Learning, Memory & the Quest for Nootropia

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Ampakines are a class of drugs that have been developed by Cortex Pharmaceuticals Inc. to improve the aberrant excitatory neurotransmission that is observed in many neurological disorders[1]. These drugs were developed because they seem to enhance memory by affecting a theoretical neurological process called long-term potentiation (LTP). LTP is regarded as the best neurobiological model of learning and memory today. This paper first overviews the events and findings that led to the discovery of this phenomenon as a prelude to the development ofAMPAKINE technology. The second part describes the efficacy of this novel class of drugs on improving cognitive abilities in both impaired and non-impaired individuals. The quest for nootropia, or enhanced cognitive ability, has effectively begun.

Part I - Historical Basis for the Development of the Ampakines

Early Learning and Memory Theory

For more than a century, scientists have been interested in the relationship between neural connections and cognitive ability. Central to cognition is the process of information acquisition, storage, and reactivation. This is more commonly referred to as learning and memory. Learning and memory are inseparable constructs that allow us to change in response to our environment, store information, and in return, use this information to affect the environment. Imagine what your world would be like if you could not learn and remember - every individual would seem like a stranger, every task would seem novel, and every spoken word would be incomprehensible.

Between approximately 1917 and 1950, Karl Lashley searched for the "engram", the storage closet for memories in the mammalian brain. In one of his well-known articles, he articulates the "principle of equipotentiality" and the "principle of mass action". The former refers to the conclusion that all cortical areas can substitute for each other as far as learning is concerned. The latter refers to the idea that a reduction in learning is proportional to the amount of brain tissue destroyed[2]. These conclusions still shape how neuropsychologists and neurophysiologists view learning and memory processes today. Out of this grew the idea that memories may involve a large number of cells, or a network. In 1949, at McGill University, Donald Hebb, arguably the most famous neuroscientist of the 20th century, published his book titled *The Organization of Behaviour*. With the work of Lashley and others in mind, he presents his "Neurophysiological Postulate" as follows:

> *When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth processes or metabolic change takes place in one or both cells such that A's efficiency,as one of the cells firing B, is increased.*[3]

The prescient notion that memories may involve changes in the strength of the connections between brain cells, put forth by Hebb, has been supported by the discovery of a phenomenon called long-term potentiation (LTP). LTP is a physiological model of how synapses may be strengthened following intense electrical activity. Currently scientists at Cortex Pharmaceuticals Inc. are basing their drug development strategies on the theory that the synaptic strengthening observed during LTP is both biologically relevant and associated with cognitive capabilities.

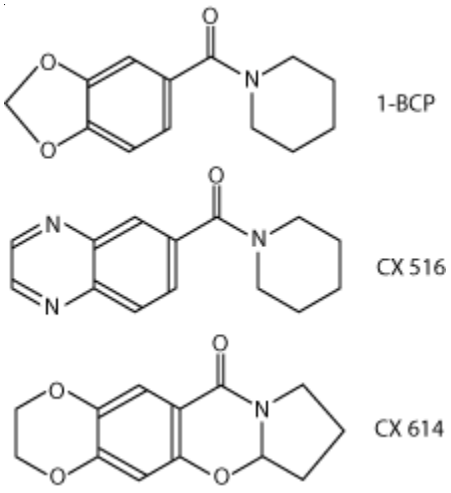

Figure 1. Ampakines seem to enhance memory by affecting the process of long-term potentiation.

The Discovery of Long-Term Potentiation

Prior tol973, when the discovery of LTP was fast published, neuroscientists searched for examples of lasting increases in synaptic transmission. In 1941, Feng showed that excitatory synapses are capable of releasing more neurotransmitter substance for a period of up to 300 ms following a transient injection of current into the presynaptic cell. He coined the term facilitation for his observation. If the presynaptic cell was repeatedly activated by injecting several pulses of current, the change in the voltage in the postsynaptic response was enhanced for several seconds. This is now referred to as augmentation[4,5,6,7]. In 1965, Spencer & Wigdor and Beswick & Conroy independently showed that high frequency activation (800 injections of current per second; 800 Hz) applied to the presynaptic cell in a simple spinal cord reflex pathway could lead to an enhanced elevation in the voltage of the postsynaptic cell lasting several minutes[8,9]. However, the implications of these results suffered tremendously from the fact that the high frequency stimulation of 800 Hz is far outside the range of electrical activity normally observed in this region[10]. This third process has been called post-tetanic potentiation (PTP). In facilitation (lasting for a maximum of 300ms), augmentation (lasting for a maximum of 40 seconds), and PTP (lasting for a maximum of 7 minutes), the postsynaptic response is enhanced.

