**Metabolomic analysis of oxytocin effects on social deficits in mice**

Brain tissue samples (Midbrain, Cerebrum, Hind brain, Fore brain and Olfactory bulb) were shipped the NIH RTI-RCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis. A total of 99 study samples were thawed on ice for sample preparation.

Each thawed tissue sample was transferred into a pre-labeled MagnaLyser tube and massed. 50% acetonitrile in water was added depending on the mass of each sample (500 uL for up to 200 mg and 1000 uL for more than 200 mg). Samples were homogenized using MagnaLyser for two 30 sec pulses and then centrifuged. A volume of each sample supernatant required to analyze the desired sample mass (20 mg for Mid, Cerb, Hind, Fore, and 10 mg for Olf) was aliquoted. For a subset of samples with excess material, a total study pool was created by mixing the remaining volume of extracted tissue. Five replicates of the total study pool were created and prepared identically to the study samples. Samples were frozen and lyophilized. Lyophilized samples were mixed with 250 μl of Master mix (0.2 mM phosphate buffer (pH 7.5) containing 10% Chenomx Internal Standard - 0.5 mM DSS-d6 and 6mM Imidazole). The tubes were vortexed for 2 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 4 min. A 200 µl aliquot of the supernatant was transferred into pre-labeled 3 mm (4”) NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of brain extract samples were acquired on a Bruker 700 MHz NMR spectrometer (located at David H. Murdock Research Institute in Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 ℃. A 1D NOESY presaturation pulse sequence (noesypr1d, [recycle delay (RD)-90°-t1-90°-tm-90°-acquire free induction decay (FID) was used for data acquisition. For each sample 64 transients were collected into 65k data points using a spectral width of 8.41 kHz (12.02 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.9 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.2 software (Bruker-Biospin, Germany). Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS-d6 signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks. NMR bins (0.70-8.30 ppm) were created excluding water (4.60-6.00 ppm), low S/N (6.20 – 7.00 ppm), and imidazole (7.14-7.80 ppm) using intelligent bucket integration of 0.04 ppm bucket width with 50% looseness using ACD NMR Processor (ACD Labs Inc, Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum.