



CanCog Technologies Final Study Report

Effect of Apoaequorin on Cognitive Function in Aged Canines

Study Number: CTI1-10039-CE

Drug generic name: Apoaequorin

Proposed indication(s): Treatment of age-associated cognitive dysfunction.

Sponsor name and address: Quincy Bioscience
301 S Westfield Rd, Suite 200
Madison, WI, United States
53717

Testing facility name and address: CanCog Technologies Inc.

Study initiation date: 2010-07-15
Study completion date: Date when final study report signed-off

Experimental initiation date: 2010-07-16
Experimental completion date: 2010-08-22

TABLE OF CONTENTS

A.	SUMMARY	3
B.	STUDY NUMBER.....	5
C.	SPONSOR.....	5
D.	OBJECTIVE	5
E.	STUDY SCHEDULE.....	6
F.	STUDY TITLE	7
G.	STUDY DESIGN.....	7
H.	EXPERIMENTAL MATERIALS	7
I.	MATERIALS AND METHODS.....	8
J.	RESULTS AND DISCUSSION.....	12
K.	CONCLUSIONS.....	20
L.	DATA INTEGRITY STATEMENT	20
M.	PROTOCOL AMENDMENTS AND DEVIATIONS.....	21
N.	INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE INFORMATION.....	21
O.	ACCURACY OF REPORT STATEMENT	21

LIST OF APPENDICES

APPENDIX 1 SUBJECT INFORMATION AND DATA

APPENDIX 2 STATISTICAL TABLES

A. SUMMARY

The purpose of this study was to examine the effect of Apoaequorin, a calcium binding protein naturally found in jellyfish, on cognitive functioning in aged beagle dogs. Twenty-four cognitively experienced beagle dogs greater than 9 years of age served as subjects. Following baseline cognitive assessment, the dogs were assigned to three groups that were cognitively equivalent on performance on a delayed-non-matching-to-position task. The groups included a control group, a group treated with a low dose of test compound approximately 2.5 mg/kg and a high dose group dosed at approximately 5.0 mg/kg. The test or control compounds were administered daily over the course of the study in 2.5 mg/kg tablets. All subjects were tested on three tasks, an object discrimination learning that assessed general learning ability, a visual search task that assessed attention and a delayed-non-matching-to-position (DNMP) task that assessed visuospatial working memory.

The discrimination learning task tested the animals' ability to learn to discriminate between two objects, one of which was associated with reward. Both treatment groups showed more accurate learning than the control group, and the difference between the low and control group was statistically significant. The visual search task involved presentation of one positive object and either zero, one, two or three identical negative objects that served as distractors. Performance on the visual search task depended on the treatment, number of distractors and accuracy of responding. In general, the greater the number of distractors, the greater the number of errors. With respect to treatment, overall, the control group performed more poorly than either of the two treatment groups. The magnitude of the group differences also varied as a function of dose number of distractors. The high dose group differed significantly from the controls when presented with two distractors (3 choices). On this task, we also looked at response latency (speed of responding), which provided a measure of processing speed. Response latency varied as a function of number of distractors, and response accuracy. In general, the greater the number of distractors the slower the response. With respect to accuracy, subjects responded significantly faster on trials in which the response was correct than they did on trials in which the response was incorrect. There was also a marginally significant interaction between accuracy and dose, with the animals in both treatment groups responding more rapidly than the control animals when they responded correctly, but not when they responded incorrectly.

The third cognitive test, the DNMP, assessed the ability of the dogs to remember the location of an object presented during the sample presentation. Performance on the task varies as a function of the delay between sample and test presentation, reflecting increased memory demands. The subjects were tested under both baseline and treatment conditions. Although there were no significant group differences, the high dose group showed significantly improved performance when baseline performance was compared with performance under the treatment condition. This effect was not seen in either the control or low dose group.

