Taken from publication

METABOLOMICS

Mouse hippocampal tissues were collected for untargeted RP-UPLC-FTMS metabolomics analysis. Tissue samples were kept in dry ice prior to storage at -70/80 0C. Metabolomics were completed by TMIC (The Metabolomics Innovation Centre).

*Metabolite Extraction*: Each mouse hippocampal sample in an Eppendorf tube was mixed with water, 5 μL per mg of the tissue, and two 4-mm metal balls were added. The tissue was homogenized on a MM 400 mill mixer at a vibrating frequency of 30 Hz for 1 min twice. After 5-s spin-down, a mixture of methanol-chloroform (4:1) was added, at 25 μL per mg tissue, to each tube. The sample was homogenized again for metabolite extraction using the same setup for 1 min twice, followed by sonication in an ice-water bath for 5 min. The tube was centrifuged at 15,000 rpm and at 10 0C for 20 min. The clear supernatant was transferred to a 1.5-mL Eppendorf tube. A 60-μL aliquot from each sample was dried down inside the same nitrogen evaporator and the residue was reconstituted in 40 μL of 80% methanol. 10 μL was injected for reversed-phase (RP)-UPLC-FTMS. Two rounds of sample injections were made, with positive- and negative-ion detection, respectively.

*RP-UPLC-FTMS Analysis*: A Dionex Ultimate 3000 UHPLC system coupled to a Thermo LTQ-Orbitrap Velos Pro mass spectrometer, equipped with an electrospray ionization (ESI) source, was used. Reversed-phase (RP)-UPLC-FTMS runs was carried out with a Waters BEH C8 (2.1 x 50 mm, 1.7 μm) column for chromatographic separations. The mobile phase was (A) 0.01% formic acid in water and (B) 0.01% formic acid in acetonitrile-isopropanol (1:1). The elution gradient was 5% to 50% B in 5 min; 50% to 100% B in 15 min and 100% B for 2 min before column equilibration for 4 min between injections. The column flow was 400 μL/min and the column temperature was 60 0C. For relative quantitation, the MS instrument was run in the survey scan mode with FTMS detection at a mass resolution of 60,000 FWHM at m/z 400. The mass scan range was m/z 80 to 1800, with a reference lock-mass for real-time calibration. Two UPLC-FTMS datasets were acquired for each sample, one with positive-ion detection and the other with negative-ion detection. LC-MS/MS data was also acquired from each sample set with collision induced dissociation (CID) at different levels of normalized collision energy.