**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.

The NMR samples were used for the neurotransmitter analysis after NMR data acquisition. These samples were lyophilized overnight followed by reconstitution in 50 µL 95:5 methanol:water. Following a final centrifugation, extracts were transferred to vials for analysis by injection (20 µL) onto the Thermo Scientific (TS) 3000 series U-HPLC system. Compound separation was achieved on a Thermo Scientific Hypersil Gold, 200 x 2.1 mm column, and 1.9 µm particle size with an isocratic flow rate of 0.400 mL/min with a column temperature of 30°C. Simultaneous detection of neurotransmitters was achieved by the TS 5600A 16 channel coulometric array ECD with coulometric cells set at incremental potentials ranging from -150 mV to 900 mV. A Standard Mix containing 14 target compounds at known concentrations was analyzed just prior to each batch to confirm the retention time and elution channel of each compound. Additionally, the mix was used as an external standard to semi-quantitate any targets found in the extracts.

An individual response factor (RF) for each target analyte was calculated using the following equation:

* + RF = peak area of known target/sample amount of the known target

The amount of a target(s) detected in a study sample was calculated using the following equation:

* + Amount of target = peak area of target in sample/RF

The 14 neurotransmitters that were included in the Standard Mix were: L-Dopa, Norepinephrine, Tyrosine, Epinephrine, Homogenistic acid, 5-Hyroxytryptophan, Dopamine, Tyramine, 5-Hydroxyindoleacetic acid, 4-Hydroxyphenylacetic acid, Serotonin, N-Acetylserotonin, Anthranilic acid, and Homovanillic acid (Table 1).

Table 1. Compound, Elution Channel and Channel Potential

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| **Compound** | **Abbreviation** | **Dominant Channel** | **Channel Potential (mV)** |
| Norepinephrine | NE | 4 | 400 |
| L-Dopamine | LD | 4 | 400 |
| Epinephrine | E | 4 | 400 |
| Homogenistic acid | HGA | 3 | 200 |
| Tyrosine | TYR | 7 | 800 |
| 5-Hydroxytrpytophan | 5-HTP | 4 | 400 |
| Dopamine | DA | 3 | 200 |
| Tyramine | TYRA | 7 | 800 |
| 5-Hydroxyindoleacetic acid | 5-HIAA | 4 | 400 |
| 4-Hydroxyphenylacetic acid | 4-HPAC | 7 | 800 |
| Anthranilic acid | ANA | 7 | 800 |
| N-Acetylserotonin | NA5HT | 4 | 400 |
| Serotonin | 5-HTP | 4 | 400 |
| Homovanillic acid | HVA | 6 | 600 |