

SAMPLE PROTOCOL FOR DOG SLEEP STUDY

The general strategy is to evaluate test compounds' effects on three different aspects of sleep function:

- activity-rest rhythms
- sleep stages
- memory consolidation

EFFECT OF TEST COMPOUND ON ACTIVITY-REST RHYTHMS

Purpose:

Dogs, like other mammals, show circadian activity cycles, being active during the day and inactive during the night. These circadian activity cycles are age sensitive.

Methods:

Activity is monitored with an Actiwatch monitoring system. The Actiwatch is a device placed inside a specially designed animal case and placed on a collar around the dog's neck that records every movement made by the animal as a binary event, as well as the chronological time of occurrence. The advantage is that subjects are allowed to follow their usual patterns of activity, rest, exercise and feeding in an undisturbed manner.

General Test Protocol:

A repeated measures design is used to compare the response to the drug with the response to a placebo.

Subject population:

The test compound's effects are compared at three time points: young to middle aged dogs - (2- 6 years of age); old dogs > 8 and less than 12; very old dogs > 12.

Assessment Parameters:

1. **Total behavioral activity over 24 hours** As illustrated in Figure 1, dogs show progressively less home cage activity with age.

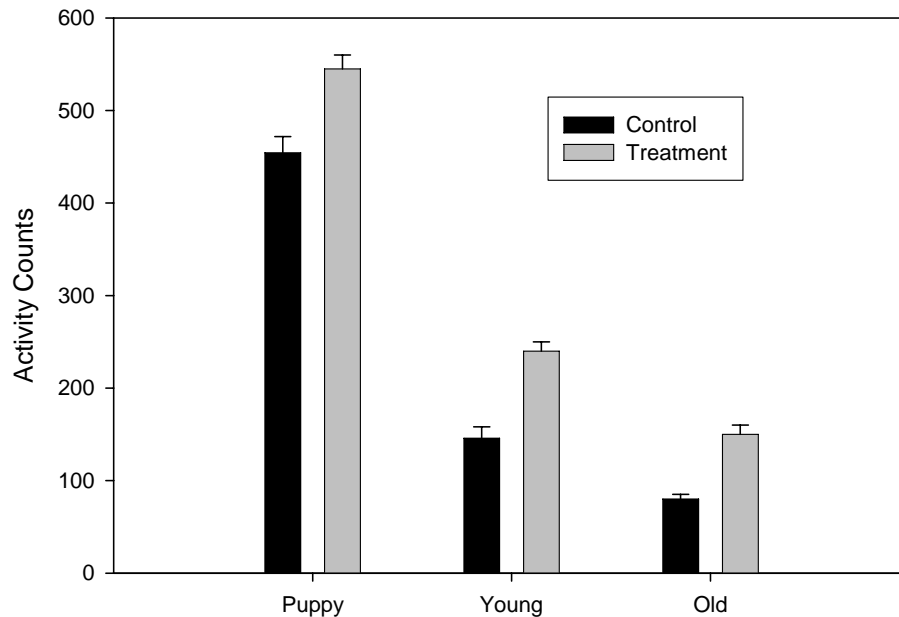


Figure 1: An example of the total activity count provided by the Actiwatch system. The treatment leads to increased levels of activity counts.

2. **24-hour Activity Profile** – This provides a pattern of activity as a function of time of day.