Overall, these results suggest that daily administration of Apoaequorin has beneficial effects seen in improved learning (assessed in discrimination learning task) and in attention (assessed in the visual search task). The results further suggest that the effectiveness varies as a function of dose, with the high dose animals performing better overall than the other two groups on the visual search and DNMP tasks. All of the animals tested in the study were aged, and showed some degree of cognitive impairment. Thus, one possible interpretation is that the treatment has the potential of reversing age-associated cognitive dysfunction.

B. STUDY NUMBER

CT11-10039-CE

C. SPONSOR

Quincy Bioscience/Quincy Animal Health
301 S Westfield Rd, Suite 200
Madison, WI, United States
53717

D. OBJECTIVE

Apoaequorin is a bioluminescent calcium binding protein that is reported to improve sleep and provide neuroprotection in human subjects and is a synthetic form of apoaeguorin, which is a naturally occurring protein, found in certain jellyfish species. This study was designed to support the use of Apoaequorin to treat age-associated cognitive dysfunction and Canine Cognitive Dysfunction Syndrome (CDS) in companion animals.

E. STUDY SCHEDULE

Date	Study Day	Key Event	Activity
7/16/2010	-5	Beginning of In-life Phase	- Individual animal identification - Beginning of twice daily animal observations
2010-07-16 to 2010-07-20	-5 to -1	Cognitive testing (baseline)	- DNMP testing (5 sessions)
2010-07-21	0	Group Assignment Beginning of Treatment Body Weights	- Randomization and allocation to treatment groups - Treatment administration will begin according to assigned treatment schedule - Animal body weights taken
2010-07-21 to 2010-07-24	0 to 3	Treatment wash-in	- Animals dosed orally once daily
7/24/2010	3	Cognitive testing (preference)	- Animals given preference test
2010-07-25 to 2010-08-03	4 to 13	Cognitive testing	- Object discrimination learning (200 trials or successful completion of Stage 2 criterion)
2010-08-04 to 2010-08-10	14 to 20	Cognitive testing	- Attention task
2010-08-11 to 2010-08-12	21 to 22	Rest days	
2010-08-13 to 2010-08-22	23 to 32	Cognitive testing	- DNMP (180 Trials or successful completion of Stage 2 criterion)
2010-08-22	32	Body Weights End of Treatment End of in-life phase	- Animal body weights taken - Last day animals are treated - End of twice daily animal observations

F. STUDY TITLE

Effect of Apoaequorin on Cognitive Function in Aged Canines

G. STUDY DESIGN

A blinded parallel group design with a total of 24 aged (> 9 years) beagles were used.

There were three treatment groups:

- a) Placebo
- b) Apoaequorin 2.5 mg
- c) Apoaequorin 5 mg

Before group assignment, all subjects were tested on the DNMP for 5 days to provide baseline measurements, which was used to assign subjects to cognitively equivalent groups.

Subjects were administered the compound (PO in tablets) from Day 0 to Day 32. During this period, and for the remainder of the study, all personnel except those responsible for drug preparation and administration were blinded.

Cognitive testing was performed on Days 3 to 32. After a 5 day wash-in, the subjects were first tested on the attention task, and subsequently on the DNMP.

H. EXPERIMENTAL MATERIALS

1 Investigational drug and control

I Test Article:

- i* **Chemical name:**
Apoaequorin
- ii* **Active ingredient:**
Apoaequorin
- iii* **Dosage form:**
The test substance was delivered as a chewable tablet.
- iv* **Doses tested:**
2.5 mg and 5 mg

- v *Lot No.:*
RD051810-2000mg
- vi *Packaging:*
The tablets are packaged in white plastic bottles each containing a minimum of 60 chewable tablets per bottle.
- vii *Drug storage during study:*
At room temperature in CanCog Technologies archive room.

