

# Cognizin

## MEMORY ENHANCEMENT

“COGNIZIN™ HAS A MEASURABLE EFFECT ON MEMORY WHICH MAY BENEFIT PATIENTS SUFFERING FROM CEREBROVASCULAR CONDITIONS SUCH AS ALZHEIMER'S, PARKINSON'S, AND STROKE AS WELL AS GLAUCOMA.”

Memory consists of the following components: **1.** implantation of information in the brain (memorization) **2.** retention and **3.** recollection. Without all three, information cannot be established as memory.

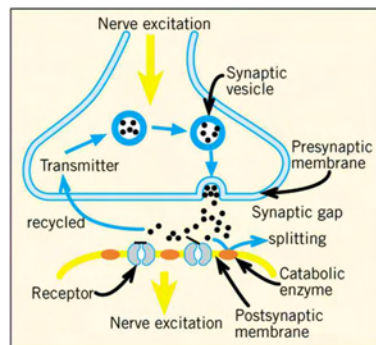


Figure 1: Synaptic structure.

At the end of the nerve where the excitation is transmitted (the presynaptic membrane), neurotransmitters (e.g. acetylcholine) are released from the synaptic vesicle into the synaptic gap. When the neurotransmitters bind to the receptors of the post-synaptic membrane, the next neuron is excited and the stimulus is transmitted along the network. (Figure 1)

Since the synaptic vesicle consists of the same components as the cell membrane, it easily fuses itself with the cell membrane and releases its contents.

Two mechanisms regulate nerve excitation: 1. the mechanism that metabolizes the neurotransmitters, thereby activating them and 2. the mechanism that restores and reuses the pre-synaptic membrane.

### ACETYLCHOLINERGIC NERVES

Memorization in the brain occurs in the hippocampus. (Figure 2) Acetylcholinergic nerves are distributed throughout the hippocampus where their activity is believed to be important for memory function. By administering scopolamine, a drug that competes with acetylcholine, to animal test subjects, it

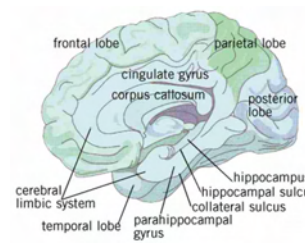


Figure 2: The human brain.

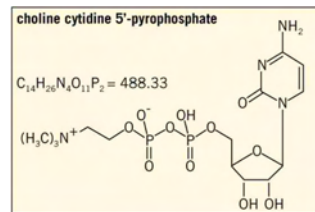


Figure 3: Cognizin™ structural formula.

is possible to create an amnesia model. Also, in Alzheimer's disease, which is characterized by dementia, it is known that the activity of the acetylcholinergic nerves is diminished. Other nerves utilizing glutamic acid as the neurotransmitter are also located in the hippocampus and play an important role in memory.

Cognizin™ is a precursor of phosphatidylcholine, a type of phospholipid, a component of cell membranes. (Figure 3) Cognizin™ is believed to stabilize the membranes and, when absorbed by the body, breaks down into uridine and choline. Cognizin™ also is converted to acetylcholine, a neurotransmitter, which inhibits the formation of free fatty acids and diacylglycerol, substances which exhibit cytotoxicity. (Figure 4, Ref. 1-7)

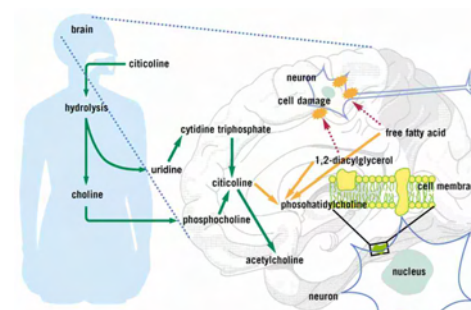


Figure 4: Cognizin™ citicoline metabolism and activity.

### STUDY

Kyowa Hakko performed the water maze study to test the impact of Cognizin™ citicoline supplementation on memory. In this study, a Morris Water Maze (Ref.8) was used to study the effect of Cognizin™ citicoline on the memory capacity of mice. (Figure 5) This device is a cylindrical pool filled with water. At a depth of 0.5 cm, a transparent acrylic platform (PF), invisible to the mice, was placed in the cylinder. Utilizing the mouse's tendency to memorize the PF location and to struggle onto it from the water, the device measures the subject's memorization and learning capabilities.