However, in all of these instances, the enhancement persists for such a limited length of time that implicating any of these processes as a mechanism for memory would have clearly been absurd as memories can span almost a lifespan. In short, a physiological mechanism that exhibits change lasting for more than minutes had not been found prior to the discovery of LTP.

In Oslo in the late 1960s, a graduate student named Terje Lomo was studying the electrophysiology of the hippocampus. The hippocampus is a subcortical brain structure related to spatial learning and memory processes. At the same time, Tim Bliss was recruited from McGill University to work with Lomo in Oslo. In the autumn of 1968, Bliss and Lomo conducted their first experiment together. They transiently electrically stimulated the perforant pathway (a set of neurons leading into the hippocampus) and the postsynaptic potential was massively enhanced. There is no question that this reflects an increase in the strength of the synapse. Minutes passed, and the magnitude of the response fell, as expected of PTP. Amazingly, the response then leveled off well above baseline. Hours passed with increasing excitement as Bliss and Lomo watched the responses on their oscilloscope remain stubbornly elevated[11]. LTP (below) had been discovered and they published their results in 1973. LTP has subsequently been recorded in the live animal for up to half of its lifespan[12]. This

phenomenon is now formally defined as a persistent enhancement of an excitatory postsynaptic potential (EPSP) following brief high-frequency stimulation of afferent pathways[11].

In 1986, Morris and colleagues published results in Nature showing that blocking of the NMDA receptor not only blocks LTP, but it also attenuates learning and memory. The administration of the NMDA antagonist APV into the hippocampus (a structure involved in spatial learning and memory) was shown to block LTP. A task was then developed whereby a test animal has to learn to escape from a pool of murky water to a hidden platform based on spatial cues in the surrounding room. Animals that received APV, the drug that blocked LTP, could not learn this task as well as controls. On the last day of testing, the platform was removed and the time that the animal searched in each quadrant was recorded. The animals that had received APV did not search in the correct quadrant as much as controls supporting the conclusion that these animals could not learn and remember as well[13].

Those who believe that LTP is a valid model of learning and memory typically point to certain facts to support their claim. First, changes in the strength of synaptic connections are observed following learning. Second, altering LTP before a task alters the learning process itself. Third, altering LTP after learning affects the memory for that information.

Critics however correctly state that a fourth criterion must be met: artificially altering LTP must induce a false memory[14]. If LTP itself is not a memory per se, but rather a model of changes to a network, then induction of a false memory would not accompany conventional LTP induction. Currently, the technology to test this latter hypothesis is absent. LTP may not be learning and memory itself, but it does appear to be a good model.

Several thousands of articles have since been published about long-term potentiation and billions of dollars have been allocated to research effort aimed at understanding this phenomenon.

The Molecular Mechanism

Excitatory signals travel through the brain primarily via glutamatergic neurons, or neurons that release glutamate. Glutamate is released by the presynaptic cell and is capable of binding to and activating two major classes of postsynaptic receptors, AMPA and NMDA. When AMPA receptors are activated by glutamate, they open and allow sodium to rush into the cell causing a depolarization. In contrast, the NMDA receptor is activated after the postsynaptic cell is both depolarized and glutamate binding has occurred. The depolarization needed to activate the NMDA receptor is accomplished by activity at the AMPA receptor. Thus, the opening of the NMDA receptor is dependent upon the number and the sensitivity of the AMPA receptors. The activated NMDA receptor allows for calcium to flow into the cell. The elevation in the concentration of intracellular calcium triggers a series of biochemical pathways that lead to at least two important outcomes. First, AMPA receptors are converted from low affinity to high affinity glutamate receptors. Second, more AMPA receptors are inserted into the membrane.