II Control Article:

- i *Dosage form:*
The test substance was delivered as a chewable tablet.
- ii *Lot No.*
RD06071010-2000 mg
- iii *Packaging*
The chewable tablets are packaged in white plastic bottles each containing a minimum of 60 tablets per bottle.
- iv *Drug storage during study*
At room temperature in CanCog Technologies archive room.

I. MATERIALS AND METHODS

1 Test System

The test system consisted of 24 aged beagles of both sexes obtained from the CanCog Technologies dog colony. Ages of the dogs ranged from 9.48 to 17.33 years at study initiation.

2 Selection and Allocation of Animals

All qualified subjects were previously trained to a criterion level of performance on the delayed-non-matching-to-position (DNMP) task and were consistent responders on the task. The 24 were selected from a larger group of animals that received 5 baseline screening sessions. In addition to reliable performers, the animals selected tended to perform at below maximal levels to allow for the possibility of seeing memory enhancement. The inclusion of study animals was approved by the Study Director.

Any animal deemed unsatisfactory for the purpose of the study such as poor health or uncooperative disposition prior to treatment was eliminated prior to allocation. The rejection of study animals was approved by the Study Director.

Subjects were placed into 3 cognitively equivalent groups with 8 animals per group. The animals were handled and cared for as similarly as possible throughout the study.

To allocate the animals to groups, subjects were ranked based on baseline performance on the DNMP. A total percent correct score was calculated. Next, the dogs were ranked such that the higher the score on the DNMP, the higher the rank (e.g. the best performing animal received a rank of 1, and the poorest performing animals received a rank of 24.) Subsequently, dogs were divided into three groups. Once this was completed, and all dogs were assigned to treatment groups, the allocation of treatment groups to experimental units was performed.

Once the dogs were assigned to a group, designated personnel randomly assigned each group to a treatment condition by the drawing of a lot. For lot drawing, three pieces of identically sized paper were used. "Placebo", "Low dose", and "High dose" was written on one of the pieces of paper and were placed into a non-transparent container. The designated personnel removed one of the papers, and assigned Group 1 to that condition. The second drawn paper was used to assign Group 2 to the condition written on the paper. The final paper was used to assign Group 3. Group assignments were recorded and this information was kept in a secured location during the study to ensure blinding of personnel collecting data. The subjects and group allocations are shown in Table 1.1 in Appendix 1.

3 Acclimation and Pre-Treatment of Test System

All animals involved in this investigation were housed in the CanCog animal facility for a period not less than three months prior to experimental initiation. Therefore, no acclimation period was needed.

4 Administration of Test Articles

Beginning on Day 0 and continuing through Day 32, dogs in the low dose (2.5 mg) group and 4 dogs from the placebo group received one tablet daily. Dogs in the high dose (5 mg) group and 4 dogs from the placebo group received two tablets daily. Treatment was performed daily from Day 0 to Day 32 inclusive.

Animals were dosed 75 minutes (± 15 minutes prior to cognitive testing.)

The test article was administered with attention to complete delivery and retention of the entire intended dose according to the following general procedure:

The person administering the test article ensured that the mouth contained no food or other object. The dog's head was tilted backward with the nose upward and the mouth was gently opened. Using the thumb and index finger or a dosing device, the test article/placebo was quickly placed on the back of the tongue. The mouth was closed and the head was kept slightly raised for a few seconds to ensure swallowing and retention.

5 Procedures and Data Recorded

I. DNMP

DNMP Testing was carried out on the days indicated in Section 7.1, Study Design Summary. Testing was performed as per Standard Operating Procedures DOG.24. Briefly, each trial began with an initial presentation of an un-baited block, followed by a delay and a second presentation with two identical blocks: a baited block in the original position covering the empty well and an un-baited block covering the reward in a novel position. The dog was rewarded for displacing the stimulus in the novel position. This study used the variable-delay paradigm in which delays of 5, 55 and 105 seconds occurred equally over 18 test trials per day, resulting in 6 trials for each delay. The delays occurred randomly within the test session and each possible position was used for each delay.

