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CHARACTERIZATION OF THE EFFECTS OF FATIGUE ON THE CENTRAL NERVOUS SYSTEM (CNS) AND DRUG THERAPIES

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14. ABSTRACT

This study aimed to establish a rat fatigue model to test fatigue countermeasures. The model focused on central fatigue. Central fatigue associated with sleep disruption may precede peripheral fatigue, and therefore may predict impaired performance earlier than peripheral fatigue. The modified flower pot method of sleep disruption was used to induce fatigue. This study utilized hippocampal dependant tasks, the radial arm maze and the Barnes maze, to characterize the effects of sleep disruption fatigue. In addition, EEG recordings were taken to detect different sleep state changes associated with 12 hours of light phase sleep disruption. Modafinil given before sleep disruption, and at least 6 hours after the beginning of the light phase during the sleep disruption tended to improve performance in the radial arm maze. Orexin receptor antagonist SB 344867 was administered at the same time points as modafinil for a total dose of 30 mg/kg in a 12 hour sleep disruption time period. SB 344867 had an effect on Barnes maze performance. In this modified flower pot method for sleep disruption, corticosterone increased significantly during the 12 hours of sleep disruption and returned to basal levels at the end of the light phase. Elevated corticosterone leads to dendritic changes in the hippocampus, which is associated with radial arm maze and Barnes maze performance.

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Characterization of the Effects of Fatigue on the Central Nervous System (CNS) and Drug Therapies

Abstract:

The primary aim of this study is to establish a rat fatigue model to test fatigue countermeasures. This model focuses on central fatigue. Central fatigue associated with sleep disruption may precede peripheral fatigue, and therefore may predict impaired performance earlier than peripheral fatigue. The modified flower pot method of sleep disruption was used to induce fatigue. This study utilized hippocampal dependant tasks, the radial arm maze and the Barnes maze, to characterize the effects of sleep disruption fatigue. In addition, EEG recordings were taken to detect different sleep state changes associated with 12 hours of light phase sleep disruption. Modafinil given before sleep disruption, and at least 6 hours after the beginning of the light phase during the sleep disruption tended to improve performance in the radial arm maze. Orexin receptor antagonist SB 344867 was administered at the same time points as modafinil for a total dose of 30 mg/kg in a 12 hour sleep disruption time period. SB 344867 had an effect on Barnes maze performance. In this modified flower pot method for sleep disruption, corticosterone increased significantly during the 12 hours of sleep disruption and returned to basal levels at the end of the light phase. Elevated corticosterone leads to dendritic changes in the hippocampus, which is associated with radial arm maze and Barnes maze performance.

Background

Using hippocampal function to measure effects of fatigue has several advantages over behavioral tests for general fatigue. However, hippocampal impairments are not an established model for fatigue. Mechanisms underlying fatigue of the central nervous system are unclear. Factors involved in the expression of serotonin and dopamine have been examined in an animal model utilizing strains selected for high endurance runners (HCR) and low endurance runners (LCR) revealing elevation of expression of 5HT1 and DR2 (Foley, TE et al., 2006). Once an animal model is established, it might be possible to study the effects of counter fatigue measures. One proposed animal model of fatigue utilized placing a rat in a cage with 1.5 cm of water covering the cage floor. Animals were then tested for fatigue with a weight loaded forced swim test (Tanaka, et al., 2003). This fatigue model measured glucose uptake by brain regions and reported reduced uptake associated with fatigue and insufficient dopamine and serotonin turnover. In addition, there is some evidence of central fatigue preceding peripheral or muscle fatigue in exercise based on studies utilizing transcranial magnetic stimulation (Davis, et al., 2007). A fatigue model which focuses on impaired brain function as the predictor of muscle fatigue could be utilized to enhance sensitivity to fatigue markers. This study will investigate the use of behavioral tests for impairments, which are measured along with EEG recordings for sleep states, corticosterone plasma levels for stress responses, and a physiological measurement for synaptic plasticity long term potentiation.

The most recent pharmacological solution to be studied by researchers affiliated with the U.S. Air Force is modafinil. Modafinil is a Food and Drug Administration approved drug for use in individuals with the sleep disorder narcolepsy. Additional research has demonstrated modafinil's effectiveness as a fatigue countermeasure in shift workers and aviators/aircrew. Based on data from these reports, modafinil is believed to be efficacious and lacks the unwanted side-effects of

amphetamine or caffeine. Despite an understanding of modafinil at the behavioral level, little is understood regarding the central nervous system (CNS) processes that underlie the drug effects.

The goal of this study was to more thoroughly establish the behavioral characteristics of modafinil using an animal model. The final step is to explore the role of the neuropeptide orexin as it has been hypothesized to be a modulator of the wakefulness stimulated by modafinil.

