**Introduction**

Many studies have shown that nicotine and cocaine are neurobiological teratogens which can produce long-term effects on brain function. Since these substances are commonly used concurrently, the effects of both drugs were investigated. Maternal cigarette smoking has been associated with premature labor, stillbirth, neonatal death, low birth weight, and SIDS\(^1\). Prenatal cocaine exposure has been linked to problems with attention and reactivity, as well as maladaptive responses to acute stress\(^2\). This study assessed the neurotoxic effects of each of these drugs on brain development using ex vivo proton \(^1\)H magnetic resonance spectroscopy (MRS).

**Methods**

In this study, experienced Sprague-Dawley females were bred in-house. Animals were kept on a 12-hour light/dark cycle and were given free access to food and water. Pregnant females were randomized into three treatment groups. From day 4 of gestation until parturition, saline, nicotine \((3\) mg/kg, bid, sc), or cocaine \((10\) mg/kg, bid, sc) were administered. On postnatal day 120, the adult offspring \((\text{saline: males } n=7, \text{ females } n=5; \text{ nicotine: males } n=6, \text{ females } n=6; \text{ cocaine: males } n=5, \text{ females } n=6)\) were sacrificed. Animals were anesthetized with chloral hydrate. The head was quickly scalped, removed by decapitation, placed in liquid nitrogen and stored at -70ºC. Brain removal, dissection, and weighing were done over dry ice to minimize metabolite degradation.

Individual brain regions were homogenized in 5 volumes \((5\) x wet weight) \(0.04\) M HClO\(_4\) \((\text{diluted from } 11.65\) M solution with D\(_2\)O) and centrifuged at 16,000 \(x\) g for 15 minutes. The supernatant was kept cold while the pellet was resuspended in HClO\(_4\) and recentrifuged. The two supernatants were combined and 3-(trimethylsilyl) propionic \([2,2,3,3, d^4]\) acid, sodium salt \((\text{TSP})\) was added as an internal standard for a final concentration of \(2.5\) mM. Two supernatants were combined and 3-(trimethylsilyl) propionic \([2,2,3,3, d^4]\) acid, sodium salt \((\text{TSP})\) was added as an internal standard for a final concentration of \(2.5\) mM.

For analysis, \(0.45\) ml was used for measurement of metabolite concentration on a Bruker ARX 500 spectrometer \((8.45\) T) with a \(5\) mm inverse broadband probe at room temperature. A standard one pulse echo experiment was applied with a presaturation of the water resonance. The acquisition parameters were: \(30^\circ\) flip angle, \(8\) µs, \(6100\) Hz spectral width, \(32\) K data points \((0.186\) Hz/point), \(120\) averages, TR \(4.0\) s. Signal areas were determined by using integrals of the peak area referenced to the internal standard \((\text{TSP})\), following Fourier transformation, phasing, and baseline correction. Regional concentrations of several major brain metabolites were determined, including N-acetylaspartate \((\text{NAA})\).

**Results**

Offspring exposed to nicotine or cocaine in utero showed significant long-term changes in some brain metabolites, in a treatment, sex, and brain region specific manner. Of primary interest were the changes in NAA levels. In males exposed to cocaine prenatally, we found significant reductions in NAA levels in left hippocampus \((-8.5\%\)\) and left striatum \((-12\%\)\) as compared to saline controls and prenatal nicotine animals. In contrast, there was a significant increase in NAA in the right striatum \((+7\%\)\) of the prenatal nicotine treated females as compared to saline controls.

**Discussion**

These data indicate that there are long-term effects stemming from prenatal exposure to nicotine or cocaine which can be measured by \(^1\)H MRS. This study also provides evidence that rats exposed to prenatal injections of cocaine have neuronal damage or dysfunction in specific brain regions, as evidenced by their lower levels of NAA. NAA is a putative neuronal marker which is reduced under conditions of neuronal damage or loss. Reduced NAA has been reported in many conditions such as hypoxia, cerebral infarction, closed head trauma, dementias, HIV brain diseases, brain tumors, neurodegenerative diseases, and during early brain maturation\(^4\). Since \(^1\)H MRS can be applied non-invasively to humans of various ages, the results might be used to develop a “fingerprint” of neuronal effects stemming from prenatal exposure to nicotine, cocaine, or other substances affecting the development of neuronal chemistry. Such studies could help determine which children might be at greatest risk for development of subsequent behavioral and learning problems, and perhaps develop strategies to minimize the damage. From a basic science perspective, \(^1\)H MRS analysis of the brain should allow us to understand more about developmental insults and brain development in general.

**References**