PS 1024 NEUROBEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF ACUTE TOLUENE EXPOSURE—IS A SHORT-TERM EXPOSURE LIMIT (STEL) OF 200 PPM SAFE?

C. van Thriel, S. Kleinbeck, M. Schäper, K. Golka, M. Blaszkewicz, M. Lehmann and M. Rottrof. *IfADo—Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany.*

The neurotoxic effects of toluene are well characterized both, mechanistically and behaviorally. However, in the working environment there are still some uncertainties about a neurobehavioral NOAEL. Due to the rapid uptake of toluene during inhalational exposure acute neurobehavioral effects are of concern when establishing STELs. Since the glutamatergic and dopaminergic system are thought to be targets of toluene, we investigated behavioral and electrophysiological measures (event-related potentials, ERPs) obtained with neurobehavioral tasks associated with these neurotransmitters.
In an environmental chamber eight male volunteers were acutely exposed to two concentrations (5 ppm, 50 ppm, including two 200 ppm exposure peaks of 15 minutes) of toluene for 4 hour each. The low concentration was used as odorous control condition, while the other simulated exposures as high as permitted by the German OEL, including two 200 ppm exposure peaks. After 20 and 160 minutes of exposure the subjects performed cognitive tasks that required response inhibition, visual change-detection (both NMDA-receptor sensitive), working memory, and task switching (both dopaminergic modulated). On the behavioral level we examined reaction times and errors, on the level of cortical processing we analyzed ERP components like the NoGo-N2.
The behavioral parameters of all four tasks were neither affected by the time-weighted average concentration of 50 ppm, nor by the exposure peaks of 200 ppm. The analyzed ERPs reflected task-related variations (e.g. NoGo-N2) but were not significant affected by the toluene exposure. However, the number of false alarm was increase and correspondingly the NoGo-N2 amplitude was decreased during high exposure condition.
The "negative results" have to be interpreted with caution since (a) no physical workload was applied and (b) the most sensitive test (response inhibition) was not performed during the exposure peak.

PS 1025 ADULT-ONSET HYPOTHYROIDISM INCREASES RESPONSE LATENCY AND LONG-TERM POTENTIATION (LTP) IN RAT HIPPOCAMPUS.

K. Sanchez-Huerta[2], M. E. Gilbert[1] and J. Pacheco-Rosado[2]. *[1]Toxicity Assessment Division, US EPA, Research Triangle Park, NC and [2]Depart de Fisiologia, ENCB, IPN, Mexico City, Mexico.*

Thyroid hormones (TH) influence central nervous system (CNS) function during both development and in adulthood. The hippocampus is critical for some types of learning and memory and is particularly sensitive to thyroid hormone deficiency. Hypothyroidism in adulthood has been associated with cognitive decline, and both morphological and biochemical alterations have been reported in hippocampus following TH deficiency. The impact of the TH deficiency on the hippocampal synaptic function has not been well studied - reports are limited and results inconsistent across laboratories. The present study was designed to assess the effects of adult onset TH deficiency on hippocampal physiology and learning. Adult male rats (PN60-80) were exposed to propylthiouracil (PTU: 0 or 10 ppm), through the drinking water for 1 month to reduce serum TH. Body weight gain was reduced and thyroid gland weight increased by PTU. Learning was assessed using a trace fear conditioning paradigm. Field potentials were recorded in the dentate gyrus under urethane anesthesia. In contrast to TH insufficiency induced during development, trace fear conditioning, amplitudes of excitatory synaptic potentials, and magnitude of inhibitory transmission were not significantly altered by PTU. However, response latencies were increased in hypothyroid animals. LTP of the excitatory postsynaptic potential was slightly increased whereas no effect was observed in LTP of the population spike. The data indicate that only modest changes in hippocampal physiology accompany TH-insufficiencies induced in adulthood. Nongenomic actions of TH or secondary effects of hypothyroidism on temperature regulation may underlie some of these observations. These data are in partial agreement with previous published reports and indicate that adult onset hypothyroidism produces milder and distinct hippocampal dysfunction compared to developmental hypothyroidism. (Does not reflect EPA policy)

PS 1026 EFFECTS OF MOBILE PHONE RADIATION ON HSP90 IN RAT BRAIN.

