American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

A Quantitative Analysis for the Expression of AMP-Glu R2 Coding Gene in BALB/C Male Mice during Modafinil Administration

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Received: 2025, 15, Dec
Accepted: 2026, 10, Jan
Published: 2026, 16, Feb

Abstract: Nootropics often known as smart medicines, brain boosters, neuro-stimulants, behavioral stimulators, and intellect enhancers, are medications, nutrients, nutraceuticals, and food supplements that supposedly boost brain abilities like intellect, memory, intelligence, enthusiasm, focus, and concentration. The modafinil, including nootropics, is able to achieve amnesia restoration caused by, for example, scopolamine, electroconvulsive stimulation and hypotension. It was shown that modafinil modifies cognitive and behavioral processes and affects many brain proteins. Nevertheless, results were not identified at the stage of compact protein complex. In the nucleus accumbens, a critical part of brain's reward systems, smart medicines including modafinil may modify the activity of AMPA-type glutamate receptor subunits. The effect of such a control on the general condition of inspiration is unknownIn the present study various concentrations of modafinil is administered to BALB/c male mice to check the expression levels of AMPA – Glu R2 sub units. Molecular and immunological approaches are utilized to check the final results of this study.

Keywords: AMPAR-GluR2 expression, Modafinil, RNA isolation, RT PCR, Agarose gel electrophoresis, ELISA

Introduction

The brain is a complex system for the body. The mind's capacity to retain and remember information (consciousness) is indeed one of the major purposes [1-2] That role is in effect regulated by the interaction of many chemicals, proteins, co-factors and nutritional throughout the brain and the central nervous system being formed, released, secreted, and activated.[3]

Nootropics is best characterized mainly by modifying the existence of molecules that regulate the transport of ions in the mind by the mind's production. These substances may be hormones, proteins, or neurons like acetylcholine, GABA and dopamine, etc.,[4] Nootropics, often known as smart medicines, been with us 1000's of years with behavioral or memory stimulants.[5]

The Chinese, who utilized extracts like ginseng, ginkgo biloba, etc., along with its various herbal tea formulations, could be attributed to the initial usage of smart medicines to boost brain power and functioning. Firstly, examples of the utilization of Nootropics via man may be shown to be these medicines that created a state of consciousness, increased libido and provided the capacity to work excessive hours [6] Although since, in the quest to create more evolved civilization activity, different drugs were used to enhance those processes regulated by the brain. The recent history underlying intelligent medicines could be traced back to the groundbreaking study of Dr. Corneliu E. Giurgea, a Romanian psychologist and chemist who in 1964 synthesized Piracetam, the very first current nootropic drug .[7] He took a bunch of groundbreaking studies on such medications and woke back with how a nootropic medication should be with his hypotheses. He suggested that there should be the preceding features of a nootropic drug: They have ability to increase learning and memory, resist to learn behaviors which can able to resist them, save the brain towards numerous physical or chemical infections and also enhance effectiveness of tonic cortical.[8]

Attributed to the reason that several chemicals regulate the function of the mental areas of the brain, various smart medicine groups can have various impacts. As well as a whole bunch of many other substances are among the compounds that facilitate cognitive function. Smart class of drugs that enhance memory and concentration. [9]

Hormones, proteins and different chemicals called neurotransmitters strongly attenuate the function of the central nervous system. By influencing and modifying the development, dissemination, operation and degradation of these neurotransmission, smart drugs exercise their behavior [10].

The numerous neurotransmitters like, Acetylcholine, Gamma-Amino Butyric Acid, Dopamine and so on. Depending on the behavior on these neurotransmission described above, a category of smart medicines can be performed.[11]. There are many other medications that perform performance enhancing acts that cannot be solely categorized as smart medicines, it must be said. Cholinergics ease the development of memory. Dopaminergics improve alertness, concentration and anti-oxidant behavior. [12]

Memory enhancements are created by an improvement in Cyclic Adenosine Mono Phosphate levels. It is also apparent that by raising the amount of such neurochemicals in the mind, smart medicines function. Nerve signals are blocked by neurotransmitters including gamma-amino butyric acid .[13-14]

Memory is affected adversely by anxiety and burnout. Smart medicines that act as anti-depressants, tension muscle relaxer and GABA inhibitors can also boost memory. In our study, we have identified the expression of AMP-Glu R2 coding gene in BALB/C male mice while the administration of modafinil at diverse concentrations [15-16].

