was made to expose the skull. Holes for anchoring screws and the cannula were drilled. A stainless steel (22 gauge, Plastics One) guide cannula extending into the third ventricle was permanently affixed to the skull by means of metal bone screws and quickly-drying dental acrylic. A removable 18 gauge obturator sealed the guide cannula when not in use. All skull openings are sealed with dental acrylic. Gelfoam or bone wax followed by skin closure with suture. By manipulating the placement of the cannula, we can also put the cannula into specific brain regions for more local injection of substances.

Experiment Series 1.2 Results

We observed little or no significant effects of modafinil (either dose) to increase anxiety-like behavior in rats. In both EPM and open-field tests, experimental rats exhibited levels of anxiety-like behavior similar to those observed in vehicle treated rats. Specifically, acute modafinil did not increase significantly the amount of time spent in the closed arm (EPM, Figure 4) or significantly increase the time spent at borders (open-field, Figure 5). Both of these measures are standards for assessing anxiety and stress in rats. However, we did observe a slight but significant effect of acute modafinil on hippocampal dependent recognition memory (Figure 6). The degree of specificity for hippocampus was indirectly assessed by passive avoidance fear conditioning, which is known to be an amygdala-dependent learning task.(Figure 7). The statistical significance of the data were analyzed by 1-way between-subjects ANOVA and Tukey's HSD post-hoc tests. Asterisks indicate statistically significant differences from vehicle treated rats.

Key Research Accomplishments:

- Chronic modafinil reduces body weight with a transient decrease in food intake.
- Chronic modafinil does not increase body adiposity.
- Acute ICV modafinil does not increase stress or anxiety levels in rats.
- Acute ICV modafinil may impair hippocampal- but not amygdala-dependent memory.

Reportable Outcomes:

1. Portions of the Year 1 data were presented at the 2006 annual meeting of the Society for the Study of Ingestive Behavior (Naples, FL USA).
2. Portions of the Year 1 data were presented at the 2007 annual European Winter Conference for Brain Research (Villars, Switzerland).
3. No patents or cells lines have been developed.
4. An animal model (mouse) of chronic variable stress based on the hypotheses generated here is currently under development in collaboration with Dr. James Herman (University of Cincinnati).
5. Derrick Choi, a student in the Benoit Lab, has selected orexin-A activation in stress and food intake for his Thesis project. This project will be officially proposed at the end of this calendar year.

Conclusions:

While, the studies of Year 1 were by design more descriptive than mechanistic, we were able to draw several important conclusions that will guide the execution of experiments proposed for the subsequent years' funding periods. First, we conclude that chronic intraperitoneal administration of modafinil does not increase body adiposity in rats. This was a key hypothesis for the overall proposal. Importantly, we are now assessing the effects of chronic modafinil administration *and withdrawal* on the rate of body weight gain and increased risk for the development of obesity. Second, we conclude that *acute* administration of modafinil directly into the central nervous systems *does not* in itself increase stress or anxiety-like behaviors. These data were critical for the correct interpretation of data to be collected in experiments during the subsequent funding periods. However, we did observe a significant reduction in hippocampal-dependent memory function, in rats receiving the highest dose of ICV modafinil. These data will be conceptually replicated by ICV administration of orexin-A and also by testing in the classic Morris water maze task. These experiments are ongoing. The next phase of these studies will assess the effects of *chronic* drug-treatment *and withdrawal*.

References:

1. Woods, S.C., Seeley, R.J., Rushing, P.A., D'Alessio, D.A., and Tso, P. 2003. A controlled high-fat diet induces an obese syndrome in rats. *Journal of Nutrition* 133:1081-1087.

Appendices:

1. Supporting Data:

See next page.

Figure 1. Daily modafinil administration significantly reduced body weight in rats.