Based on this information, the model for LTP is as follows: first, baseline levels of excitation from the presynaptic cell induce the release of a relatively small amount of glutamate molecules. AMPA receptors, and not NMDA receptors are activated in this situation and a small, transient change in the postsynaptic potential is observed. Then, when the presynaptic cell is excited to a greater degree, a larger amount of glutamate is released, and a larger postsynaptic potential is observed. This increase in depolarization due to the activity of the AMPA receptors initiates the involvement of the NMDA receptor and calcium can now enter the cell through the pore of the NMDA receptor. Calcium is normally very tightly regulated in the neuron and even slight changes can drastically change its behaviour. Biochemical pathways triggered by the rise in calcium concentration leads to both an increase in the gross number of AMPA receptors and the sensitivity of those receptors to glutamate. Initial low levels of electrical stimulation can now lead to a much larger postsynaptic response. In other words, the synapse has been potentiated[15].

Long-term potentiation is the leading biological model to explain how learning and memory works, and has been for over thirty years. Efforts to understand the molecular mechanism(s) that underlie LTP are based on the assumption that this knowledge

would lead to an explanation of memory storage. An additional motivation to study LTP is to establish a set of synaptic learning rules based on Hebbian notions of synaptic changes in the strength of multiple synapses. A third, and perhaps less emphasized rationale for this investigation, is to ultmately gain the ability to develop new classes of drugs that enhance memory[16].

The Epicenter of LTP

The molecular mechanism underlying LTP, as previously described, involves many components. In an attempt to discover the most important component in the induction and expression of LTP, the effects of manipulating individual components have been assessed. The first component that was hypothesized to be of paramount importance was the presynaptic cell's ability to release glutamate. When this was tested, it was found that manipulations that increased the probability of release do not influence LTP[17]. LTP, in contrast to facilitation, augmentation and PTP, is now considered primarily a postsynaptic phenomenon. It was next hypothesized that LTP may be due to an alteration in the general resistance of the postsynaptic cell. This hypothesis was also proven to be false[18]. Finally, focus shifted to the glutamate receptors located on the postsynaptic cell. Vanderklish *et al*. found that changes to the subunit composition of AMPA receptors changed the expression of LTP.[19] This is what one would expect if these proteins are in fact the agents of LTP expression. Further support for the pivotal role of the AMPA receptor came from two important pieces of evidence. First, LTP is accompanied by changes in the waveform of synaptic responses which are largely mediated by AMPA receptors[20,21]. This finding has been replicated by two independent groups[22,23]. Second, LTP is paralleled by alterations in the pharmacology of AMPA receptors[24,25,26]. The above results suggest that the epicentre for LTP is the AMPA receptor. The above series of studies have culminated into a foundation for the development of the ampakines by Cortex Pharmaceuticals.

Part II - Ampakines as a Family ofNootropics

Nootropics

A nootropic is both a memory enhancing drug and a Holy Grail for many neuroscientists. Almost all major neurological disorders seem to involve, at least in part, a dysfunction in the realm of fast excitatory neurotransmission. Cortex Pharmaceuticals, Inc. is a company that has generated over 700 compounds to date that are aimed at improving learning and memory deficits in Alzheimer's disease, mild cognitive impairment, depression, schizophrenia, attention deficit disorder, Parkinson's disease, and spinal cord injuries, to name but a few[1]. The current therapies for these disorders are considered inadequate by all health professionals. The foundation for this company is the ampakine technology developed by Dr. Gary Lynch at the University of California at Irvine. The chief executive officer for Cortex Pharmaceuticals claims that the market for these drugs was worth approximately 40 billion dollars worldwide in 2001, and has been, and continues to be growing at a rate of 17% per year. He emphasizes that this percentage will only grow as the "Baby Boom" generation enters their later years. In short, ampakines have the potential to be a multi-billion dollar blockbuster[1].

The Cellular Effects of Ampakines

It was originally hypothesized by Dr. Gary Lynch that if the size and duration of the postsynaptic response is increased via modulation of the AMPA receptor, then calcium influx through the NMDA receptor would be promoted[16]. At this point, it had been established that AMPA receptors play the major role in allowing the postsynaptic response to reach the threshold necessary to activate the NMDA receptor. With Hebb's theory, the mechanism for LTP, and data on the critical role of AMPA receptors to LTP in mind, over 100 drugs were isolated that possess the ability to increase the current through AMPA receptors, enhance LTP, and freely cross the blood-brain- barrier[16]. CX 516 has been the most heavily investigated of the ampakines. Both desensitization and deactivation are inhibited by CX 516 indicating that this drug allows more sodium ions into the cell by keeping the pore open for a longer

been shown to facilitate eye-blink and fear conditioning, two classic examples of long-term memory[36,37]. In a two odor discrimination task, Larson *et al*.[34] found that animals administered an ampakine require fewer trials to reach criterion. Interestingly, these animals were previously well trained on this learning problem suggesting that ampakines can improve learning above and beyond the natural ceiling in cognitively healthy individuals.