Orexin is a hypothalamic peptide important in the sleep wake cycle. Orexin neurons are localized to the perifornical region of the hypothalamus and send projections to the hippocampus (Brisbare-Roch, C et al. 2007) Orexin neurons increase firing during alertness and decrease during sleep (Lee, MG , et al., 2005). SB 344867 has been shown to reverse the effects of orexin administered intra cerebral ventricular (Duxon, MS et al., 2001). The lateral hypothalamus has been historically implicated in reward and motivation. Orexin neurons which are localized to the lateral hypothalamus project to the reward associated brain regions such as the ventral tegmentum (VTA) and the limbic system including the nucleus accumbems (Peyron, C. 1998, Fadel, J. 2002). In an experiment using place preference, there was enhanced activation of the orexin neurons as measured by *cfos* expression after place preference conditioning (Harris, Glenda 2005 Nature). In addition, administration of the orexin receptor antagonist SB 344867 reduced place preference. In addition orexin receptor antagonist are being tested in clinical trials as drugs to treat the ill effects of sleep disruption in shift workers.

The experimental design of this study is to train animals in tasks, and then sleep disrupt them to test for the effects of sleep disruption on performance of a learned task. The behavioral test in this experiment were the radial arm maze and Barnes maze, which are designed for use with rats. The radial arm maze and Barnes maze were the behavioral tests used for assessing aspects of memory acquisition and consolidation in task performance. Animals were trained to criterion in the radial arm maze task. On the morning after an animal reached criterion, animals were sleep disrupted on the flowerpot. This experiment will assess the ability of a non-sleep deprived animal to acquire and perform a task that requires training and memory consolidation, and the effects of sleep disruption after training to criterion. Animals were trained in the Barnes maze to escape to a target box by having four trial a day for four consecutive days. On the fifth day, animals were sleep disrupted and tested for impairments in finding the target box at the conclusion of the twelve hours of sleep disruption.
The second phase of this experiment was performed in animals that have been sleep deprived for a given period of time per day and treated with drugs which are used to ameliorate the effects of sleep disruption. This experiment assessed the ability of a sleep deprived animal to perform tasks when aided by the effects of modafinil. Modafinil is a putative dopamine transporter inhibitor, which accounts for its ability to enhance wakefulness. Modafinil's wakefulness requires an intact adenosine system, and serotonin system. Modafinil anecdotally may be addictive because it increases productivity in overtasked workers. The reward of accomplishing goals may be modulated by increased dopamine modulation.

The working hypothesis is that modafinil works through orexin system to enhance wakefulness and maintiain performance of learned tasks.

The second set of experiments will address mounting evidence that the neuropeptide orexin-A is an effecter neuropeptide of modafinil. Orexigenic neurons are hypothesized to mediate arousal (wakefulness) and motor activity. Orexigenic neurons have been demonstrated to be in a state of activation following administration of modafinil. Furthermore, the orexin receptor has been found in the hippocampus, and in areas of the brain involved in reward for food

cues. To the degree that orexin may modulate reward, and therefore an orexin receptor antagonist may dampen reward seeking behavior.

Behavioral Tests:

1. **Eight arm radial maze**

Animals: Male Sprague-Dawley rats weighing 175-199 g.
1 Eight arm radial maze manufactured by Lafayette Instrument Company. The maze is 72 inches in diameter and 36 inches above the floor and constructed of black painted stainless steel with Plexiglas walls on the arms and hub. Each arm is 10 inches across. The doors on the hub are controlled by levers behind curtains.

2. A laptop with HVS software to collect tracking data on the eight arm radial maze (RAM).

RAM as a tool to test reference memory. The radial arm maze was developed by Dave Olton to test the ability of a rat to remember a list of spatial locations. Several experiments demonstrated the rat discriminating between arms based on a map in its memory, not response strategies such as choosing alternating arms or intra-maze cues such as food or trail odors. Extra-maze spatial cues were used. In this study all eight arms were baited with one half of a Froot loop. In addition, animals were given one trial a day, in the afternoon between 3 pm and 6 pm. Animals were trained to criterion, which is eight correct choices out of the first eight choices. On the morning following an animal's criterion trial the animal was either left in the home cage during the light phase, 7 am to 7 pm, or placed on an inverted flowerpot to induce REM sleep disruption. The flowerpot model is created with a circular platform approximately 2.3 inches in diameter and then inverted in pool of water at least 12 inches deep. The platform allows for slow wave sleep, but not REM sleep because REM sleep causes loss of muscle tone and the animal would fall into the 1 foot deep pool of water.

At the end of the light phase, 7 pm, the animal was given a final maze trial. A small error number is the expected outcome for a cage control. In pilot studies sleep disrupted animals made significantly more errors on the post sleep disruption trial.

Initially, animals were anesthetized with urethane to measure long term potentiation immediately after the maze trial. However, these animals were succumbing to anesthesia. To decrease the morbity rate animals were permitted 12 hours of recovery before attempting to anesthetize for physiological measurements.

Protocol for 8 arms baited:

Maze training was:

1. Day 1, 10 minutes with all doors to arms open, exploration. No bait.
2. Day 2, the arms have ½ a Froot loop, and the animal is allowed up to ten minutes to visit each arm. The trial is stopped after 10 minutes or when the animal has visited each arm at least once.
3. Day 3, one trial a day consists of baiting all the arms, and watching for the animal to visit each arm, the trial was stopped once the animal had gone to the bait and returned to the hub of the maze for each arm. The time was called the duration.

Mistakes were counted when an animal revisits an arm by going at least 1/3 of the length of the arm.
4. The animal reached criterion by visiting each arm just once.
5. Once the animal reached criterion, the animal was placed in one of two groups for sleep disruption the next day.
6. One group of animals was placed on a small flowerpot platform to induce REM sleep deprivation, and the other group remained in the home cage. The time spent on the flowerpot platform was 12 hours, during the light phase.
7. The animals had a maze trial immediately after the 12 hours of sleep disruption or in the case of cage controls after 12 hours in the home cage.

2. Barnes Maze:

The Barnes maze was built at UTSA, it is a circular tabletop 48 inches in diameter with 20 holes 4 cm in diameter cut along the perimeter. The Barnes maze behavioral test does not require food restriction, as does the radial arm maze. The Barnes maze was another behavioral test that detects hippocampal impairments in finding the target box for escape. Food restriction increases corticosterone and orexin, which are increased by sleep disruption. Based on the literature and preliminary trials the protocol is as follows:

1. Animals were given four trials a day with a 10 minute rest period between trials.
2. All the surfaces were wiped with a Sani cloth plus between each trial.
3. The first day the rat was habituated to the Barnes Maze.
4. If the animal failed to discover the target box within the three minutes allotted for each trial, the animal was be placed into the box, for one minute.
5. Once in the target box the animal remained in the target box for 1 minute during all phases of training and testing.
6. On the day following habituation, animals were placed in a cylindrical Plexiglas start tube in an orientation random to the target box for 30 seconds.
7. The start tube was raised by hand to start the trial.
8. Rats were returned to home cage between trials for a 10 minute interval.
9. Latency to entering the target box was recorded in seconds. Number of errors defined as sniffing over or poking around an incorrect hole, was also recorded.
10. AnyMaze software (Stoetling Co.) was used to measure latency to target box, visits to false boxes, create maps of the path taken and to generate graphs.

The room containing the Barnes maze is relatively small; all four walls have differing black and white patterned cues. The Barnes maze on the first trial of each daytime test, tested reference memory, the subsequent 3 trials tested a combination of working and reference memory.

Trials were administered for four days; animals were then assigned to one of three groups. One group was sleep disrupted for 12 hours during the light phase with no injection. The second group was sleep disrupted for 12 hours during the light phase and injected with 0.25% methyl cellulose at 7 am and again at 3:00 pm intraperitoneally. A subset of the second group

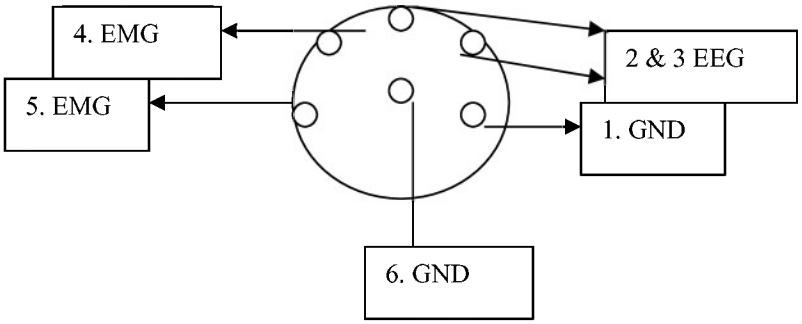
was sleep disrupted and given a dose of 150mgk/kg of modafinil dividing the dose into two parts, the am and the pm injection. A third group was sleep disrupted for twelve hours during the light phase and given a 10mg/kg body weight orexin antagonist SB 3344867 suspended in 0.25% methyl cellulose at 7am and 3 pm intraperitoneally. Animals were tested for four trials as was done during training in sets of two, and given a ten minute rest between each of the four final trials. All animals were given four trials after the conclusion of the light phase on day five of the training week.