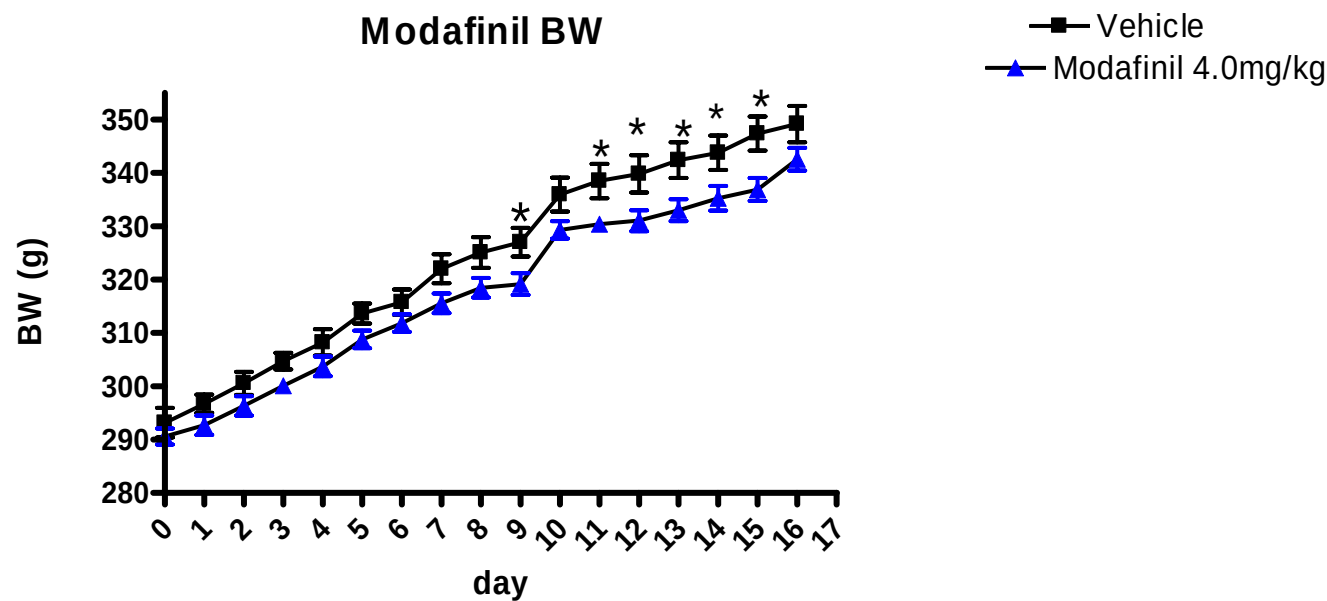


Figure 2. Daily modafinil transiently decreased cumulative food intake in rats.