E. Cuevas[1], M. E. Wyde[3], S. M. Lantz[1], B. R. Robinson[1], W. R. Hamilton[1], P. C. Howard[2], N. J. Walker[3], M. G. Paule[1] and S. F. Ali[1]. *[1]NEUROTOXICOLOGY, NCTR/FDA, Jefferson, AR, [2]Office of Scientific Coordination, NCTR/FDA, Jefferson, AR and [3]NTP, NIEHS, Research Triangle Park, NC.*

Mobile phone users are exposed to specific types of radiofrequency (RF) energy and previous studies have reported that biological changes are associated with RF exposure. This study assessed the effects of specific absorption rates (SAR) of mobile phone RF radiation on the rat brain. Adult male and female Harlan Sprague-Dawley rats were exposed to Global System for Mobile Communication (GSM) or Code Division Multiple Access Interim Standard 95 (CDMA IS-95) modulation at 3, 6, or 9 W/Kg. Animals were exposed 18.67 hours /day (10 min on, 10 min off cycle) for 7 days a week during gestation, lactation, and during the last week of exposure, and 5 days a week from PND 21- 42. Control rats were housed in similar chambers without RF exposure. After the last exposure, rats were sacrificed by decapitation and their forebrains were dissected and immediately frozen for subsequent western blot analysis probed with a heat-shock protein 90 (HSP90) antibody. Brains were also sectioned and analyzed for astrogliosis (GFAP) and stress markers (RAGE and HSP90 antibodies). GSM exposures did not produce any significant changes in HSP90 levels in either males or females. CDMA IS-95 exposures significantly increased HSP90 at 3, 6, and 9 W/Kg in females but only at 9 W/Kg in males. At 9 W/Kg in both sexes, exposure to both GSM and CDMA modulation increase astrocytosis and number of RAGE- and HSP90-positive cells. These preliminary studies indicate that the effects of different types of mobile phone RF are dose- and gender-dependent. Studies are underway to further examine the observed gender differences and to evaluate whether RF exposure might have any potential neuronal damaging effects in specific brain regions. (FDA/NCTR IAG # 224-070-0007) (NIH IAG # Y1ES1027).

PS 1027 EFFECTS OF METHAMPHETAMINE AND MPTP ON THE RETINAL DOPAMINERGIC SYSTEM IN MICE.

R. Hamilton, W. J. Trickler, B. L. Robinson, M. G. Paule and S. F. Ali. *NCTR/FDA, Jefferson, AR.*

This study was designed to examine the effect of multiple doses of methamphetamine (METH) and MPTP on the retinal dopaminergic system. Six month old C57BL/6 mice were injected i.p. with either a low-dose (LD - 2 doses of 5 mg/kg) or high-dose (HD - 3 doses 10 mg/kg) of METH or MPTP or equivalent amount of saline as a control. Mice were sacrificed 1 day after treatment by cervical dislocation; retinas were removed using the Winkler technique and immediately frozen. Retinas were thawed on ice, homogenized by sonication in 300 µl of 0.2 µM perchloric acid and 1 µM DHBA as internal standard, and centrifuged at 13,000 x g for 10 minutes at 4oC. The supernatant was filtered through a 0.45 µm membrane and a 25 µl aliquot was analyzed using HPLC for dopamine (DA) and its metabolites, 3,4-Dihydroxyphenylacetic acid (DOPAC) and Homovanillic acid (HVA). METH produced no significant changes in DA, or its metabolites in the retina. LD MPTP produced no change in DA level, but significantly decreased DOPAC and HVA 19% and 39% respectively. HD MPTP significantly decreased DA, DOPAC and HVA levels 26%, 28% and 30% respectively. Additionally, LD MPTP significantly decreased the DOPAC/DA and HVA/DA ratios 17% and 44% respectively. Mice did not show any significant changes in DA or its metabolites when exposed to multiple doses of METH. Although, METH is known to cause a release of DA, this is likely a transient phenomenon, and levels quickly return to normal after treatment. Where as MPTP, a selective, dopaminergic neurotoxin used to model Parkinson's disease, caused a significant decrease in DA and its metabolites. MPTP also decreased DA utilization as evidenced by significantly decreased DOPAC/DA and HVA/DA ratios. Taken together these results suggest that inhibition of the DA metabolizing enzymes MAO or COMT may take place at lower doses of MPTP treatment; conversely, higher doses of MPTP may cause decreases in DA, DOPAC and HVA through apoptotic cell death cell death in the retinal dopaminergic system.