Surgical Protocols

EEG implants:

Electrode construction:

1. The day before surgery prepare the electrodes and pedestal as follows:
    a. Use stainless steel wire Teflon coated from AM systems cat # 792500. 0.010 bare, .013 coated, half hard.
    b. There are six holes in the pedestal (Plastics One MS 363 2298 six pin)
    c. The order of the electrodes inserted into the large holes of the two sides of the pedestal is 1. ground 2 & 3 EEG electrodes 4 EMG 5 EMG 6. ground



2. To prepare the **Ground** electrodes two pieces were cut out of the stainless steel Teflon coated wire (AM systems #792500). Scrape the Teflon coating off one end with a single stroke of the scalpel on each side of the wire. Using curved forceps bend the tip into a hairpin hook. Insert this hook into electrode socket (Plastics One E363/0). Connecting the wire to the electrode socket may be done in two ways, solder, which is time consuming, or by use of a crimping tool (DMC, crimper AFM8). Recently acquired crimper tool has allowed for ease, so I will no longer use soldering. Remove the Teflon coating off the other end of the wire with two strokes of a scalpel. This exposed wire end will be wrapped around right and left bone screws in the frontal bone.
3. To prepare **EMG** electrodes, cut two pieces of wire. Scrape the Teflon coating off one end with a single stroke of the scalpel on each side of the wire. Using curved forceps bend the tip into a hairpin hook. Insert this hook into electrode socket. Scrape off the Teflon coating on the other end of the wire. Place a cuff over this end. Bend the exposed tip into a hairpin loop and fasten the loop with the cuff. The cuff is stainless steel and devised from a piece of needle 20 gauge. Once the cuff has fastened over the loop crimp with needle nose pliers.
4. To prepare the hippocampal electrode, cut one wire of sufficient length to allow for the twisted bipolar electrode to be lowered into the brain 3.5 mm. Place the electrode sockets as directed above on each end of the wire. The wire is bent into half and twisted. The twisting compresses the two wires so that insertion through the skull hole drilled by hand is facilitated. Once the wires are tightly twisted in a braid, blunt cut the end of the

loop leaving on the straight tips exposed. The tips may be twisted away from each other to increase the field potential.

5. Once all the electrodes are prepared insert the expandable part of the electrode socked into the pedestal. The socket will not be flush with the surface of the pedestal. To press the socket too deeply into the narrow region of the pedestal will result in a pedestal, which may not accept the pins of the cable.
6. Once the six electrodes are in the pedestal check connections with a voltmeter.
7. Small volumes of dental acrylic AM systems (525000) may be added to secure the electrode to the pedestal.

Implant surgery:

8. Sterilize all surfaces, instruments, and containers. Cidexis a good agent. If not available, use 70% ethanol. All instruments should be flamed immediately before surgery.
9. Anesthetize the animal with 0.8 ml ip injection of a cocktail
    a. 6 ml sterile saline in a bottle
    b. 2.5 ml of Ketamine (100gm/ml) (light sensitive, so cover container in foil)
    c. 1 ml acepromazine (0.5 g)
    d. 0.5 ml xylazine
10. Wet the dorsal portion of the rats head with betadine, using sterile gauze pads.
11. Using a new scalpel blade, shave the rat's head from in front of the eyes to behind the ears.
12. Place rat in the ear bars on the stereotaxic frame.
13. Using a scalpel make a midline incision from the eyes to the neck. When cutting skin over muscle, reposition the skin to the bone to avoid cutting muscle which causes excessive bleeding.
14. Using two small hemostats carefully clip the fascia, not skin, which will die if clamped, and retract the skin from the wound.
15. Immerse the exposed skull in hydrogen peroxide to destroy all cells. Dry the bone with sterile gauze.
16. Immerse the bone with sterile saline to clean the bone. If the bone is bleeding stop the bleeding by scraping, which induces vasoconstriction, cauterize or use adrenaline.
17. There will be two screws in the frontal bone, 0.5 laterals.
18. There will be two screws in the parietal bone for anchoring the dental acrylic.
19. Level the skull by comparing the dorsal ventral coordinates of lambda and Bregma.
20. Always keep the bone as dry and clean as possible to ensure the bonding of dental acrylic
21. Using a manual drill (Plastics One, drill bit #56 8J60DrillIHSS) drill the holes for the screws (#81010121201f) and hippocampal electrode. When removing the bit as if unscrewing a bolt to avoid damaging to maintain the thread.
22. Hold the screw in place with curved forceps and turn the screw until enough of the shaft is exposed to wrap the ground wire.
23. First place the EMG electrodes as follows:
    a. One electrode goes on the surface of the superficial muscle bundle
    b. The other electrode goes on the surface of the deep muscle bundle.
    c. The two EMG electrodes must never touch each other.
24. The ground electrodes are attached to the bone screw, which are tightened once the exposed tips are wrapped around the screw.
25. Small amounts of dental acrylic are painted onto the screws to insulate them and secure the wires in place.
26. Dental acrylic is mixed in a watch glass and applied with a spatula.

27. The setting of the acrylic is determined by pokes with a bent needle.
28. The hippocampal electrode is lowered into brain with the following stereotaxic coordinates:
    a. DV 2.5mm, this is the dorsal surface of the hippocampus
    b. L= -0.25cm
    c. P=-0.4cm
29. Once all the electrodes have been anchored with dental acrylic, the entire headset is enclosed in dental acrylic to ensure no wires are exposed. Use at least two sutures to close the wound. Apply Neosporin topically to the incision, inject 0.2 ml of penicillin sub cutaneously, and give the animal Rimadyl tablets.
30. Allow the animal 5 days for recovery, and begin recording by connecting the animal to the cable, which connects to the commutator.
31. ICELUS developed by Dr. Mark Opp at the University of Michigan was used for data acquisition and analysis.

Results:

EEG:

The modified flowerpot method of sleep disruption differed in the EEG experiment and maze experiment. The EEG animals were sleep disrupted while having an implant connected to a computer. The primary difference was the depth of water, which was approximately one inch in a plexiglass cage. The decreased depth of water might induce a less fatiguing sleep disruption. The differences in per cent recording time in REM sleep within the first five hours after sleep disruption was finished indicate a trend for both sleep disrupted and non sleep disrupted animals to not have REM sleep. The animals that were sleep disrupted and treated with 30mg/kg of SB 344867, an orexin receptor antagonist experienced the most REM sleep within the first five hours after the completion of 12 hours of sleep disruption during the light phase. Animals sleep disrupted and treated with 150mg/kg of modafinil in two injections initially had more REM sleep than control animals, but after four hours began to be at the same level as control animals. The greatest difference between sleep disrupted and non sleep disrupted animals was in per cent time in NREM sleep. Sleep disrupted animals spent almost all the five hours post sleep disruption in NREM sleep. Animals sleep disrupted and treated with modafinil or SB 344867 after the first two hours has a per cent NREM in a range between 60 and 80 per cent. Sleep disrupted animals treated with modafinil had a latency to first REM sleep which averaged less than ten minutes. Sleep disrupted animals treated with SB 344867 had an average latency to first REM of over 80 minutes

Maze performance:

The radial arm maze was used to test for hippocampal function in performing a learned task after sleep disruption, which is associated with impaired learning and memory. The cage controls had almost no errors on the trial the day after criterion was met. However sleep disrupted animals has the highest error rate, averaging two visits to previously visited arms before visiting all eight arms at least once. Sleep disrupted animals treated with modafinil did not differ significantly form cage controls, suggesting modafinil did improve performance of sleep disrupted animals in the radial arm maze. However, sleep disrupted animals treated with SB 344867 averaged fewer errors than sleep disrupted animals treated with modafinil, although the difference was not significant.

The Barnes maze was used to test for hippocampal function after sleep disruption and dose not require food restriction. The Barnes maze test spatial memory and is based on the animals motivation to escape bright lights and an open field into a dark target box in a constant position in one of 20 holes on a circular platform. Animals were given four trials after completion of sleep disruption. The latency of the fourth trial was significantly lower for sleep disrupted animals treated with SB 344867. The cage control animals averaged 20 seconds before entering the target box sleep disrupted animals averaged almost 80 seconds, sleep disrupted animals treated with modafinil averaged 20 seconds, and sleep disrupted animals treated with SB 344867 averaged 5 seconds. The sensitivity of the Barnes maze to fatigue is supported by the observation that the greatest variance in treatments occurs on the fourth trial after sleep disruption. During training animals often explore more on the fourth trial, after establishing the position of the target box. One clear effect of SB 344867 is the absence of the tendency to explore on the fourth trial. In addition, the animals were able to maintain a consistent behavior of going directly to the target box all four trials after sleep disruption. The same animals did not behave this way on the fourth training day when the position of the box was fully learned.