In the present study, we used five sessions for the baseline phase, and ten sessions for the treatment phase.

II. 2-Choice Discrimination

2-Choice Discrimination testing was carried out on the days indicated in Section 7.1, Study Design Summary. Testing was performed as per Standard Operating Procedures DOG.36, with the following protocol-specific instructions:

There were 10 trials per session, and there were two test sessions per day for all subjects. Subjects will be trained with two new objects that they have no previous familiarity with. All subjects will have one 10-session preference test on the last day of wash-in. Training on the 2-choice discrimination will be twice daily, in the morning and afternoon, with 10 trials per session. The object preferred by the subject in the preference test will be rewarded in the 2-choice discrimination test. Training will be complete after the subjects either pass a two-phase criterion or complete 200 trials.

III. Attention Task

The Attention Task was a visual search task in which the subjects are required to select the correct object when presented with up to four alternatives in order to obtain food reward. The testing was carried out on the days indicated in Section 7.1, Study Design Summary. Testing was performed as per Standard Operating Procedures DOG.38, with the following protocol-specific instructions:

The objects were the same objects used in the 2-choice discrimination Test. The positive object was the rewarded object from the 2-choice discrimination test. The negative object was present in triplicates and served as the distractor object. All animals were given one session per day and 16 trials per session. Four trials 0 distractors, four trials had 1 distractor (two choices), four trials had 2 distractors (3 choices), and four trials had 3 distractors (4 choices).

IV. Health Observations

The dogs were observed twice daily for any signs that would not be expected in normal dogs, including but not limited to, muscle tremors, coughing, nasal discharge, sedation, rapid or laboured breathing and convulsions. An observed abnormality will be checked off as “abnormal” and briefly described under “comments” on the Daily Animal Observation Record by the person making the observation. All daily observations were recorded by blinded personnel.

V. Adverse Events

An Adverse Event (AE) is any undesirable experience occurring to an animal, whether or not related to the investigational product. Thus any post-treatment indication of abnormal (or other than normal) any place in the data is an adverse event. All adverse events, as well as all similar findings pre-treatment, were tabulated in the final study report. The Study Director reported all serious AE to the sponsor as soon as practical, even if the event did not appear to be drug-related. Abnormal findings on the Daily Animal Observation Record were recorded on the Adverse Event Report

6 Blinding of the Study

The treatment given to each animal was not revealed to any personnel involved with data collection. The study was blinded to all study personnel with the exception of the person(s) preparing and administering the test and control articles and the person responsible for performing the allocation.

Treatment conditions were assigned to 1 of 3 treatment groups (refer to Section 7.1.5.2) and this information was kept in a secured area over the duration of the study.

The cognitive testers carrying out the assessment were blinded. The code assignments, treatment records and any other documents that would reveal treatments to people collecting data remained in a secured location at CanCog until all data was collected.

7 Calculations and Statistical Analyses

The data were statistically analyzed using analyses of variance. Dunnett’s test was used to compare performance of each treatment group with the controls. Total errors to complete a criterion level of performance were used as the dependent variable on the discrimination learning task.

For the attention task, the dependent variable was number of correct responses at each level of distraction. The attention task continued over 7 consecutive days, with 4 trials daily at each level of distraction. All animals responded correctly when there were no distractors, so the analysis was restricted to cases when there was at least one distractor present. We also analyzed the latency data. To do prevent outlying data from having disproportionate effects,

for each animal at each level of distractor, the mean latency and standard deviation were calculated and all scores >3 SD from the mean were dropped and this process was repeated until there were no outlying scores. We also discarded all scores ≥ 4.0 S.

On the DNMP, results at each delay were represented as percent correct responses.