PS 1028 *IN VIVO* STRESS AND CHRONIC GLUCOCORTICOID EXPOSURE INFLUENCE THE NEUROINFLAMMATION AND DOPAMINERGIC NEUROTOXICITY ASSOCIATED WITH METHAMPHETAMINE.

D. B. Miller[1], K. A. Kelly[1], J. F. Bowyer[2] and J. P. O'Callaghan[1]. *[1]CDC-NIOSH, Morgantown, WV and [2]US FDA-NCTR, Jefferson, AR.*

Upregulation of proinflammatory cytokines/chemokines in brain ("neuroinflammation") accompanies brain injury/disease and systemic infections. Following nerve terminal damage after acute exposure to dopaminergic neurotoxicant,

methamphetamine (METH), we documented elevated neuroinflammation, which may serve as a modulator or mediator of astroglial/microglial activation. Activated glia (associated with all types of brain injury) may be neuroprotective or exacerbate neural damage. Our prior genetic and pharmacological interventions have resulted in partial suppression of METH induced neuroinflammation without affecting neurotoxicity/astrogliosis. Here, mice were pretreated with the stress hormone corticosterone (CORT;400 mg/L drinking water) for 1 week or repeated *in vivo* stress for 5d, consisting of social reorganization and cage shaking. METH administration alone (20mg/kg,s.c.) caused significant increases in proinflammatory cytokine (TNFα,IL6,CCL2,IL1β,LIF,OSM) mRNA expression in striatum at 12h. By 72h marked astrocytic hypertrophy (GFAP protein/immunohistochemistry(IHC)), microglial activation (isolectin IHC) and dopaminergic nerve terminal damage (TH protein /IHC) was observed in striatum. Chronic CORT pretreatment caused exacerbated inflammation, astrocyte hypertrophy and microglial activation after METH exposure in the striatum, hippocampus and cortex. Of note, chronic CORT pretreatment exacerbated METH-induced decreases in TH protein (to 10% of control) in striatum. However, repeated *in vivo* stress exposure completely blocked striatal dopaminergic neurotoxicity and reduced the neuroinflammatory response to METH. As the levels of chronic CORT approached or exceeded those associated with high physiological stress, our data suggest that chronic CORT therapy or sustained physiological stress sensitizes the CNS neuroinflammatory and neurotoxic responses to METH. Also, more severe or prolonged *in vivo* stressor application may be required to produce priming of the CNS similar to exogenous CORT.

PS 1029 THE MODULATION OF 3, 4-(±)-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED NEUROTOXICITY BY CATECHOL-O-METHYLTRANSFERASE.

J. M. Herndon, A. B. Cholanians, L. E. Lizarraga, S. S. Lau and T. J. Monks. *Pharmacology/Toxicology, University of Arizona, College of Pharmacy, Tucson, AZ.*

