3031 New Insights into the Role Microglia Play in the Etiology of Gulf War Illness

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Gulf War Illness (GWI) is a multi-symptom disorder with symptoms: persistent headaches, chronic fatigue, memory loss/confusion, skin and GI problems. These features are characteristic of persistent sickness behavior, known to result from underlying microglial neuroinflammation. Chronic exposure to corticosterone (CORT), at levels associated with high physiological stress, can prime the CNS to mount an exacerbated neuroinflammatory response (increase in proinflammatory cytokines/chemokines) following systemic exposure to neurotoxicants/inflammagens. When we administered CORT (200 mg/L 0.6% EtOH in drinking water) for 7 days prior to exposure to sarin surrogate, diisopropyl fluorophosphate (DFP; 4 mg/kg, i.p.), a heightened neuroinflammatory response was observed without astrogliosis or neurodegeneration 6-72 hours after DFP exposure. While these observations recapitulated the underlying early symptoms of GWI, they did not address the persistent episodic bouts of sickness behavior that characterize the long-term nature of GWI. As the phenotype is punctuated by symptom flare-ups, systemic exposure to lipopolysaccharide (LPS - a bacterial mimic; 0.5 mg/kg, s.c.) was used to challenge the GWI phenotype 2d following DFP treatment. CORT pretreatment primes the neuroinflammatory response to produce augmented LPS-induced inflammation and a single dose of DFP significantly exacerbated this effect. Here pretreatment with CSFR1 inhibitor Pexidartinib (PLX) (290 mg/kg chow *ad libitum* over 28d), a compound that has been demonstrated to "eliminate" or reduce microglia in brain, was used to investigate the role microglia play in the pathogenesis of this neuroinflammatory disorder. The well documented reduction in DFP-induced mortality by CORT pretreatment was further reduced by PLX in CORT DFP (7%-36%) groups and eliminated in the DFP alone group (0%-71%). CORT pretreatment caused significant thymic involution in both control (35%) and PLX (47%) groups with PLX groups showing reduced thymic weights (11% and 27%, respectively). Brain cytokine/chemokine levels measured by qPCR revealed large increases in IL6 & OSM in response to LPS in PLX pretreated groups (LPS: 129% & 107%; CORT LPS: 178% & 181%; CORT DFP LPS: 184% & 194%, respectively) revealing a potentially proinflammatory effect of PLX. Findings potentially point to not only a non-microglial origin of these cytokines in this model, but also a protective role of microglia in certain neuroinflammatory conditions.

3032 Doxorubicin-Induced Neuromotor Impairments in Male Athymic NCr Mice: Partial Protection by Phenylaminoethyl Selenide

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There is considerable research characterizing cardiotoxicity of the antitumor drug doxorubicin (DOX), while far fewer studies have focused on neuromotor and cognitive impairment. DOX has been shown to induce fine-motor deficits in humans and motor neuron degeneration, *in vitro*. The athymic T-cell deficient NCr (*nu/nu*) mouse is a frequently used model of cancer growth and chemotherapeutic treatment, but there have been few studies assessing neuromotor function in this strain and none investigating DOX-induced motor impairments and neuroprotection afforded by the antioxidant phenylaminoethyl selenide (PAESe). Male NCr mice were initially tested on rotarod and voluntary wheel running. After initial testing, they were assigned to exposure groups to ensure that groups did not differ prior to treatment. Mice were then administered four weekly i.v. doses of 5mg/kg DOX, 10mg/kg PAESe, a DOX+PAESe cocktail, or equivalent volume of Saline control. Mice were tested on rotarod and voluntary wheel running 17 and 31 days following final dose administration and on a functional observation battery (FOB) 32 days after final dose administration. Rotarod was sensitive to both DOX-induced neuromotor impairment and partial recovery by PAESe. Although PAESe provides protection against DOX impairment, it alone did not afford any benefit to motor function beyond Saline. Independent of exposure, latency to fall from rotarod decreased for all groups following dosing, suggesting that motor coordination in this strain is affected by age or anesthesia required for dosing. Total distance run in voluntary wheel sessions similarly decreased following treatment, but this measure was not sensitive to the specific treatments. Finally, some measures of the FOB were sensitive to treatment, especially those related to hindlimb function. In sum, DOX impairs motor coordination and hindlimb function, while co-administration of PAESe partially rescues this function. Further research is necessary to determine whether PAESe affects effectiveness in tumor shrinkage and whether this drug is protective against other side-effects of DOX, such as cognitive impairment.