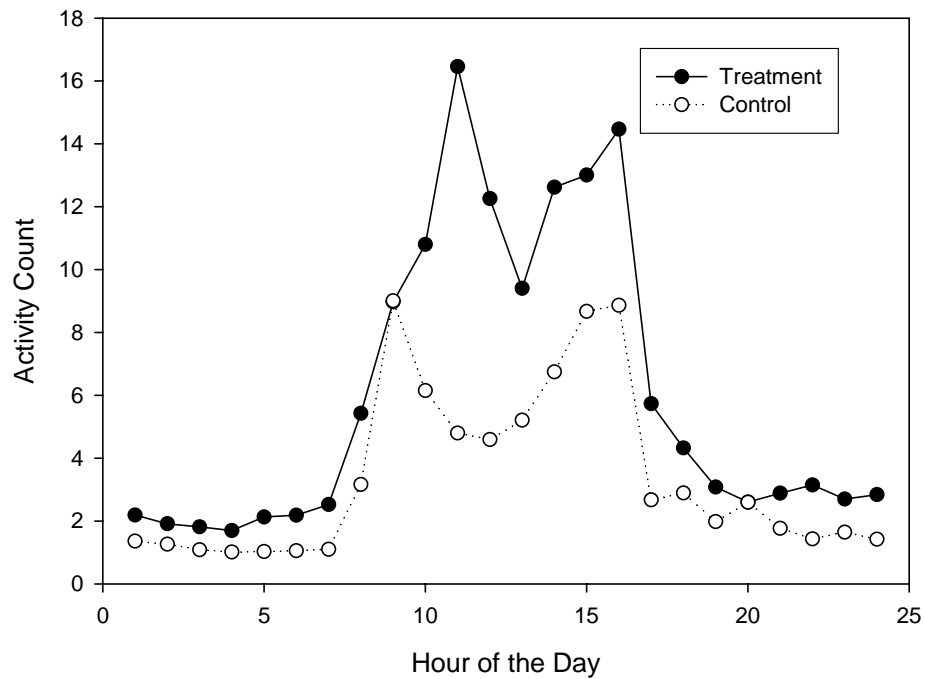


Figure 2: Example of a 24-hour activity profile. These data provide information about how long after dosing the drug begins to take effect and how long the increase in activity will last.

3. Activity Onset- Activity onset is defined as the number of minutes between the time that the environment lights come on and the time of activity onset. This is calculated from the Actiware-Rhythm software. Activity onset is considered to be the first bout of activity lasting for a minimum of 30 minutes. Activity bouts beginning prior to lights on are assigned a negative value for the number of minutes.

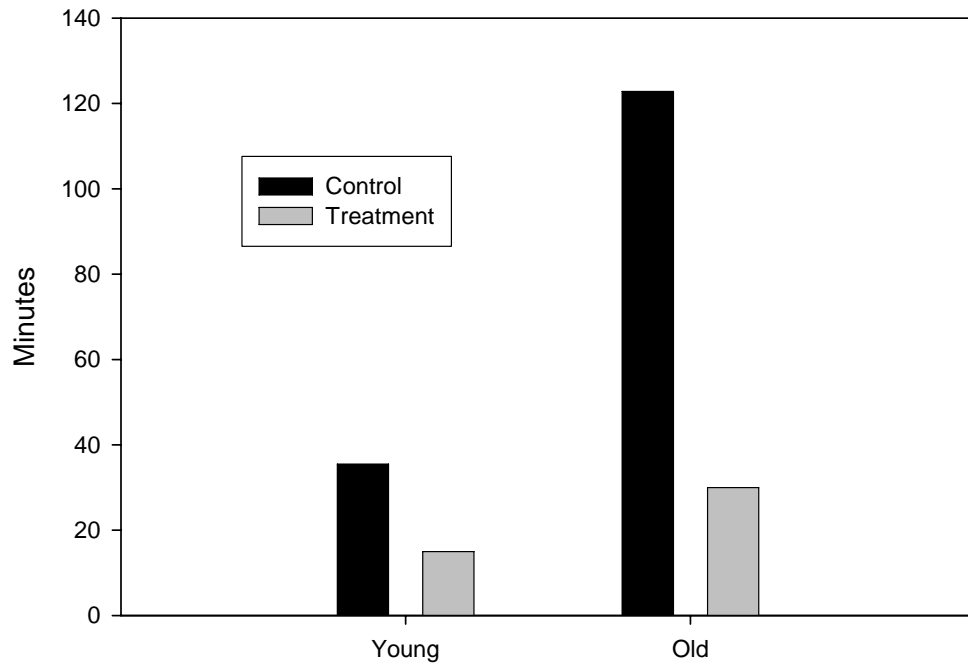


Figure 3: The number of minutes between the time the room lights turn on and when the dog becomes active. A test compound may reduce this time.