Materials and methods

Animals and drug administration

BALB/c male mice was chosen for our study. In selected mouse, Modafinil is soluble in 5 percent dimethyl sulfoxide and afterwards dissolved in distilled H_2O and 5 percent Cremophor EL until subcutaneous injection. In this analysis, three separate quantities containing various concentrations of modafinil were carried out. Animals were observed for the expression of AMP-Glu R2 gene at different concentrations.

Isolation of RNA

The RNA was extracted from the mice brain tissues. A 100 mg of the brain tissues were used and it was frozen with 1x SDS-buffer. One ml Trizol reagent was added to the tissue sample, incubate at room temperature for 5 mins and immediately spin the tissue for 5 mins at 10000 rpm. Further add 200 µl chloroform/1 ml Trizol followed by shaking and kept incubation for 5 mins. Repeat these steps 5 to 7 and add 0.7 volume of isopropanol followed by centrifugation, and then re-suspended in 11 µl of 10 mM Tris at a pH of 7.5 [17, 18].

Reverse transcriptase polymerase chain reaction

The isolated RNA samples were taken for RT PCR study. The primers were designed and the reaction mixture of 0.5 µL of guanidine thiocyanate buffer was utilized for the lysing process. Further, 2 M sodium acetate, 0.5 mL of phenol and a ratio of 49:1 chloroform and isoamyl alcohol were added. The cDNA synthesis process was executed at final 30 µL solution. Taq DNA polymerase was used for denaturation and the reaction carried out at 96°C for 15 sec, annealing at 55°C for 30 sec and the extension process at 72°C for 3 mins. A “hot start” reaction was performed by heating the mixture to 80°C before the addition of polymerase and eventually the cycle was carried out for 35–40 times. Eventually, real-time PCR products were analyzed through 2% agarose gels followed 1× TBE staining of ethidium bromide and subsequently the process was visualized under UV light [19, 20].

Agarose gel electrophoresis

The movement of DNA has been observed using agarose gel electrophoresis techniques. The step-by-step protocol is given below:

Agarose gel of 1.2% in TBE buffer were taken through dissolving in a 0.24 gm agarose in a 20 ml TBE buffer. Let the agarose mixture to by using heating in a microwave. Once the agarose is completely dissolved, add a 20 µl concentration of ethidium bromide at 55°C for staining a buffer. Allow the gel to cool down at incubation period of 65°C H_2O bath. Place the gel in a casting apparatus and place a comb into the gel mold to form the wells. Load various samples in a diverse wells and then switch on the power supply and further the electrophoresis was carried out at 100 Volts. Once it’s done, switch off current when the dye tracking started moving to the ¾ of the gel. Eventually, the gel was removed for visualization [21, 22].

Enzyme Linked Immuno-Sorbent Assay

The U-bottomed ELISA wells were occupied with 100 µl of 0.1 M of sodium carbonate and bicarbonate buffer at a pH of 9.8. The mixture was incubated for 2 hour at room temperature and kept at an overnight period of 4°C, further the wells were wash away 3 times with a PBS buffer of 0.15 M PBS-TWEEN at a pH of 7.4. Further, 3 g of Bovine serum albumin in 100ml of ELISA coat buffer was added. After the incubation period of 10 mins at a room temperature, wash the wells with PBS-TWEEN buffer. Then add the 300µl of sodium chloride solution was added to the reaction mixture and incubated a plates for 10 mins at RT in the dark area. Eventually, the absorbance was measured at 450 nm by employing an ELISA reader. Each tissue samples were assayed in triplicate and further the mean absorbance values were evaluated [23-25].

