Supplementary materials

Amphora Coffeaeformis Algal

Acetone Algal Extract

Algal biomass was homogenized in acetone solution (95%) and then kept overnight at 4 ºC in the dark [1]. The bleached algal cells were obtained through several repetitions of the extraction steps. Consequently, the extract was centrifuged at 10,000 rpm for 5 min and then filtered. Next, algal extracts were filtered and then dried entirely by the evaporation under vacuum at 40 ºC [2].

Determination of Fatty Acids

Methylation of fatty acids: The fatty acids were converted to methyl esters using methyl alcohol solution. Specifically, methyl alcohol solution was prepared using a 3% sulfuric acid in methanol (a 3 mL of H_2SO_4 was added to 97 mL methanol). The obtained oil extract was reflexed at 90 ºC for 3 hours and re-extracted with *n*-hexane. Besides, the extract was then kept overnight with sodium sulfate and then concentrated by a rotary evaporator before the injection into Gas Chromatography (GC). Perkin Elmer Auto System XL GC equipped with flame ionization detector (FID), fused silica capillary column DB-5 (60 x 0.32 mm) was used to measure unsaturated and saturated fatty acids. Particularly, oven temperature in GC was initially maintained at 150 ºC and then programmed at a ramping rate of 3 ºC min^{-1} from 150 to 240 ºC. The GC was preconditioned and maintained at 230 ºC injector temperature, 250 ºC detector temperature, and a flow rate of helium (carrier gas) at 1 mL min^{-1}.

Neurobehavioral Assessment:

Barnes maze (BM)

Apparatus & settings: A circular black colored wooden arena about 150 cm in diameter and 19 holes (each is 10 cm in diameter) was used. The arena was set 50 cm high from the floor with a source of light directly centered above it. The holes were secured by false bottoms to avoid the escape and falling of animals out of the maze. The wooden escape cage is (25* 19 in diameter) was placed at the same place all over the experiment. Black cues (2 black cues 50 cm length and 5 cm in diameter) were placed at the same place, all over the experiment, on the wall directly next to the maze. The experimenter chair was set about 122 cm from the nearest rim of the maze. Cardboard with the number of each rat and its group

was used to allow the later offline analysis of the recorded videos. The arena and the box were cleaned with 70% ethanol to avoid odor cues.

Experiment: the experiment was done throughout 4 days: initial training day (to allow the animal to acclimate to the arena), 2 training days (to allow the animal to learn the site of the escape box) and a probe day (to test the reference memory and learning). The animals were placed in the examination room in their home cages 1 hour before the experiment. At the beginning of each trial, the direct light above the maze is turned on. Next, white noise is turned on to mask the surrounding sounds, and a digital camera is used to record the experiment. The time of the beginning of the procedures was recorded manually by the experimenter, and a stopwatch was used to count down the time allowed for the rat. Animals were allowed to explore the arena for 5 minutes on each training day. The rats were placed at the center of the arena and covered with a container (20 cm in diameter, 30 cm in height). At the first training trial only, the container was transparent to allow the animal to see the arena. After that, a dark container was used throughout the experiment. After 15 seconds, the container was removed, and the animals were allowable to explore the arena freely. If the rat did not find the escape box, it was guided by the experimenter. Animals were left in the escape box for 2 minutes to acclimate. The training was repeated 2 times in each training day with at least 15 minutes apart between the trials. In the probe day (the fourth day of the experiment), the same procedures were repeated. However, the escape box is closed by a false bottom and the time taken by the animals to reach the site of the box was recorded by the experimenter.

Analysis of the results: offline analysis was done through the recorded videos. The time elapsed to reach the box (latency), the number of errors (nose dipping in wrong holes), and the number of quadrants visited by the animals were analyzed [3].

Open Field Maze (OFM)

This test was used to assess anxiety-like behavior and motor activity of animals.

Apparatus & settings: A white cubic arena made of nonporous plastic material was used. Its dimensions were 100*100 cm in length for the floor, and the walls were 35 cm in height. The floor of the arena was divided into even grids using an oil-based marker pen; each is 5*5 cm in diameter. The central area was marked in red color (25*25 cm in diameter).