J. RESULTS AND DISCUSSION

1 Baseline Data

Table 1.2 in Appendix 1 shows the individual animal data during the baseline condition. To assure that the groups were cognitively equivalent, the data were analyzed with a repeated measures ANOVA with delay (5, 55 and 105) as a within subject variable and group placement as a between subject variable. The results revealed a statistically significant effect of delay ($p=0.002$), and no other significant effects or interactions (see Table 2.1 in Appendix 2). As summarized Table 2.2 (Appendix 2), the delay effect was due to performance at the 5 second delay being significantly more accurate than at the 55 second or 105 second. The grouped baseline data are shown in Figure 1.

Group Performance at Baseline on DNMP

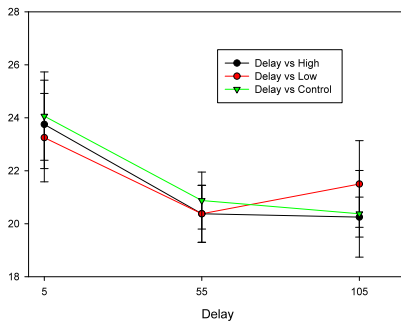
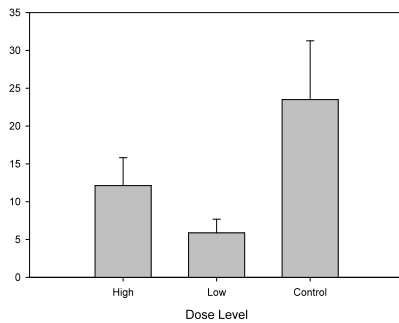


Figure 1. Baseline Performance on the DNMP. Each animal was given 30 trials at each delay over 5 baseline sessions. The y-axis indicates total correct responses at each delay.

2 Discrimination Learning

The data were analyzed with a one way analysis of variance comparing the treatment groups on both errors and percent correct out of total responses. The results are shown in Tables 2.3 and 2.4 and indicate a significant effect of treatment. As illustrated in Figure 2, the two groups receiving the test compound performed better than the controls on both of the measures. When the groups were compared with the control using the Dunnett test, the low dose group learned with significantly fewer errors ($p=0.042$) and had a significantly higher percent accuracy ($p=0.026$). The high dose group did not differ significantly from the controls ($p=0.218$ for error measure; $p=0.115$ for percent accuracy measure).



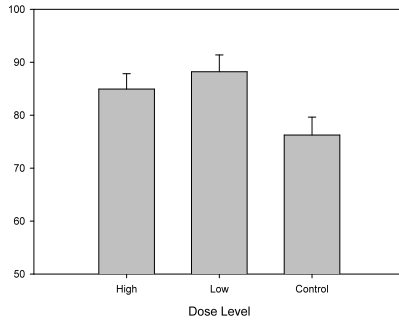


Figure 2. Effect of Apoaequorin on two different measures of performance on the discrimination learning task. The top shows the effect on errors to criterion measure of learning. The bottom shows percent correct for each of the groups.

3 Attention Task

Performance Accuracy

All animals responded correctly when there were no distractors. The analysis, therefore, was restricted to the performance in the presence of 1, 2 and 3 distractors only. The data were first analyzed with a repeated measures analysis of variance with number of distractors as a within subject variable and treatment as a between subject variable. The results, which are summarized in Table 2-5 revealed a significant effect of number of distractors ($p < 0.01$) and a marginally significant effect of treatment ($p = 0.071$). The distractor effect results from the task becoming progressively more difficult the greater the number of distractors. These results are illustrated in Figure 3. Further, performance with one distractors (two objects) differed significantly from performance with two ($p < 0.01$) and three ($p < 0.01$) and performance with 2 distractors differed significantly from performance with three ($p = 0.011$) – see Table 2.6.

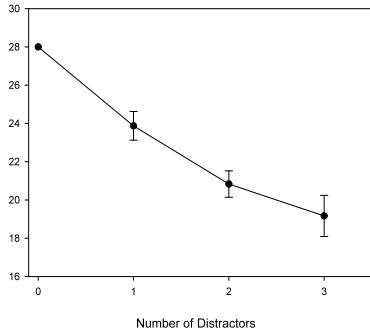


Figure 3. Performance on the attention task as a function of number of distractors. The data reflect combined data from all three groups.