Results and discussion

For this analysis, BALB/c male mouse were acquired from approved animal breeders. The mouse was housed in a 12 dark or light period 22°C light and T controlled room and had been given free access to experimental chow and drinking water. The standards given by the committee for the control and monitoring of animal studies were implemented quite narrowly in the whole process.

PCR study

PCR requirements were configured as per the specification above. PCR is done in 3 separate cycles in which the 1^{st} and 3^{rd} cycles are provided as additional in this unique project as the second phase is replicated for 30-35 by employing Thermo-cycler (Figure 1).

The PCR conducted throughout this study focuses on the RNA models in particular. However the first objective is to produce the cDNA from either the RNA template by employing reverse transcriptase enzymes throughout the PCR but instead subsequently the cDNA that is being obtained as a product following reverse transcriptase PCR would then be employed as a template in thermocycler and afterwards replicated into numerous copies utilizing regular standardized PCR procedures as described above.

Figure 1. Thermo-cycler is utilized for executing RNA reverse transcriptase

Afterwards reverse transcriptase PCR, successful amplification of substances and also an amplified RT PCR product are preserved at -20°C before the process of agarose electrophoresis is carried out.

Agarose Gel Electrophoresis study

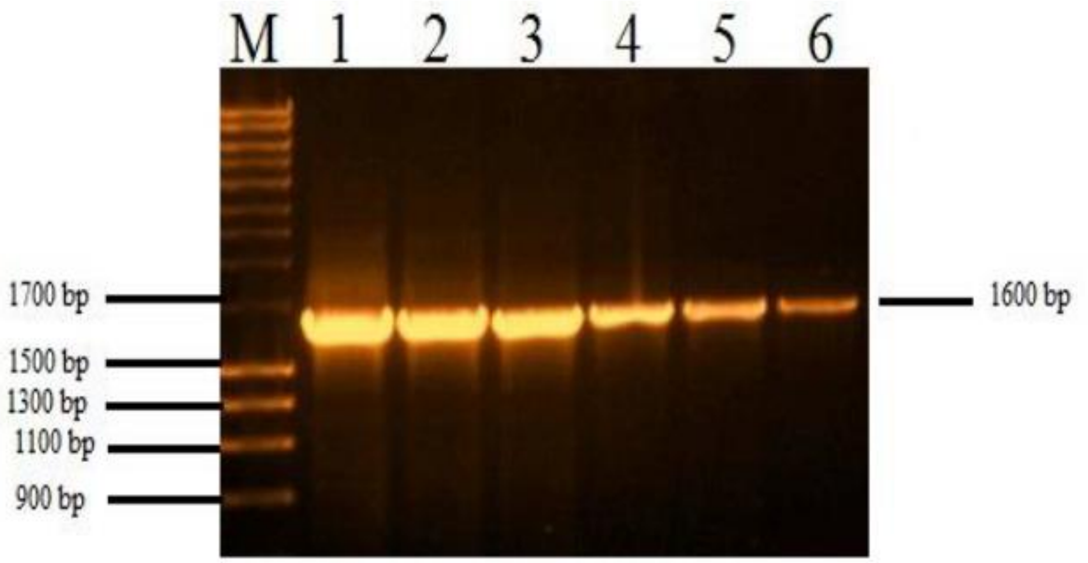


Figure 2. A gel post electrophoresis picture. The agarose gel was showing to Ultra Violet light and this image was taken by a gel documentation method

ELISA analysis

Visual color change observed after performing quantitative ELISA technique as exemplified in Figure 3.