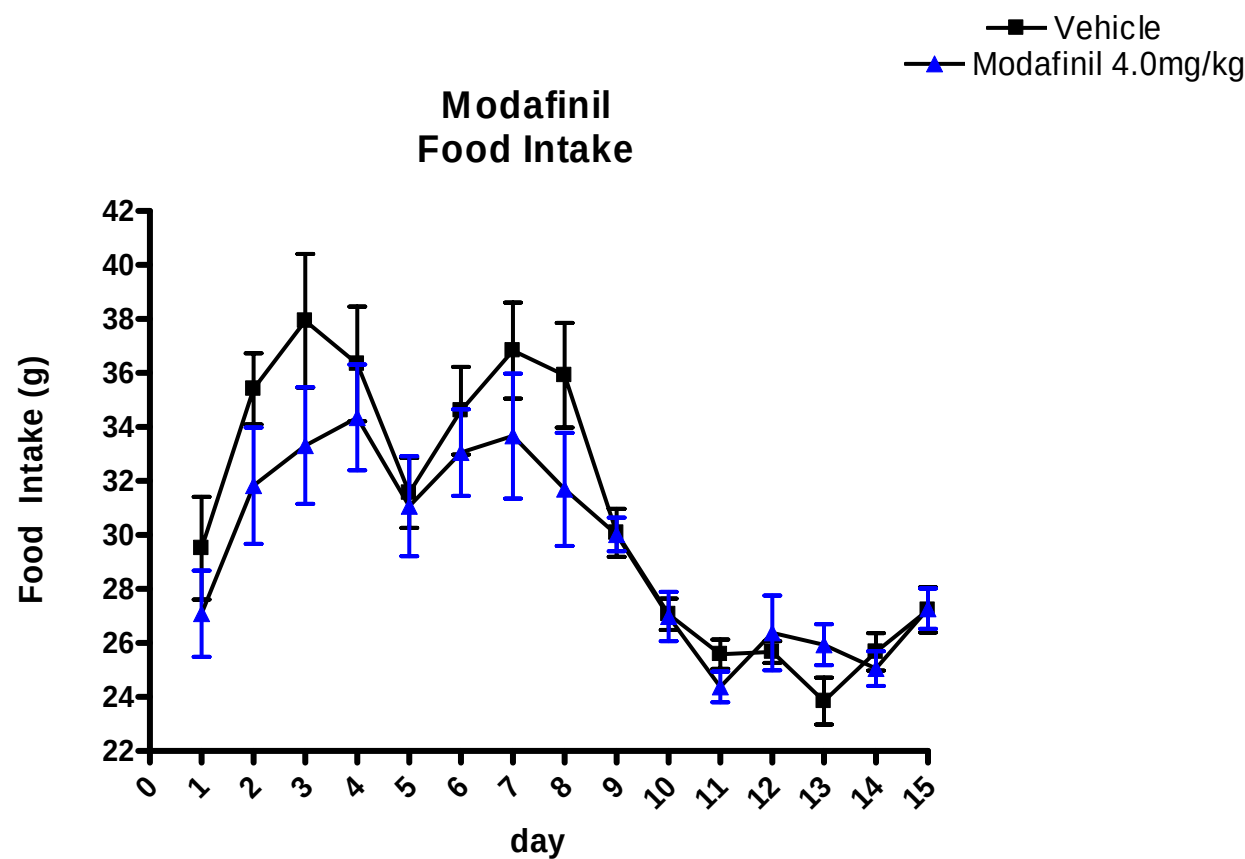


Figure 3. Chronic modafinil transiently reduced body adiposity as measured by NMR.

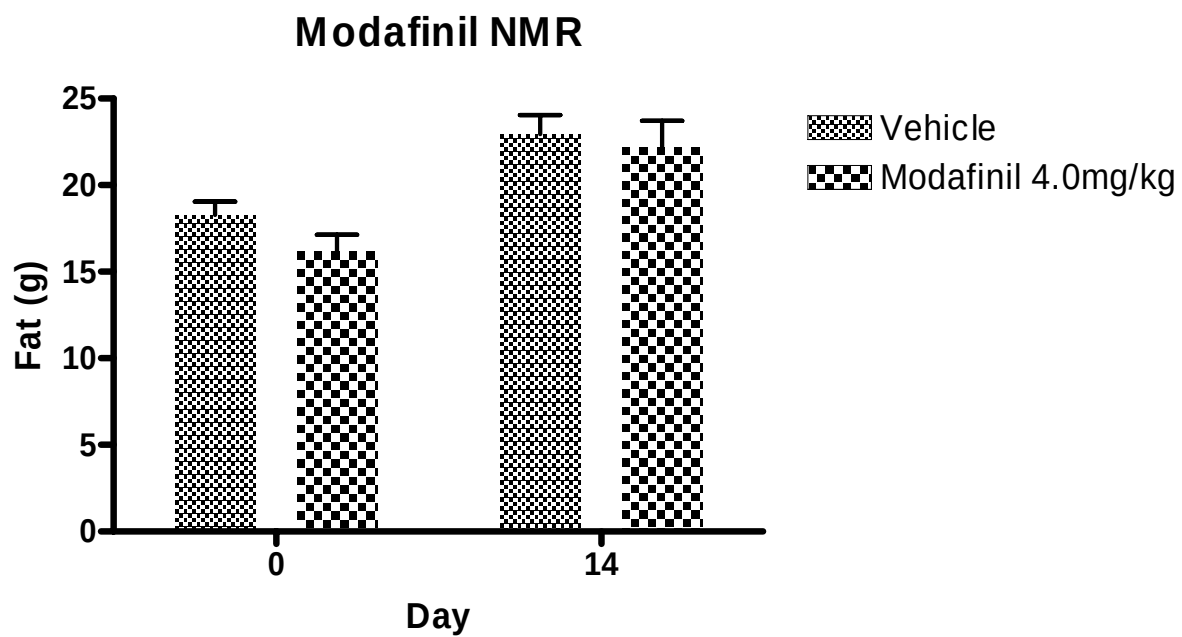


Figure 4. Acute modafinil does not significantly increase anxiety as measured by elevated plus maze (EPM) test.