The marginally significant effect of treatment overall reflects superior performance by the two treatment groups over the control group, with the difference between the high dose and control being marginally significant ($p=0.062$) and the difference between the low dose and control approaching significance ($p=0.113$). These data are illustrated in Figure 4, which also shows that the group differences were greater when tested with three distractors than when tested with 1. To further understand this effect, separate one-way ANOVA's were carried out at each level of distractor. The results of the analysis are summarized in Tables 2-7 to 2-9 and revealed a statistically significant effect ($p=0.0250$) when the groups were tested with two distractors. The Dennett test revealed a significant difference between control and high dose group ($p=0.02$) and a marginally significant difference between the control and low dose group ($p=0.067$).

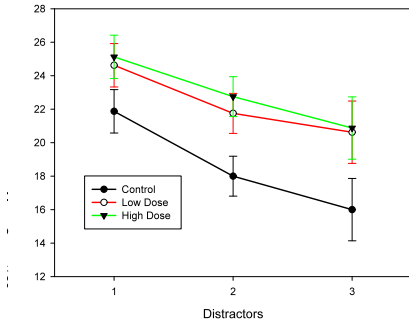


Figure 4. Performance on the attention task as a function of number of distractors and treatment condition.

II. Latency Analysis:

The latency analysis examined response latency independently of response accuracy. The data were first analyzed using a repeated measures ANOVA with number of distractors as the within subject analysis and treatment group as the between subject analysis. The results, which are summarized in Tables 2-10, revealed a statistically significant effect of number of distractors ($p < 0.01$), and no other significant main effects or interactions. Overall, latency increased with increased numbers of distractors. Although the treatment effect was not significant, the control animals showed a trend in responding more slowly with increasing numbers of distractors (See Figure 5). Comparing latency at each level of distractor (Table 2-11) revealed that latency at 0 distractors was significantly faster than all conditions with more than one distractor. In addition, subjects responded significantly faster with 1 and 2 distractors than they did with 3.

Latency as a Function of Treatment

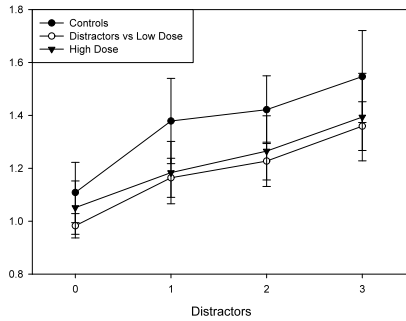


Figure 5. Latency as a function of treatment and number of distractors, independently of response accuracy.

The next analysis took into consideration response accuracy by separating correct from incorrect scores and for each animal calculating the mean score. The data were then analyzed with a repeated measures ANOVA with accuracy and distractors as within subject variables and dose as between subject variable (Note four animals, 2 at high dose and 2 at a medium dose, were not included in the analysis because they had no incorrect responses at 1 distractor; see Table 1.5). The results of the analysis are summarized in Table 2-12. They revealed a statistically significant main effects of accuracy ($p < 0.01$) and number of distractors ($p = 0.001$). There was also a significant interaction between accuracy and number of distractors ($p = 0.001$) and a marginally significant interaction between accuracy and dose ($p = 0.074$).



Figure 6. Latency as a function of accuracy, number of distractors

Figure 6 shows that the significant effect of accuracy reflects slower responding overall when the responses were incorrect than when they were correct, that latency increases with increasing numbers of distractors. Figure 6 also shows that the marginally significant interaction between dose and accuracy reflects faster responding by the two treatment groups when the response was correct compared to the controls, but no difference between the groups when the response was incorrect.