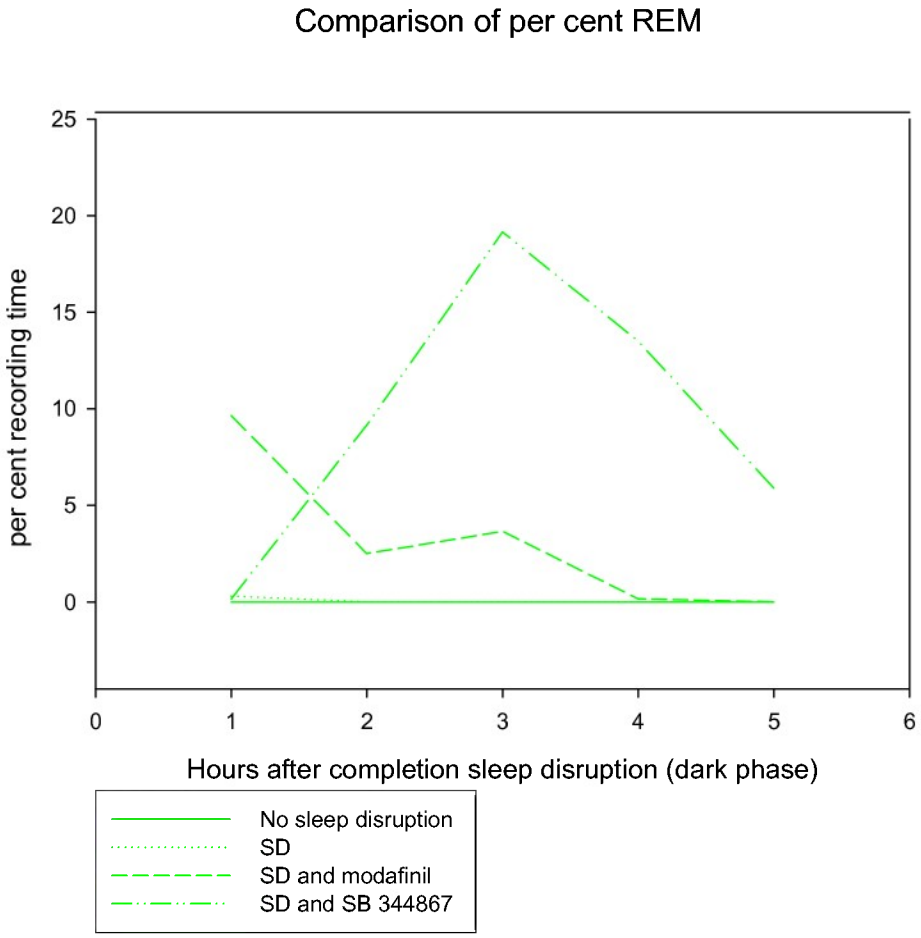


Figure 1. The per cent recording time of REM sleep in the initial five hours after completion of sleep disruption. The animals were returned to the home cage with bedding at 7:00 pm. In the no sleep disruption group, n=1, sleep disruption n=1, sleep disruption treated with modafinil 150 mg/kg in two doses n=2, and sleep disrupted treated with SB 344867, an orexin receptor

antagonist, n=2. The Kruskal-Wallis One way ANOVA on Ranks had a p0.05 for the difference between control and sleep disrupted and sleep disrupted treated with SB 344867.