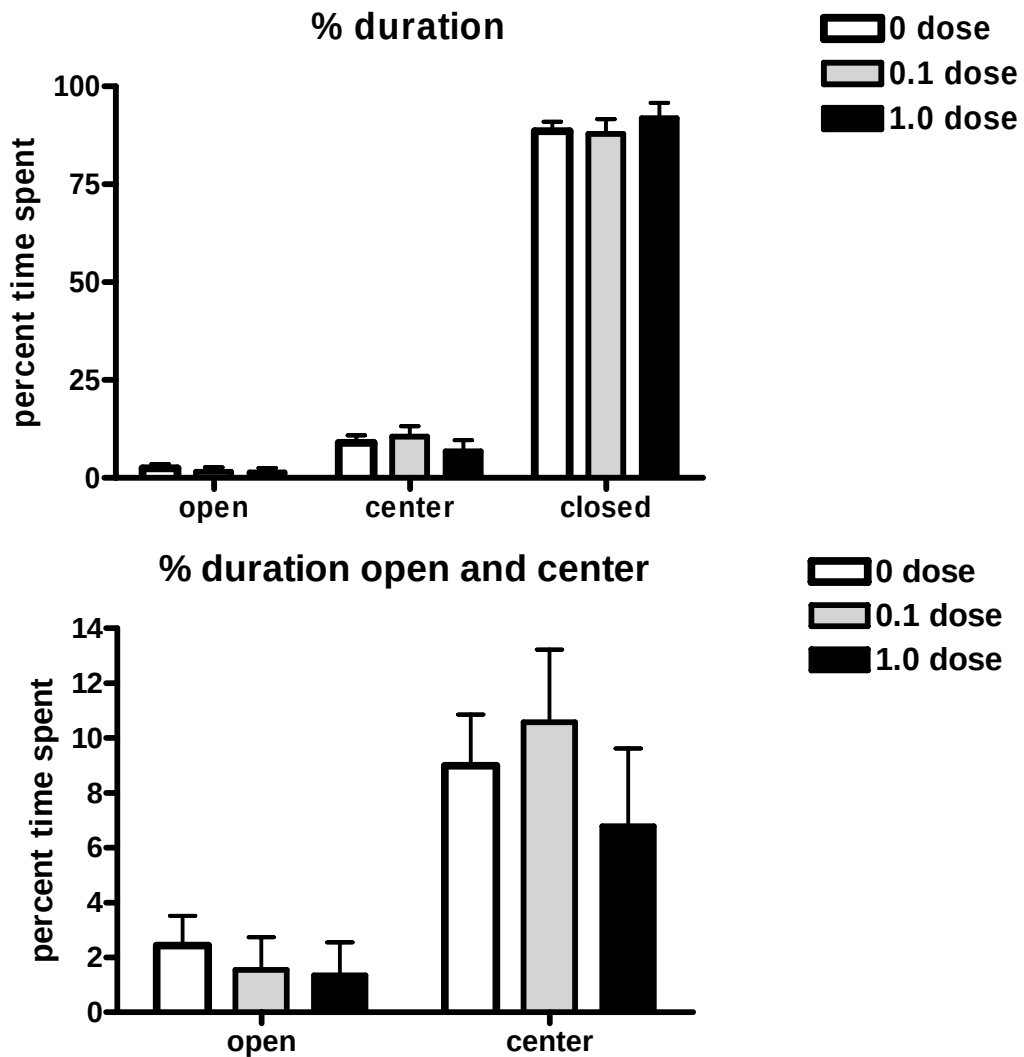


Figure 5. Acute modafinil does not significantly increase anxiety behaviors as measured by open field.