Experiment: The test protocol was according to the guidelines of [4]**.** The animals were brought to the test room one hour before the beginning of the test to acclimate. The room was lit with an upright even

light to avoid the escape of the animal to the unlit portion of the arena. The animals were placed in the center of the arena and allowed for free movement for 10 minutes. The movement and behavior of each animal across the arena were videos recorded by a digital camera for later offline analysis. The experimenter recorded the number and group of tested subjects manually. The subjects were returned to their home cages at the end of the test, and then the fecal pellets were counted. The arena was cleaned after each test trial.

Analysis of the results: The parameters used to assess the animal performance in OFM were: number of lines crossed by the animal, visits of the center zone, grooming, rearing behavior, number of fecal pellets, and passage of urine [5]**.**

Target Gene Expression by Real Time PCR:

Table (SI) thermal cycling protocol for StepOne™ Real-Time PCR System

Step	**Temperature**	**Time**	**Number of cycles**
Initial denaturation	95°C	10 min	1
Denaturation	95°C	15 S	40
Annealing	56°C	30 S	
Extension	72°C	30 S	

*It was followed by a melting curve analysis to exclude primer dimer

❖ **Gene Set Enrichment Analysis according to String database:**

Table (SII): Enrichment of molecular function of four target gene products:

Go term ID	Molecular function	observed gene count	matching proteins in your network (labels)
GO:0005102	signaling receptor binding	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0008066	glutamate receptor activity	2	Grin2b,Grm5
GO:1990782	protein tyrosine kinase binding	2	Grm5,Ntrk2
GO:0004888	transmembrane signaling receptor activity	3	Grin2b,Grm5,Ntrk2
GO:0005126	cytokine receptor binding	2	Bdnf,Grin2b
GO:0001664	G protein-coupled receptor binding	2	Grin2b,Grm5
GO:0046983	protein dimerization activity	2	Grin2b,Ntrk2

Table (SIII): Enrichment of cellular component of four target gene products

GO term ID	Cellular component	observed gene count	matching proteins in your network (labels)
GO:0098793	presynapse	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0098794	postsynapse	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0030425	dendrite	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0043679	axon terminus	3	Bdnf,Grin2b,Ntrk2
GO:0043197	dendritic spine	3	Grin2b,Grm5,Ntrk2
GO:0014069	postsynaptic density	3	Grin2b,Grm5,Ntrk2
GO:0043025	neuronal cell body	3	Bdnf,Grin2b,Ntrk2
GO:0043195	terminal bouton	2	Bdnf,Grin2b
GO:0042734	presynaptic membrane	2	Grin2b,Grm5
GO:0005887	integral component of plasma membrane	3	Grin2b,Grm5,Ntrk2
GO:0043204	perikaryon	2	Bdnf,Ntrk2
GO:0044425	membrane part	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0044422	organelle part	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0045211	postsynaptic membrane	2	Grin2b,Grm5
GO:0009986	cell surface	2	Grin2b,Ntrk2
GO:0005622	intracellular	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0031410	cytoplasmic vesicle	2	Bdnf,Ntrk2
GO:0005576	extracellular region	2	Bdnf,Grin2b
GO:0044444	cytoplasmic part	3	Bdnf,Grin2b,Ntrk2

Table (SIV): Enrichment of biological process of four target gene products:

Go term ID	Biological process	observed gene count	matching proteins in your network (labels)
GO:0048169	regulation of long-term neuronal synaptic plasticity	3	Bdnf,Grin2b,Grm5
GO:0050804	modulation of chemical synaptic transmission	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0007612	learning	3	Bdnf,Grin2b,Ntrk2
GO:0001932	regulation of protein phosphorylation	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0032270	positive regulation of cellular protein metabolic process	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0048170	positive regulation of long-term neuronal synaptic plasticity	2	Bdnf,Grm5
GO:2000324	positive regulation of glucocorticoid receptor signaling pathway	2	Bdnf,Ntrk2
GO:0007166	cell surface receptor signaling pathway	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0009612	response to mechanical stimulus	3	Bdnf,Grin2b,Ntrk2
GO:0010647	positive regulation of cell communication	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0023056	positive regulation of signaling	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0010996	response to auditory stimulus	2	Bdnf,Ntrk2
GO:0007268	chemical synaptic transmission	3	Bdnf,Grin2b,Grm5
GO:0007399	nervous system development	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0007616	long-term memory	2	Grin2b,Ntrk2
GO:0009966	regulation of signal transduction	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0050769	positive regulation of neurogenesis	3	Bdnf,Grm5,Ntrk2
GO:0071363	cellular response to growth factor stimulus	3	Bdnf,Grin2b,Ntrk2
GO:0043408	regulation of MAPK cascade	3	Grin2b,Grm5,Ntrk2
GO:0065009	regulation of molecular function	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:0007215	glutamate receptor signaling pathway	2	Grin2b,Grm5
GO:0034097	response to cytokine	3	Bdnf,Grin2b,Ntrk2
GO:0001934	positive regulation of protein phosphorylation	3	Bdnf,Grm5,Ntrk2
GO:0051966	regulation of synaptic transmission, glutamatergic	2	Grm5,Ntrk2
GO:0006952	defense response	3	Bdnf,Grin2b,Ntrk2
GO:0021987	cerebral cortex development	2	Grin2b,Ntrk2
GO:0051602	response to electrical stimulus	2	Bdnf,Grin2b
GO:0065008	regulation of biological quality	4	Bdnf,Grin2b,Grm5,Ntrk2
GO:1990090	cellular response to nerve growth factor stimulus	2	Bdnf,Ntrk2
GO:0016310	phosphorylation	3	Bdnf,Grm5,Ntrk2
GO:0060079	excitatory postsynaptic potential	2	Bdnf,Grin2b
GO:0009967	positive regulation of signal transduction	3	Bdnf,Grm5,Ntrk2
GO:0043524	negative regulation of neuron apoptotic process	2	Bdnf,Ntrk2
GO:0050770	regulation of axonogenesis	2	Bdnf,Ntrk2
GO:0072347	response to anesthetic	2	Bdnf,Grin2b
GO:0044093	positive regulation of molecular function	3	Bdnf,Grin2b,Grm5
GO:0071356	cellular response to tumor necrosis factor	2	Bdnf,Ntrk2

GO:0071495	cellular response to endogenous stimulus	3	Bdnf,Grin2b,Ntrk2
GO:0050806	positive regulation of synaptic transmission	2	Grin2b,Ntrk2
GO:0043200	response to amino acid	2	Bdnf,Grin2b
GO:0010941	regulation of cell death	3	Bdnf,Grin2b,Ntrk2
GO:0009416	response to light stimulus	2	Bdnf,Ntrk2
GO:0050790	regulation of catalytic activity	3	Grin2b,Grm5,Ntrk2
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	2	Bdnf,Ntrk2
GO:0007204	positive regulation of cytosolic calcium ion concentration	2	Grin2b,Grm5
GO:0043410	positive regulation of MAPK cascade	2	Grm5,Ntrk2
GO:0006954	inflammatory response	2	Bdnf,Ntrk2
GO:0001666	response to hypoxia	2	Bdnf,Grin2b
GO:0010468	regulation of gene expression	3	Bdnf,Grm5,Ntrk2
GO:0007568	aging	2	Bdnf,Ntrk2
GO:0010038	response to metal ion	2	Bdnf,Grin2b
GO:0048666	neuron development	2	Bdnf,Ntrk2
GO:0071407	cellular response to organic cyclic compound	2	Bdnf,Grin2b
GO:0071417	cellular response to organonitrogen compound	2	Bdnf,Grin2b
GO:0006468	protein phosphorylation	2	Grm5,Ntrk2
GO:0048523	negative regulation of cellular process	3	Bdnf,Grm5,Ntrk2
GO:1903530	regulation of secretion by cell	2	Grin2b,Ntrk2
GO:0031667	response to nutrient levels	2	Bdnf,Grin2b
GO:0051336	regulation of hydrolase activity	2	Grin2b,Ntrk2
GO:0043085	positive regulation of catalytic activity	2	Grin2b,Grm5
GO:1901564	organonitrogen compound metabolic process	3	Bdnf,Grm5,Ntrk2
GO:1901701	cellular response to oxygen-containing compound	2	Bdnf,Grin2b
GO:0035556	intracellular signal transduction	2	Grin2b,Grm5
GO:0033993	response to lipid	2	Bdnf,Grin2b
GO:0009725	response to hormone	2	Bdnf,Grin2b
GO:0044238	primary metabolic process	3	Bdnf,Grm5,Ntrk2
GO:0006355	regulation of transcription, DNA-templated	2	Bdnf,Grm5
GO:0051252	regulation of RNA metabolic process	2	Bdnf,Grm5

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