

The art of neuromodulating! by David Laflamme

Life sciences

Experiment

Senior 1 Age 17

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Presented at the Science Fair in 2000

Project summary

Experiment to test the neuromodulator properties (ability to influence the intensity of synaptic transmission between neurons) of *Ginkgo biloba* on rats subjected to the temporary effects of an inhibitor of synaptic transmission as in Alzheimer's patients. The goal is to restore synaptic transmission.

Project Report

"Cerebral activity is a changeable geography in which the major sites and their connections are unstable, flexible, nomadic." (Eric Fottorino, *Voyage au centre du cerveau* [Journey to the centre of the brain, free translation])

Our brain is made up of a million billion connections called synapses. These connections occur between our 100 billion neurons. When we memorize something, the synapse between two neurons is strengthened and messages circulate more easily. In Alzheimer's disease, synaptic transmission is no longer adequate. Certain so-called positive neuromodulators, however, can restore synaptic transmission. The goal of this project is to test the neuromodulator properties of extract of *Ginkgo biloba* (Egb 761), one of the most powerful *in vitro* neuroprotectors.

Problem

Neurons are continually communicating with each other by sending chemicals called neurotransmitters across a space between each neuron at the synapse. There are different types of neurotransmitters, each one used in a specific region of the brain. Cholinergic neurons, which use acetylcholine as a neurotransmitter, are found in the hippocampus, the brain's memory centre. They allow us to store long-term memories throughout the brain. Alzheimer's disease attacks these cholinergic neurons in the hippocampus. The disease results in the accumulation of deposits of a negative and toxic neuromodulator called β -amyloid around the neurons. This substance causes two major problems: 1) first, it reduces the amount of acetylcholine present by up to 90 percent, preventing these neurons from communicating with others elsewhere in the brain, thus making it impossible to memorize information; 2) second, it attacks and destroys millions of cholinergic neurons in ways we do not yet understand. Recent studies have proven that the strange accumulation of β -amyloid could be caused by a lack of acetylcholine. Three quarters of all seniors present a significant reduction in the amount of acetylcholine present in the brain. In severe cases, the accumulation of β -amyloid also reduces the amount of acetylcholine present. One promising hypothesis involves positive cholinergic neuromodulators. These substances promote cholinergic synaptic transmission by, for example, facilitating the increased release of acetylcholine

or by helping to prevent its degradation. More acetylcholine means fewer cognitive deficits and less β -amyloid.

There are currently fewer than ten known substances with positive cholinergic neuromodulator properties. None of them acts on the second problem caused by Alzheimer's disease: neuron death caused by the toxicity of β -amyloid. In addition, there is currently no test relying exclusively on memory to evaluate potential cholinergic neuromodulators.

Goal

In this project, I wanted to determine whether extract of *Gingko biloba* has positive cholinergic neuromodulator properties by testing its ability to restore synaptic transmission in rats previously subjected to the temporary effect of an inhibitor of cholinergic synaptic transmission (scopolamine).

Hypothesis

Some vegetable extracts such as millepertuis and nicotine have considerable positive neuromodulator properties (on dopamine and acetylcholine, respectively). Extract of *Gingko biloba*, the most powerful *in vitro* neuroprotector known to counteract β -amyloid toxicity, could also possess positive cholinergic neuromodulator properties.