4. Main Activity Bout Length-The length of the main bout of activity is the number of hours that the longest consolidated bout of activity lasts, based on its appearance on an actogram generated by the Actiware-Rhythm software.

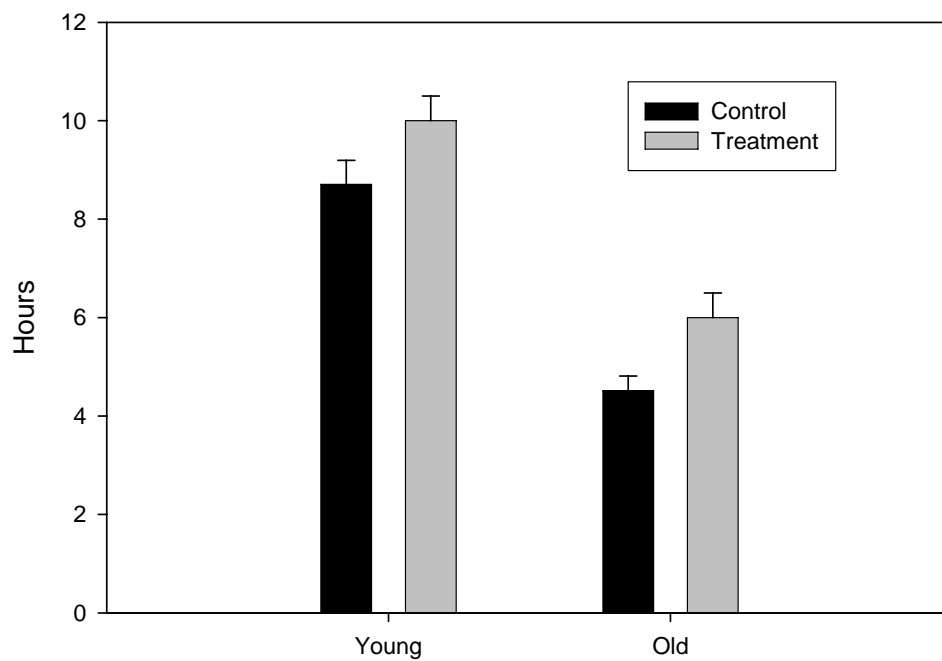


Figure 4: The length of the main activity bout may be increased by drug treatment.

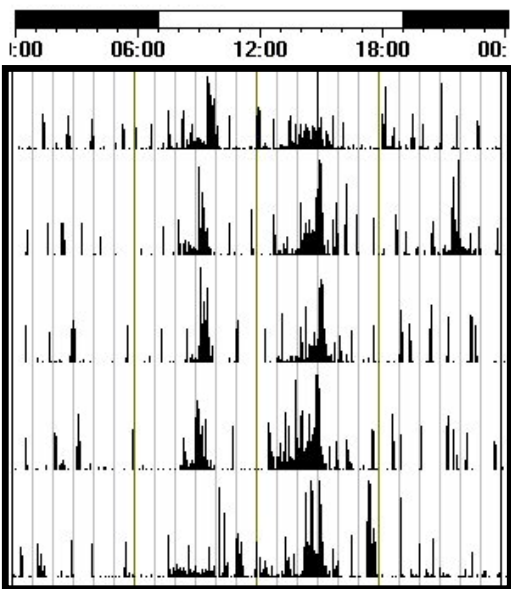
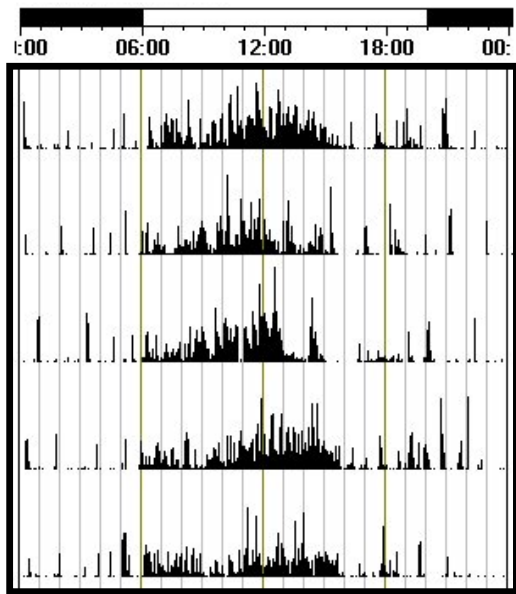