4. Effect if Apoaequorin on DNMP performance

The DNMP data were converted to a percent correct score and were first analyzed with a repeated measures ANOVA with dose (control, low dose and high dose) as between subject variable and both treatment (control vs. test) and delay as within subject variable. The results, which are presented in Table 2-13. showed a significant effect of delay ($p < 0.01$), and no other significant effects or interactions. As indicated in Figure 7, the delay effect is due to all groups performing more accurately at the short delay than at either of the two longer delays.

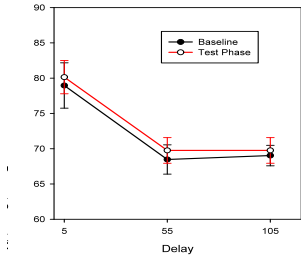


Figure 7. Accuracy as a function of delay at baseline and when retested in the treatment phase. The data shown are the combined data for all of the groups.

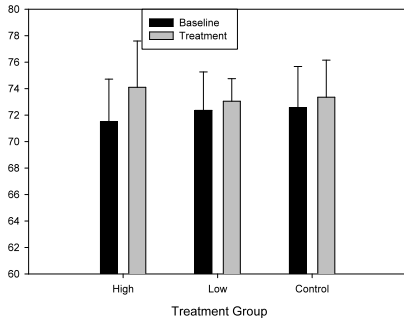


Figure 8. Percent correct on DNMP under baseline and test conditions as function of treatment. The percent correct score was calculated by combining performance at all delays.

Although there were no significant treatment effects, comparing the individuals baseline with performance under treatment condition indicated greater overall improvement in the animals in treatment groups, particularly in the high dose group. Comparing mean performance of the high dose under treatment with control condition revealed marginally significant improvement ($p=0.075$ with a two-tailed t-test and $p=0.0375$ with a one-tailed t-test). The Group comparisons between baseline and treatment conditions are shown in Figure 8. By contrast the p value for the control group was 0.73, and for the low dose group it was 0.72. Thus, these results point to a clear trend of improved performance over baseline in the high dose, while the low dose and controls performance was indistinguishable from baseline.

K. CONCLUSIONS

1. Apoaequorin significantly improved performance on a discrimination learning task at 2.5 mg/kg. At 5 mg/kg the improvement was marginally significant.
2. Performance on a visual search task that provides a measure of attentional ability varied as a function of number of distracters and treatment. In general, the greater the number of distracters, the greater the number of errors. Subjects treated with apoaequorin made fewer errors than controls and the high dose of apoaequorin was more effective than the low dose.
3. The difference between control performance and performance under apoaequorin also varied as a function of number of distracters. The high dose differed significantly from controls when tested with two distracters. Smaller differences were seen with one or three distracters.
4. Response latency varied as a function of response accuracy and number of distracters. The greater the number of distracters the longer the latency. With respect to accuracy, overall latency was significantly slower when the animals responded incorrectly than when they responded correctly.
5. Subjects treated with apoaequorin responded more rapidly than controls when the response was correct. There were no differences between the groups when the response was incorrect.
6. These results suggest that apoaequorin is effective in improving learning and enhancing attention.
7. There was no evidence of apoaequorin enhancing working memory on the DNMP. However, overall performance on the task was improved in the high dose group, which could also reflect enhanced attentional processes, or put differently improved ability to focus.

L. DATA INTEGRITY STATEMENT

There were no unforeseen circumstances that affected the quality or integrity of the data.

M. PROTOCOL AMENDMENTS AND DEVIATIONS

Protocol Amendments, Deviations, and Incident Reports are contained in Appendix 2: Protocol-Related Documents. None of these changes from the study protocol had an impact on the study.

There were no amendments or deviations to the final study protocol

N. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE INFORMATION

CanCog Technologies' Internal Animal Care and Use Committee approved the protocol, without reservation, on 2010-07-14.

O. ACCURACY OF REPORT STATEMENT

I certify that this report is a complete and accurate representation of all study observations.