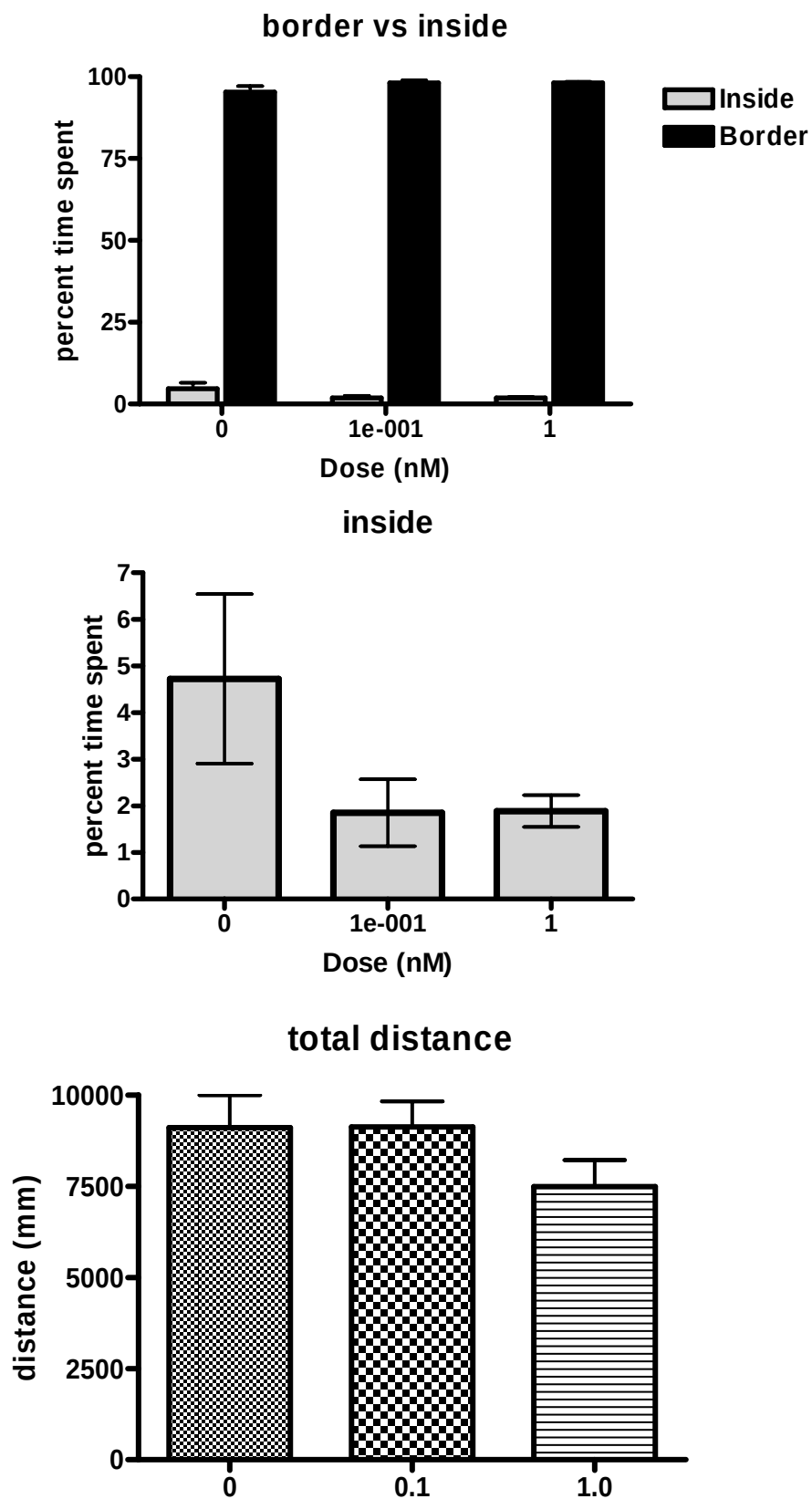


Figure 6. High dose of acute ICV modafinil significantly impairs object recognition memory.

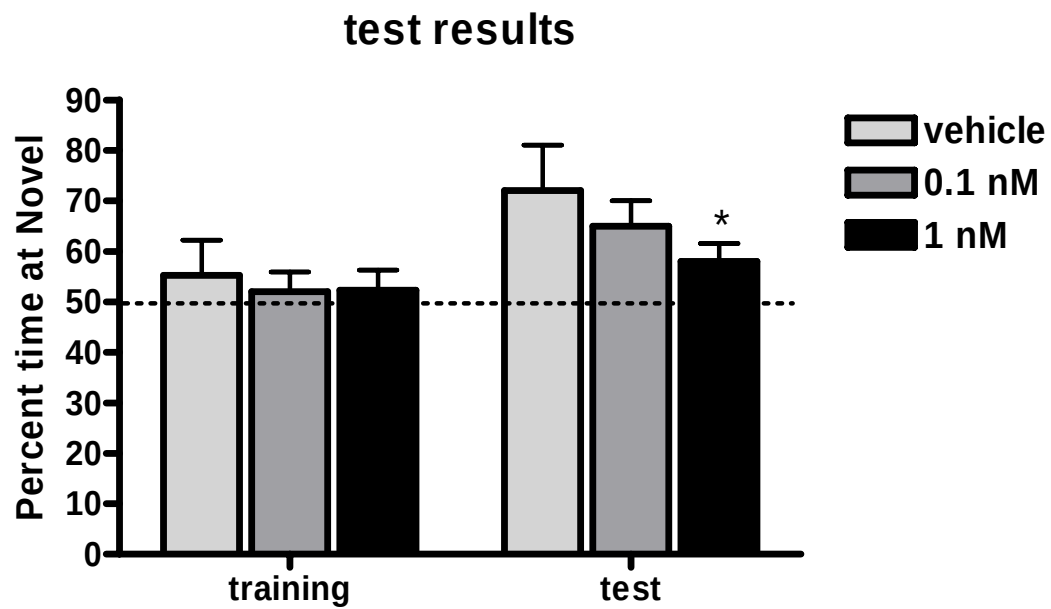


Figure 7. Acute ICV modafinil does not impair amygdale-dependent fear learning, as assayed by the passive avoidance task.