3,4-(±)-Methylenedioxymethamphetamine (MDMA) abuse remains a significant problem worldwide. Systemic administration of MDMA to rodents and nonhuman primates causes neurotoxicity, and evidence suggests that it is also neurotoxic in humans. Metabolism of MDMA appears necessary for MDMA neurotoxicity. Thus, prior work from our laboratory indicates that cytochrome(s) P450 (specifically, CYP 2D6) inhibition attenuates MDMA-induced neurotoxicity, probably by decreasing the metabolism of MDMA to N-methyl-α-methyldopamine (N-Me-α-MeDA). Because N-Me-α-MeDA is a substrate for catechol-O-methyltransferase (COMT), we examined the effect of COMT inhibition on MDMA-induced neurotoxicity. COMT converts the N-Me-α-MeDA (a catechol) to 4-hydroxy-3-methoxy-methamphetamine (HMMA), thereby limiting the oxidation of N-Me-α-MeDA to the reactive ortho-quinone. Pharmacological and genetic models were used to determine the role of COMT in MDMA-induced neurotoxicity. Adult female Sprague-Dawley rats were pretreated with the COMT-inhibitor, Ro 41-0960 (40mg/kg, ip) followed by a neurotoxic dose of MDMA (20mg/kg, sc). In the genetic model, COMT+/- and COMT+/+ wild-type mice were dosed with either MDMA (30mg/kg X3 at 3 hour intervals, sc) or saline. In both models, neurotoxicity was determined one week after dosing via determination of neurotransmitter concentrations. In the pharmacological model of COMT inhibition, MDMA-induced neurotoxicity was potentiated. The data from the genetic model was ambiguous, probably because the COMT activity in the heterozygous animals is sufficient/borderline to process the O-methylation of N-Me-α-MeDA. Studies with homozygous COMT-/- mice are ongoing. The findings suggests that enzymes involved in the formation (CYP2D6 et al) and further metabolism of N-Me-α-MeDA (COMT) are important contributors to the individual susceptibility to MDMA-mediated neurotoxicity, especially given the polymorphic distribution of these enzymes in the humans. (Supported by NIDA Award DA023525)

PS 1030 PHARMACOLOGIC INHIBITION OF THE VESICULAR MONOAMINE TRANSPORTER 2 ATTENUATES 3, 4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED HYPERTHERMIA, LOCOMOTOR ACTIVITY, AND NEUROTOXICITY.

L. E. Lizarraga, A. B. Cholanians, J. M. Herndon, S. S. Lau and T. J. Monks. *Pharmacology/Toxicology, University of Arizona-College of Pharmacy, Tucson, AZ.*

3,4-Methylenedioxymethamphetamine (MDMA, Ecstacy) is a ring substituted amphetamine derivative with potent stimulant properties. MDMA exerts biphasic pharmacological effects on the brain resulting in opposing acute and long-term effects. During the acute effects, MDMA causes major monoamine release into the synapse, primarily of serotonin (5-HT), inducing hyperthermia and hyperactivity (5-HT syndrome). Conversely, long-term serotonergic neurotoxicity manifests as a prolonged depletion in 5-HT, and structural damage to 5-HT nerve terminals. The molecular mechanisms for both the acute and long-term effects remain unclear. The vesicular monoamine transporter 2 (VMAT2) is involved in the transport of monoamine neurotransmitters, in particular dopamine and 5-HT, into intra-neuronal storage vesicles. As such, VMAT2 is critical in maintaining neuronal health by preventing neurotransmitter oxidation within the cytosol. We therefore investigated the effects of the pharmacological inhibition of VMAT2, using Ro 4-1284, on MDMA-mediated acute and long-term neurotoxic effects. Sprague-Dawley rats pretreated with the VMAT2 inhibitor (10mg/kg, ip) displayed a significant increase in 5-HT content in the cortex and striatum compared to rats treated with MDMA (20mg/kg, sc) alone. Ro 4-1284 pretreatment delayed and attenuated total horizontal movement distance and mean velocity in animals dosed with MDMA. MDMA-mediated elevation in core body temperature was also significantly reduced in Ro 4-1284/MDMA-treated rats compared to those treated with MDMA alone. Thus, pharmacologic inhibition of VMAT2 appears to attenuate MDMA-mediated 5-HT/5-HIAA depletion, locomotor activity, and hyperthermia in rats. In summary, VMAT2 plays an important role in regulating the acute and long-term neurotoxic effects of MDMA. (Supported by NIDA Award DA023525).