Figure 5: The change in length of the main activity bout is observed on the Actiware-Rhythm generated actograms.

5. Daytime Rest and Nighttime Activity-The number of dips in daytime activity (when the actogram returns to zero) and the number of bouts of activity during the night (when the actogram shows at least a brief period of increased activity) are counted.

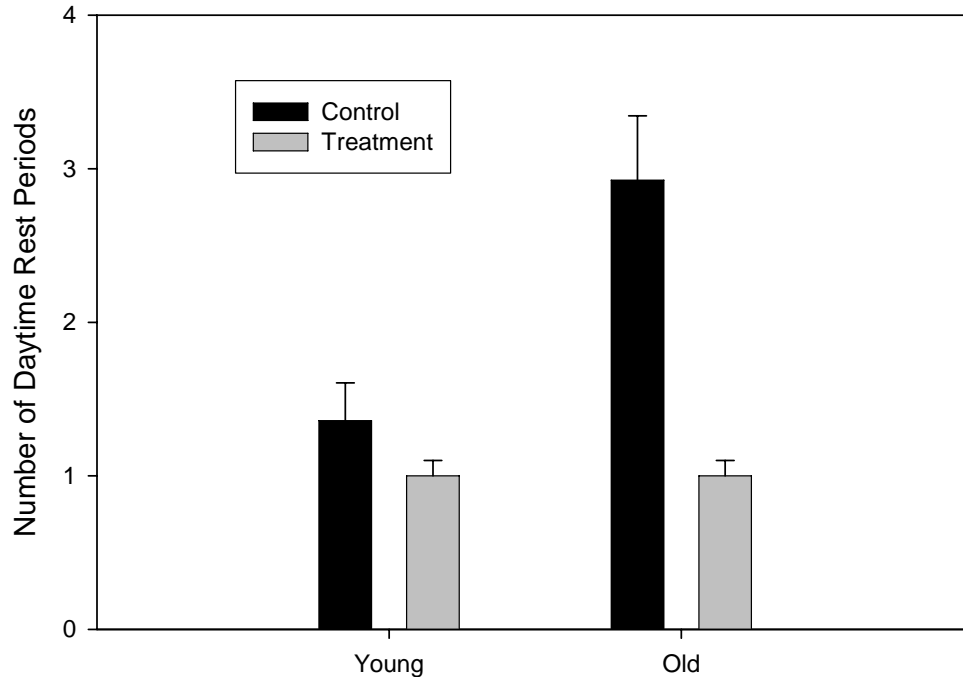


Figure 6: The number of times during the day that an animal will rest is decreased by drug treatment.

Effect of Test Compound X on EEG Activity

Purpose:

The effects of a test compound on sleep patterns (as defined by electroencephalographic and electromyographic activity) are examined.

Methods:

Electrodes are surgically implanted into the cortical surface of the brain using skull screws. Animals are also prepared with chronic EMG electrodes in neck muscle. Dogs are allowed to fully recover and are acclimatized to the testing facility before and after surgery.

Electrophysiological Recording Options:

EEG and EMG are continuously recorded for 24-48 hours for baseline measures, for each dose level of the test compound, and for a post-treatment phase to determine if there is a return to baseline levels. REM duration, slow-wave sleep, and waking periods are quantified according to their characteristic EEG and EMG patterns.