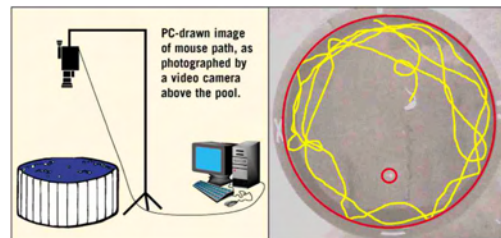


Figure 5: Overview of Morris Water Maze.

with a stainless steel mesh cover, measuring 225 mm (W) x 338 mm (D) x 140 mm (H). The mice were exposed to a conventional environment (12-hour light-dark cycle at  $23 \pm 1^\circ$  C temperature) and freely ingested (drinking) water, along with their feed.

The pool of the Morris Water Maze measured 150 cm (diameter) x 45 cm (depth). A 12 cm (diameter) transparent acrylic platform (PF) was provided at a water depth of 0.5 cm. A video camera was used to record the mice swimming and the data was processed using a Windows 98-based PC and Smart CS animal behavior software (Panlab Co., Spain).

Mice were trained to memorize the PF location and how to swim to the PF by the following method: Mice were placed in the pool and allowed to swim for up to two (2) minutes per trial. Mice that reached the PF were left there for 20 seconds. Those that failed to reach the PF within two (2) minutes were manually placed there and left for 20 seconds. By training the mice six (6) times the first day and 12 times the second day, most mice were able to learn how to reach the PF within 20 seconds. Individual mice that were able to reach the PF within the allocated 20 seconds two (2) consecutive times were considered trained and training was discontinued.

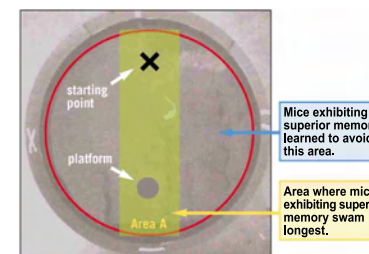


Figure 6: Swimming path divisions of mice.

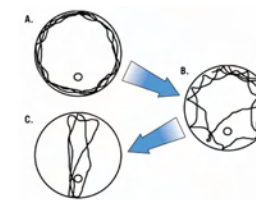


Figure 7: Swimming path changes as the mouse learns.

At the start of the training, the mice exhibited a tendency to swim around the edge of the pool. However, as the training progressed, they began to swim to the center. Eventually, they swam directly to the PF from the drop point. Generally, the mice exhibited a path change as is shown in Figure 7 (a-b-c). Repetition of the learning trials gradually decreased the time required by the mice to reach the PF.

The trained mice were divided into two (2) groups and it was verified that both groups swam the same number of times during training. The control group was fed a commercial powdered diet (CD2, CLEA Japan, Inc.). The second group was fed CD2 containing 2% Cognizin™ citicoline. Each group was raised on the respective diets for four (4) weeks.

After the 4-week period, the PF was removed from the Morris Water Maze and the mice were made to swim for one (1) minute. Their swimming path was recorded as an image (Figure 5) and the distance and time each mouse swam in Area A was analyzed using Smart CS. (Figure 6)

### RESULTS

The study clearly determined that the group of mice which had ingested the diet containing 2% Cognizin™ citicoline, swam longer in Area A compared to those mice fed the control diet. (Figure 8) The difference was statistically significant. Also, in the mice fed Cognizin™ citicoline, the following tendencies were evident: the time required to struggle to and swim across the platform location was shorter (Figure 9); and the number of times the mice crossed the platform within 60 seconds was greater than for the control group. (Figure 10)

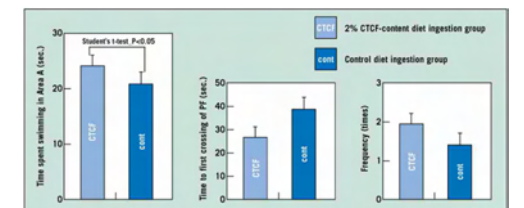


Figure 8: Time swimming in Area A.

Figure 9: Time to first crossing of PF.

Figure 10: Time across PF location in 60 sec.

### CONCLUSION

The study results demonstrate that the mice that ingested Cognizin™ citicoline remembered how to reach the submerged platform better than the control mice, exhibiting enhanced memory-related abilities. Moreover, because both groups of mice swam at the same speed during the tests, it can be determined that Cognizin™ citicoline did not affect motility.

### REFERENCES

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