Although the above behavioural studies are convincing, the ultimate goal of these drugs is to improve memory in humans. Humans commonly think of memory, not as an ability to be conditioned but rather, as an ability to remember complex and explicit events. To address this issue, a relatively complex behavioural task called the Delayed-Non-Matching-to-Sample (DNMS) task was employed. All animals were trained on this task until asymptotic levels of performance were obtained. Half of the animals then received an ampakine every other day, just prior to testing. The performance of these animals increased substantially over the subsequent two weeks. In addition, this enhancement persisted for seven days after cessation of the drug. Behavioural improvements were paralleled by reductions in neural activity associated with errors on this task[38,39]. The above experiment was repeated with aged animals that normally show impairments on this task. Ampakines improved memory performance to levels comparable to those seen in young control rats[16].

Ampakines for Humans

To date, a limited number of clinical trials with ampakines have been conducted. The most significant results were obtained in a study of delayed recall for nonsense syllables in 65 to 75 year-old subjects. The highest dose led to more than a two-fold improvement in performance[40]. The second study tested lower doses of the drug on younger individuals. Improvements in the retention of complex visual associations following a 24 hour delay, recognition of odors after a 45 minute delay, and improvements on a visuospatial maze across days were observed in the groups that received ampakines. Trends towards improvement were also obtained in several other period of time. This will make the postsynaptic cell reach threshold for the NMDA receptor more quickly, and more often than normal. The short pulse used is reflective of the transient pulses commonly seen in both cortical and sub-cortical regions of the brain[27,28,29].

Importantly, ampakines such as CX 516 can influence whole networks of synaptic connections and can do so at relatively low doses[30,28]. It is thus possible, that these drugs may influence longer chains of synaptic transmission before they impact simpler, but no less important, reflex-like circuits[16]. When investigators administered CX 516 to freely moving animals equipped with implanted electrodes, excitatory synaptic responses were again prolonged and LTP was again enhanced[31,32]. To assess the effects that ampakines have on more complex circuits, the expression pattern of immediate early genes across multiple brain regions was evaluated in a subsequent study. The expression of immediate early genes is an indicator of neuronal activity. Using this technique, it was shown that ampakines cause a shift in activity away from sub-cortical regions such as the hippocampus to cortical regions of the brain[33].

The Behavioural Effects of Ampakines

Seizure activity is thought to result from too much excitation, or perhaps more accurately, too little inhibition. Therefore, it is not surprising that high doses of ampakines induce seizures in rats. Lower doses however have appeared to have more subtle effects on behaviour. Behavioural tests of response latency, attention, motivation, and fine motor control produced null results[34]. In contrast, when animals were tested on memory tasks, improvements have been observed. The initial study employed a within-subject design in which rats were given drug or vehicle, allowed to collect rewards on a radial arm maze, and then tested several hours later. Retention of the spatial location of the rewards was significantly better in rats that had received CX 516 (also known as BDP-20) [31,32]. This study has been replicated and a similar task using olfactory instead of spatial cues has produced comparable results[32]. In addition, more potent ampakines appeared to have larger behavioural effects[35] supporting the relationship between AMPA receptors, LTP, and memory. Ampakines have also

domains related to memory[41]. The intent of the aforementioned studies was not to assess the efficacy of ampakines on memory, but rather to simply test the safety of ampakines in humans. Approval for further testing will use higher doses that are more comparable to those that improved memory in rats and more subjects will be employed[16].

Development of the ampakine drugs has resulted from decades of pure research into the neurobio logical underpinnings of learning and memory. It is an example of how biotechnology can utilize research aimed at simply understanding the dynamic nature of the human body to develop novel therapeutic interventions for disorders that plague millions of individuals in our society.

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