PS 1031 NEUROTOXIC EXPOSURES TO AMPHETAMINE CAN RESULT IN LIPOPOLYSACCHARIDE (LPS) PRESENCE IN CIRCULATING BLOOD, ORGAN DAMAGE, AND IMMUNE ACTIVATION.

J. F. Bowyer[1], M. M. Vanlandingham[2], R. E. Patton[3], J. P. Hanig[4] and M. S. Levi[1]. *[1]Neurotoxicology, US FDA/NCTR, Jefferson, AR, [2]Biochemical Toxicology, US FDA/NCTR, Jefferson, AR, [3]Toxicology & Pathology Associates, US FDA/NCTR, Jefferson, AR and [4]US FDA/CDER, Jefferson, AR.*

Immunoactivation, organ damage and release of factors such as LPS were observed in rats exposed to environmentally-induced hyperthermia (EIH) or amphetamine (AMPH). Serum levels of substances signaling these events were determined 3hr and 22 hr (1d) after AMPH or saline (EIH and control groups). EIH was induced by a hot (39°C) environment and AMPH neurotoxicity by 4 injections, 2 hr apart, of 5, 7.5, 10 and 10 mg/kg D-AMPH. At 3 hr, creatine kinase was ≥2-fold in 50% of the EIH (n=7) rats and 100% of the AMPH (n=9) rats while creatine kinase-MB isoenzyme was elevated in 55% (AMPH) and 29% (EIH). At 1d, the enzymes were still ≈2-fold control in 20% of the EIH and 50% of the AMPH animals. Liver enzymes ALT and AST were up ≥2-fold in 57% of the EIH and 75% of the AMPH animals at 3hr. These enzymes returned to control levels in the EIH group but not in all the AMPH animals at 1d. Creatinine and BUN levels were significantly elevated 2-fold by EIH and 3-fold by AMPH at 3hr but returned to control levels by 1 d indicating kidney function may be only transiently affected. Detectable LPS was present in only 12% of the control animals but ≈50% of the EIH and AMPH animals had detectable levels at 3hr and 1d. White blood cell levels were not elevated by either EIH or AMPH at 3hr. However, micro array analysis on whole blood indicated a significant increase in the mRNA expression for cell surface proteins related to macrophages (Il1b), eosinophils (Cd52) and T-cells (Ccl5, Cd8a). In summary, both EIH and AMPH can result in multiple organ dysfunctions, particularly in muscle, heart, kidney and liver after AMPH exposure. The neurotoxicity produced by AMPH, however, may not be dependent on the severity of organ damage. The presence of LPS in the blood raises the possibility that it is involved in the immune response and neurotoxicity produced by AMPH.

PS 1032 THE EFFECTS OF FLUORIDE ON THE NEUROTRANSMITTERS IN DISCRETE BRAIN REGIONS OF ICR-DERIVED GLOMERULONEPHRITIS MICE BY EXPOSURE VIA THEIR DRINKING WATER.

M. Tsunoda[1], T. Kido[1], R. Ikeuchi[1], C. Sugaya[1], Y. Kodama[2], Y. Sugita-Konishi[2] and Y. Aizawa[1]. *[1]Preventive Medicine, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan and [2]National Institute for Health Sciences, Tokyo, Japan.*

Fluoride (F) has been known as an environmental pollutant. Although homovanillic acid (HVA) in the hypothalamus was altered in the BALB/c mice exposed to F in our previous study, the effects of F on the central nervous system had not been considered a serious health problem. Since a target organ of F is the kidney and F is filtered from the blood by the kidney, the accumulation of F in animals with renal insufficiency may enhance its toxicity. ICR-derived glomerulonephritis (ICGN) mice have been used as a model for idiopathic renal insufficiency. We evaluated whether or not the administration of F via the drinking water induces neurotoxicity in ICGN mice, in which F accumulates, by using neurotransmitters in discrete

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