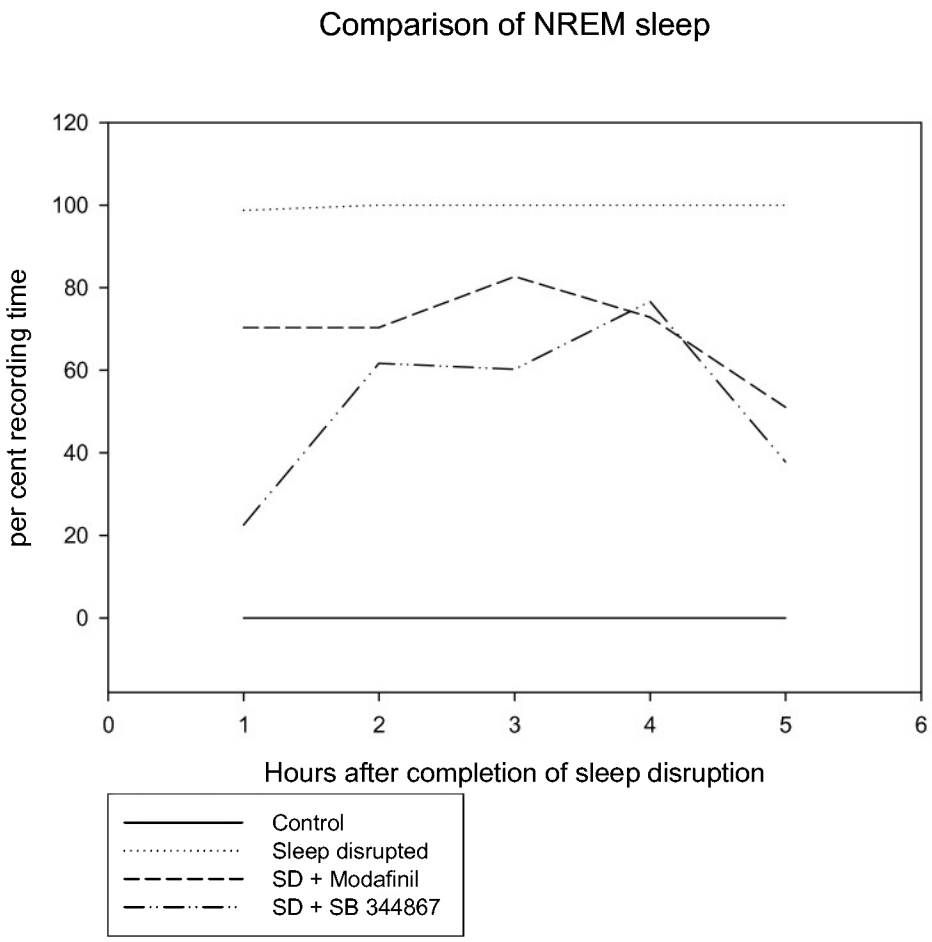


Figure 2. Comparison of per cent recording time in NREM sleep. Control and sleep disrupted (SD) had n=1, sleep disrupted treated with modafinil n=2, and sleep disrupted treated with SB 344867 n=2. There was a significant difference between sleep disrupted and control p, 0.05 using the Tukey test for significance.

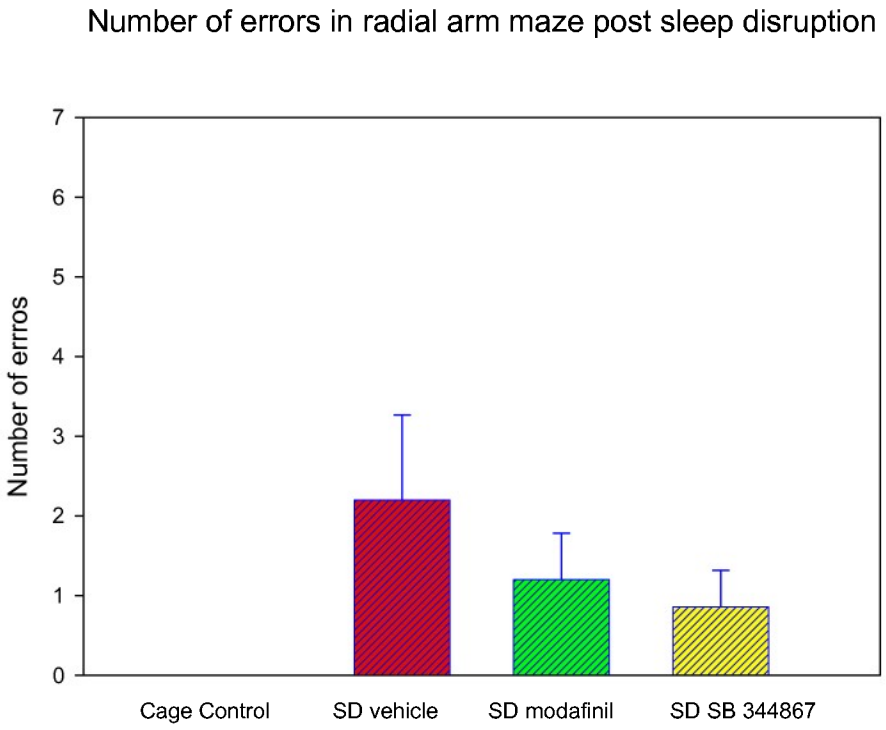


Figure 3. Comparison of errors in radial arm maze. The treatment groups are cage control (cc) with no injection, sleep disrupted with vehicle injections, and sleep disrupted with modafinil injections. The modafinil was administered intraperitoneally at 7:00 am before the beginning of the sleep disruption and at 3:00 pm. The total dose per animal was 150 mg/kg. The cc group n=5, sd vehicle n=6, and sd modafinil n=6. There is a significant difference between cage control number of errors and sleep disrupted vehicle. There is no significant difference between sleep disrupted modafinil and cage control or sleep disrupted vehicle treated.