Measurements:

1. Power spectral analysis using a Fast Fourier Transformation is conducted to calculate the power contained in the frequency bands.

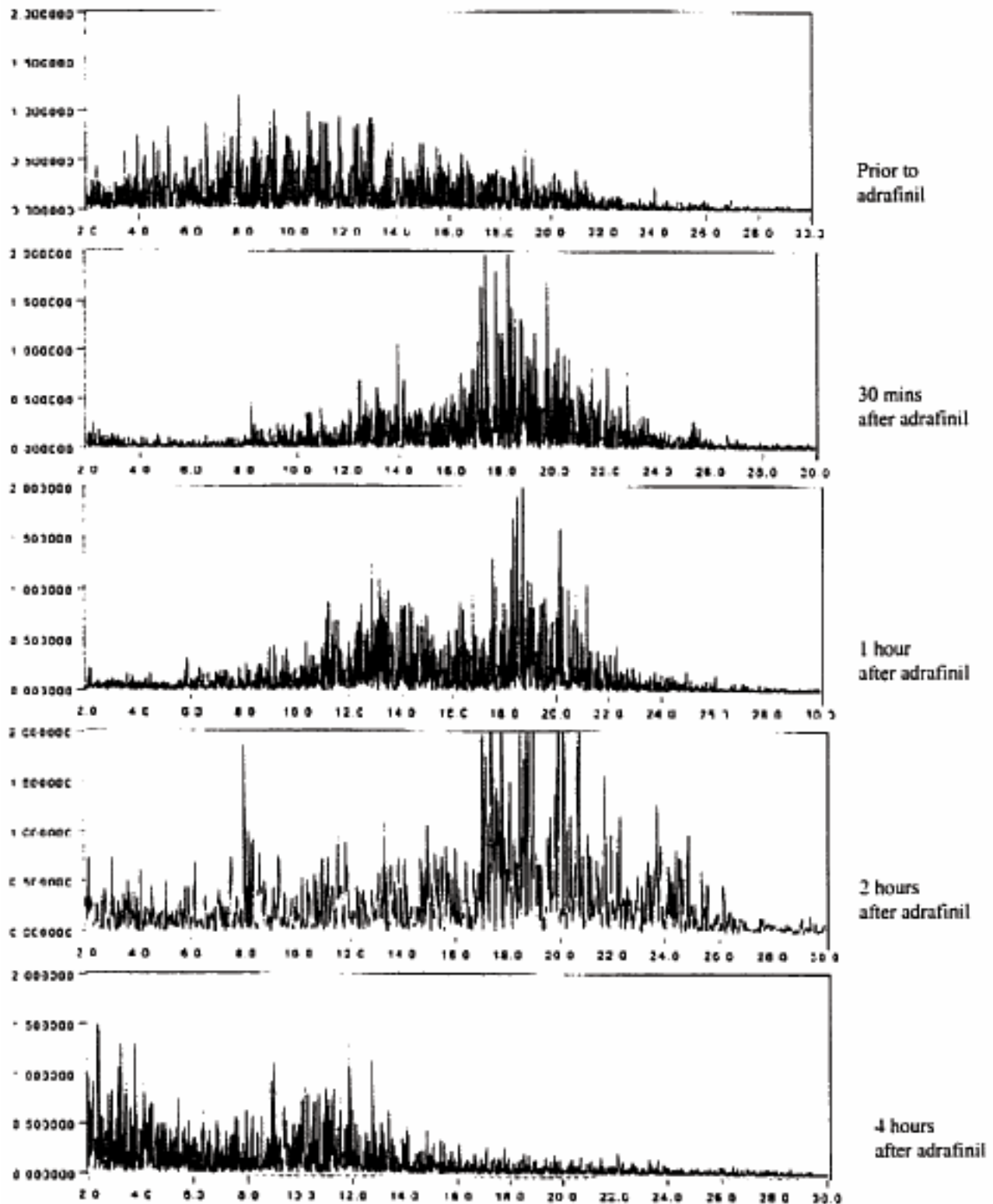


Figure 7: Power spectrum for effect of a treatment on EEG.