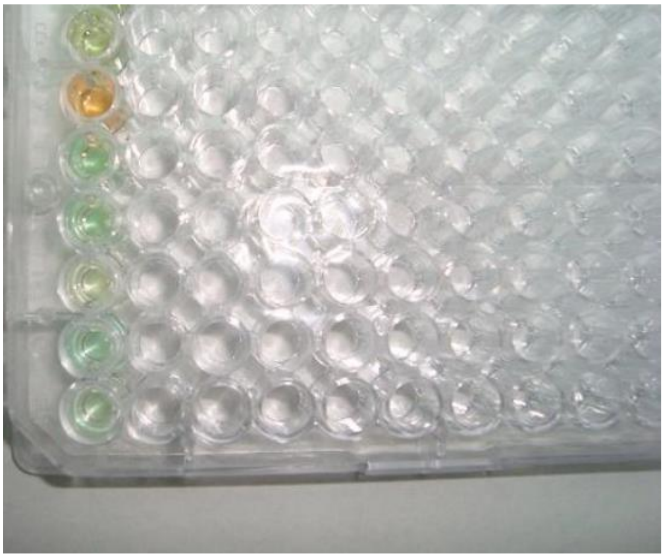


Figure 3. A color change was observed by employing ELISA method

All the results are recorded and respective concentrations are calculated using respective formulations and final results are obtained as mentioned in the below Table 1.

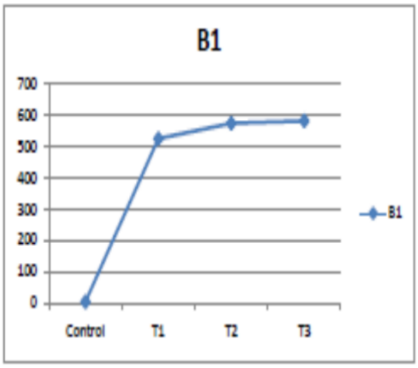
Table 1. Various concentrations of drugs were calculated using ELISA technique

	Control (ng/ml)	T1 (ng/ml)	T2 (ng/ml)	T3 (ng/ml)
B1	4	525	574	582
B2	6	615	563	642
Average	5	570	568.5	612

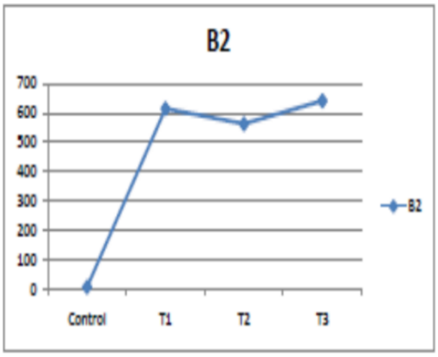
Modafinil is a nootropic that increases alertness and energy. Modafinil is utilized to combat persistent drowsiness triggered by sleep apnea, epilepsy, or rotating shifts sleep disease. Modafinil is often employed alongside breathing systems or other medications to avoid prolonged sleep deprivation accompanied by disruptive sleep apnea or hypopnea disorder.

Modafinil is now in a group of drugs known wakefulness enhancing substances. It operates by adjusting the quantities of many organic products in the region of the brain that regulates sleeping and wakefulness. From the Table 1, where, T1 is 30 µg/ml of Modafinil, T2 is 20 µg/ml of Modafinil and T3 is 10 µg/ml of Modafinil.

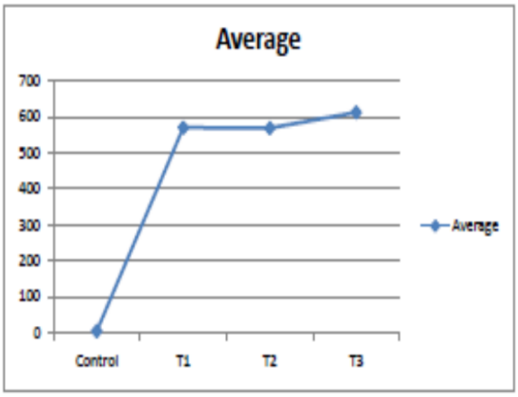
B1 and B2 are the batches which are used for the protein expression analysis. Average of these two batches are taken the highest amount of protein expression was observed in T3 which is the protein extract from the mice administered with 10 µg/ml Modafinil.