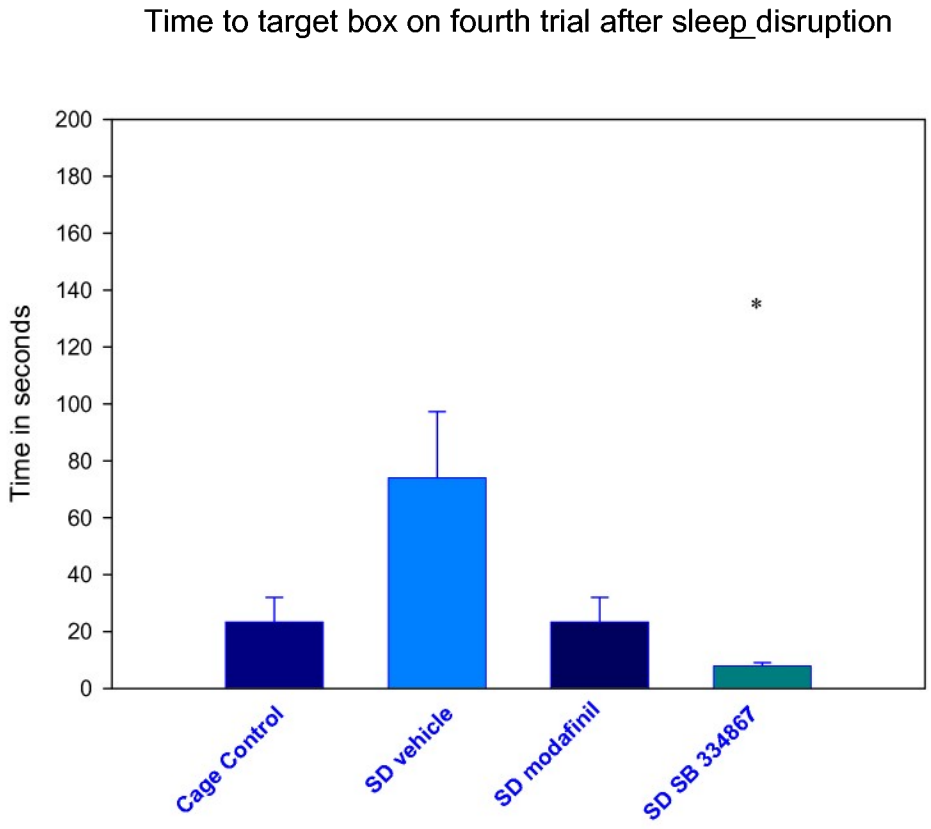


Figure 4. Barnes Maze performance comparison of latency to target box on the four trials after treatments. The treatment groups were cage control no injection, sleep disrupted modafinil at 150mg/kg, and sleep disrupted SB 344847 30 mg/kg. The cage control groups n=6, modafinil treated n=3, and SB 344847 treated was n= 4. The latency of the SB 344847 treated animals was the smallest, and significantly different from cage control latency. Each point represents the average latency for fourth trial post sleep disruption for all the animals in that treatment group.

Discussion:

EEG:

The effects of drugs on sleep disrupted animals sleep architecture was evident in the relative low number of animals tested. The modafinil appeared to have little effect on the NREM sleep after sleep disruption, while the orexin antagonist appeared to enhance REM sleep after sleep disruption. Orexin is associated with increasing wakefulness, so the antagonist may compensate for the elevation of orexin associated with sleep disruption. The shorter latency to REM sleep and the increased per cent recording time suggest orexin modulates REM sleep by reducing the occurrence in rebound sleep. Modafinil did not differ in REM after the initial two hours of rebound sleep. Given the relationship between REM sleep and maze performance, the orexin antagonist appears to modulate the sleep state more likely to affect memory and performance. Modafinil and SB 344867 had similar effect on NREM sleep state after the initial two hours, and the this effect was to decrease the per cent recording time relative to the vehicle treated sleep disrupted animal. How this effect translates into awake performance was explored with two behavioral tests.

The modified flower pot protocol for sleep disruption is a suitable model for fatigue because the effects of fatigue are detectable in sleep recordings, and in maze performance impairments. The use of the radial arm maze is of value as a comparison to other data on impairments, however the sensitivity to fatigue induced in this model may be limited by the margin of errors in sleep disrupted animals. The use of 12 hours of sleep disruption on the flowerpot is not as severe as many sleep models, and the animals remains in a state that permits behavioral testing immediately after the completion of sleep disruption. The impairments could be reversible, especially since NREM sleep begins immediately at the completion of sleep disruption on returning to the home cage.

The Barnes maze is also a good behavioral test because it does not require food restriction which increases orexin and corticosterone two hormones known to influence maze performance. The hippocampus has receptors for both orexin and corticosterone, and is a target for the elevated levels. With four trials, the Barnes maze permits a pattern of performance to be revealed after sleep disruption, rather than a single trial as is used in the radial arm maze. The behavior of sleep disrupted animals treated with SB 344867 is very interesting. The use of orexin antagonist to ameliorate the impairment of sleep disruption seems worthy of future investigations. The animals were consistent over four trials, after 12 hours of sleep disruption. The animals' behavior did differ from cage controls; however the difference resulted in greater efficiency. Perhaps orexin elevation due to sleep disruption increases a foraging drive, which creates more exploration. Blocking the effects of elevated orexin appears to enhance performance after sleep disruption.

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