2. Total number of episodes and percentage of time spent in each sleep state per 24 hours.

Comparison of SWS, PS (or REM) and wakefulness activity. The data are divided into epochs and the percent time spent in each stage is calculated.

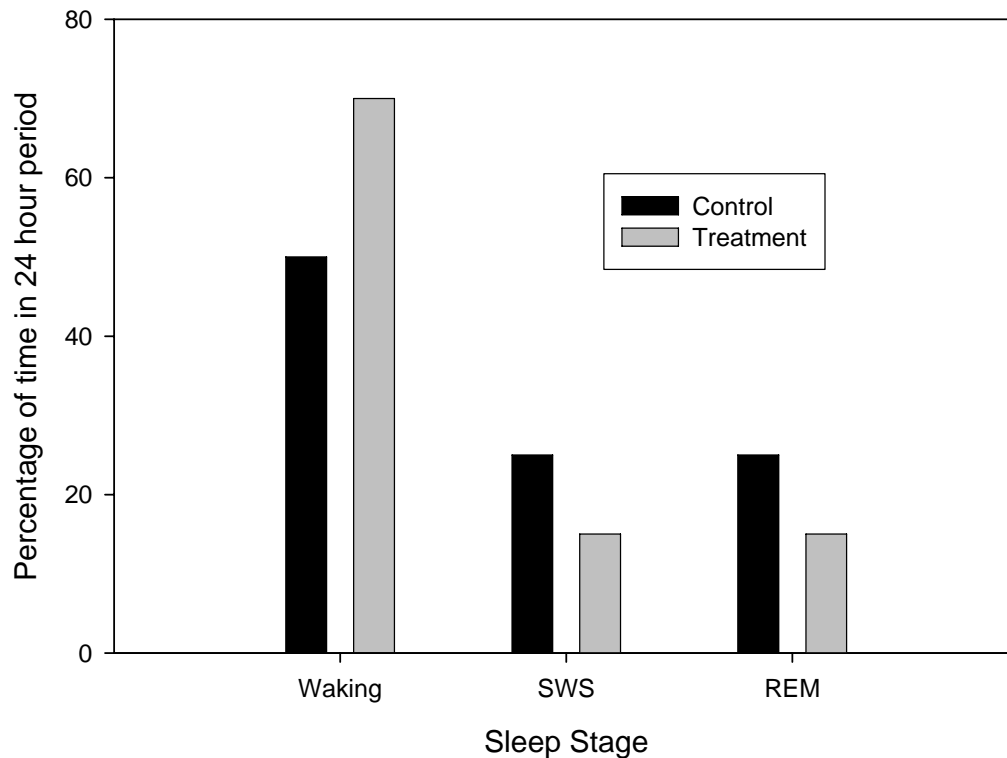


Figure 8: Percentage of time spent in each sleep stage during a 24 hour period before and during treatment.

Effect of the Test Compound on Memory Consolidation

Purpose:

The effect of a test compound on memory consolidation, using a discrimination learning paradigm, is assessed. Aged-dogs are used, as they are markedly impaired in acquisition of relatively complex shape discrimination tasks. Furthermore, these deficits are at least partly linked to a deficit in memory consolidation, evidenced by significant age differences in inter-session forgetting simultaneous with no deficits in intra-session learning. Age-related disruption in sleep rhythms is thought to contribute to this deficit in memory consolidation.

Experimental Design:

Subjects are aged beagle dogs (10 years or greater) which are divided into two cognitively equivalent groups:

Group A is administered the test substance daily

Group B is administered a placebo control

Both groups are tested on a series of oddity discrimination learning tasks.



For additional information, including pricing, references, and timelines, contact us as follows:

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