(a)



(b)



(c)

Figure 4. A graph of B1, B2 and average batches utilized for protein expression analysis

AMPA-Receptor is a protein for the neurotransmitter Glutamate. AMPAR is connected to neural plasticity throughout the specific form of Longer Term Potentiation‖ Ampakines were mediators of AMPA proteins, they intensify the impact of the initiator on the protein. Modafinil boosts the rates of histamine in the brainstem and enhances the action of dopamine as well. Orexin neurons are activated by Modafinil. Orexins were neuropeptide excitability hormones which enhance wakefulness. Orexin deficiency is the source of narcolepsy.

In the above Figure 4 (a), the highest amount of the protein expression is seen in T3 which has 10 µg/ml Modafinil and has shown the reading of 582 ng/ml. Glutamate Receptor 1 (AMPA subtype) antibody catalog number ab31232 is taken against the antigen present in the plasma which is AMPA R – Glu2 subunit. It is artificial peptides which is resulting from the C-terminus residues of 850 of the human. AMPA-type glutamate receptors (AMPARs) plays a role in memory formation. In this Figure 4 (b), the batch 2 is taken. In this the highest levels are observed in T3 which is from the mice sample which is administered with 10 µg/ml Modafinil. The lowest level is observed in control which is from the sample of the mice which is not administered with Modafinil and used as a control.

Glutamate, the predominant excitability neurotransmitter in the optic nerve, acts mainly via AMPA proteins. GluR2-immunoreactive puncta was dispersed in the grey material. Glutamate proteins activate excitatory signal transduction in the CNS but are essential with most neurotransmitter systems, like synaptic plasticity, neuronal growth, and many neuropsychological diseases. From the Figure 4 (c), the average of the batch one and batch 2 of the protein expression is taken the highest and the lowest were recorded in T3 with the reading of 612 ng/ml and control with 5 ng/ml.

Discussion

Out of all the life forms present on the planet earth only human being procures a specific ability of using his brain more than ten percent which in turn helps him to utilize all his mental abilities for his better survival which gave fruit of civilization and which gave the ability of cognitive intelligence. a variety of drugs are being hypothesized and brought into the market which are known as nootropics also known as smart drugs which increase the ability of the person to acquire far more perfection especially cognitive intelligence or creative intelligence in the present days. One such drug modafinil has attracted the present generation attention in its ability to increase various types of abilities which are concerned with brain especially those abilities involve cognitive intelligence, attention, decrease in paradoxical sleep. Various studies are done on the various effects of the brain and as well as its side effects which can be dangerous if not monitored or not been researched. There is a threat of losing a generations of health if we are not checking the side effects on the brain as well as its side effects which can be dangerous if the modafinil side effects which is used by any person by any subject or patient. The levels of AMPA- Glur2 are studied in this project. the main part of the project concentrates on the levels of the protein expressed rather than the molecular element of the gene example mRNAs because there is a strong proof that even though the mRNA is expressed there are so many post transcriptional parameters which can hider/alter the translational kinetics of this protein. In this project the concentration is laid on the particular product (protein) which comes from the transcribed gene so Bal/B - c mice is selected for this study and is grown under CPCSEA guidelines. Modafinil is administered in to the mice in three different batches. Certain observations are chosen to account for further study and the mice are dissected and the brain is taken and brain samples are collected as mentioned in the protocol. Further RNA isolations are being performed to identify whether the gene is transcribed or not. The molecular assay performed in this study is only qualitative and not quantitative as the main agenda of the molecular study is to see whether the particular transcription of the particular gene is been transcribed in vivo in mice or not. Then the quantitative study is performed using the antibody against AMPA r-GluR2 and quantitative ELISA in performed to know the quantity of the protein expressed.

Conclusion

The combinational analysis using immunological and molecular studies has given very perfect results which can easily prove that the administering modafinil has increased the expression of AMPA R-GluR 2 receptor which can be easily identified by the antibody assay from the proteins isolated from brain samples. On the other side of the story where the molecular assay such as agarose electrophoresis with the DNA samples which have been amplified can clearly mention that the transcription the particular gene is evident for all this hyper activity which is caused by taking modafinil by various subjects reported worldwide. Moreover studies were necessary to endorse an exact activity of this particular receptor when modafinil administration is taken.

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