Experiment

The experiment took place at the Douglas Hospital, which is affiliated with McGill University. It was an object recognition test administered to rats.¹ The rats experienced no stress, since the test made use of their innate need to explore. Since this was the first time the test was administered in this way, I had to establish parameters. This first phase was crucial and represented 90 percent of the experiment. The test was performed as follows. Each rat in a group of 24 is given one day to get used to a small 45-cm x 45-cm room (housing phase). The next day, two identical objects are placed in the room and each of the rats has two minutes to study them (learning phase). One hour later, one of the two identical objects is replaced by a new object and each of the rats again has two minutes to study both objects (recognition phase). The time the rats spend studying the objects is measured. Rats whose memory is intact definitely spend more time studying the new object during the recognition phase. More than 50 variables must be controlled in order to guarantee the reliability of results: the rats' age; odour saturation; the time of day; the lighting; the duration of the housing, learning and recognition phases; the colour of the objects; the size of the room; the presence or absence of the experimenter, etc. I repeated this step nine times with a control group (rats that did not receive an injection) in order to establish adequate test conditions. I then proceeded with the *Gingko biloba* test. I performed the same test on a group of 24 new rats divided into three subgroups. Groups 1 and 2 were given an injection of 0.2 mg/kg of scopolamine 30 minutes before the learning phase. This alkaloid, derived from the plant *Scopolia carniolica*, temporarily inhibits cholinergic synaptic transmission as in Alzheimer's disease and prevents memorization for a short period. Group 2 was also given an injection of 200 mg/kg of *Gingko biloba*. Group 3, the control group, was given an injection of salt water. The *Gingko biloba* test was performed three times in order to validate the results. The data obtained confirmed my hypothesis. In order to more accurately determine the degree of effectiveness of the positive cholinergic neuromodulator properties of *Gingko biloba*, I consolidated two of the parameters: the dose of scopolamine and the interval between injection and the learning phase. During the *Gingko biloba* test, the dose of 0.2 mg/kg of

¹ The rats were treated in accordance with the *McGill University Animal Care Committee* protocol.

scopolamine 30 minutes before the learning phase did not totally block memorization: cholinergic synaptic transmission was not totally inhibited. I want to make sure that cholinergic transmission in all the rats is temporarily 100 percent blocked by the scopolamine. Total control of these two elements in the final *Gingko biloba* test will provide more accurate results with respect to the degree of effectiveness of its neuromodulator properties.

Results and analysis

The test with the control group was repeated nine times in order to establish the protocol and the necessary parameters. For example, once the variables were controlled, the rats spent as much time in the learning phase studying the object on the left as they did the one on the right (Graph 1), which indicates that they had no spatial preference. If they had, biasing agents might have come into play in the recognition phase; for example, the rats might have studied the object on the left for a longer period of time. Also, once the variables were adjusted, the control rats spent more time (± 8 seconds) studying the new object during the recognition phase regardless of its location, size, colour, etc., which indicates that the test is valid. Then, the *Gingko biloba* test was repeated three times. Slightly more than half the 24 rats injected with scopolamine only (0.2 mg/kg) demonstrated significantly impaired memory: they spent as much time on both objects during the recognition phase. The rest of the group demonstrated less evident impairments, suggesting that the dose was not exactly right. The 24 rats injected with both scopolamine and *Gingko biloba* (200 mg/kg) showed no memory impairments (with respect to the control group), which suggests that the *Gingko biloba* succeeded in completely reversing the negative cholinergic neuromodulator effect of scopolamine with its positive neuromodulator properties. It therefore restored the synaptic transmission of cholinergic neurons in the hippocampus, either by facilitating the release of acetylcholine, by helping prevent its degradation or by promoting its reception. These very interesting results confirm my initial hypothesis and achieve the goal of my research. Currently, tests to determine the optimum dose of scopolamine and the ideal interval between injection and learning are under way. It appears that 0.2 mg/kg of scopolamine injected one minute prior to the learning phase or 0.4 mg/kg injected 30 minutes prior would cause more serious amnesia than that obtained with 0.2 mg/kg 30 minutes prior. This confirms the very short-term amnesic effects of scopolamine. Once these factors are completely mastered and validated, the final *Gingko biloba* test will provide the precise degree of effectiveness of the neuromodulator properties of the substance established by the initial test.

Conclusion

The role of neuromodulators in communication between neurons is absolutely fascinating! Each neuromodulator has a particular art of neuromodulating. In this project, I wanted to demonstrate that extract of *Gingko biloba* acts as a neuromodulator by testing it against the effects of an inhibitor of synaptic transmission. These results are the first to demonstrate *in vivo* the proneuronal properties of extract of *Gingko biloba*. I am very satisfied with the results, which: 1) make it possible to add this substance to the short list of known neuromodulators; 2) provide a reusable test to evaluate other possible neuromodulators and perhaps even 3) in the longer term, use a complex of molecules from different neuromodulators and neuroprotectors working together to develop a drug to treat Alzheimer's disease.

“Everything you can imagine is real.”
— Pablo Picasso