3033 Using Public Data to Develop an Open-Access Computational Knime Workflow to Identify Any Cholinergic Compound

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One goal of high-throughput data mining in the life sciences is to align compounds with potential mechanistic targets for further in-depth study. As proof-of-concept using two-dimensional structural scaffolding, we sought to identify any unknown compound that might specifically interact with the cholinergic nervous system. The sympathetic and parasympathetic cholinergic nervous systems encompass the foundation of signaling at the mammalian neuro-muscular junction. Neurotransmission is mediated by acetylcholine (ACh) via nicotinic (nAChR) and muscarinic (mAChR) ACh receptors and acetylcholinesterase (AChE). Given the extent of public data from prior studies undertaken on these target proteins, we hypothesized that the cholinergic potential of any novel compound could be computationally predicted using big data mining based on the presence of structural motifs (scaffolds) shared across the known universe of cholinergic agents. Herein we show the development of a computerized workflow to capture and cluster virtually all (~19,000) compounds known to target the cholinergic system. From these, we identified 453 scaffolds that explain the structural diversity of known cholinergics. Using the Tanimoto similarity of these scaffolds to a compound as a covariate within a random forest machine-learning algorithm, we can predict the likelihood a novel compound will target any component of the cholinergic system (nAChR, mAChR or AChE targets) with exquisite sensitivity (99.3%) and high accuracy (94%). By building similar models for each separate component of the cholinergic system, we can additionally predict the likelihood of a compound interacting with a specific target (nAChR, mAChR or AChE) with similarly high sensitivity and accuracy (>96%). In conclusion, our findings demonstrate the utility and feasibility of implementing computerized workflows to gather public data, identify key structural scaffolds that describe the known cholinergic agents, and develop automated prediction models to calculate the likelihood of a previously uncharacterized compound interacting with the cholinergic nervous system.

3034 Neurotoxic Mechanisms of the Novel Psychoactive Substance Methoxetamine in Rat and Human *In Vitro* Models

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The use of new psychoactive substances (NPS) is steadily increasing. One of the commonly used NPS is the ketamine analogue methoxetamine (MXE). Although adverse effects have been reported for MXE, there is limited data available on its neurotoxicological mechanism(s) of action. To increase insight in the neurotoxicological profile of MXE, the aim of this study was to investigate the effect(s) of ketamine and its analogue MXE on calcium homeostasis, neuronal activity and monoamine transporters in different rat and human *in vitro* models. We therefor investigated the effects of ketamine and MXE on several neurotransmitter receptors and voltage-gated calcium channels (VGCCs) using single cell intracellular calcium $[Ca^{2+}]_i$ imaging in rat primary cortical cells, human SH-SY5Y cells and human induced pluripotent stem cell (hiPSC)-derived neurons. We also investigated effects on neuronal activity in rat primary cortical cells and hiPSC-derived neurons grown on micro-electrode arrays (MEA). Finally, effects on monoamine transporters were assessed in human embryonic kidney (HEK293) cells transfected with human monoamine transporters. In human SH-SY5Y cells, MXE (10 µM) slightly inhibited the K^+- and acetylcholine-evoked increase in $[Ca^{2+}]_i$. In rat primary cortical cells, MXE (10 µM) increased the glutamate-evoked increase in $[Ca^{2+}]_i$, whereas ketamine (10 µM) was without effect. MXE and ketamine did not affect VGCCs, but inhibited spontaneous neuronal activity with IC_{50} values of 0.5 µM and 1.2 µM, respectively. In hiPSC-derived neurons, MXE (10 µM) only slightly reduced the ATP-evoked increase in $[Ca^{2+}]_i$, and inhibited spontaneous neuronal activity with IC_{50} values between 10 - 100 µM. Fiinally, MXE potently inhibited uptake via monoamine transporters (DAT, NET and SERT) with IC_{50} values in the low micromolar range. Our combined *in vitro* data provide an initial neurotoxicological profile of MXE that can aid the risk assessment of MXE and provides a rapid screening approach that is also useful to assess the hazard of other emerging NPS. *This work was funded by the Dutch Poisons Information Center and the Faculty of Veterinary Medicine (Utrecht University).*

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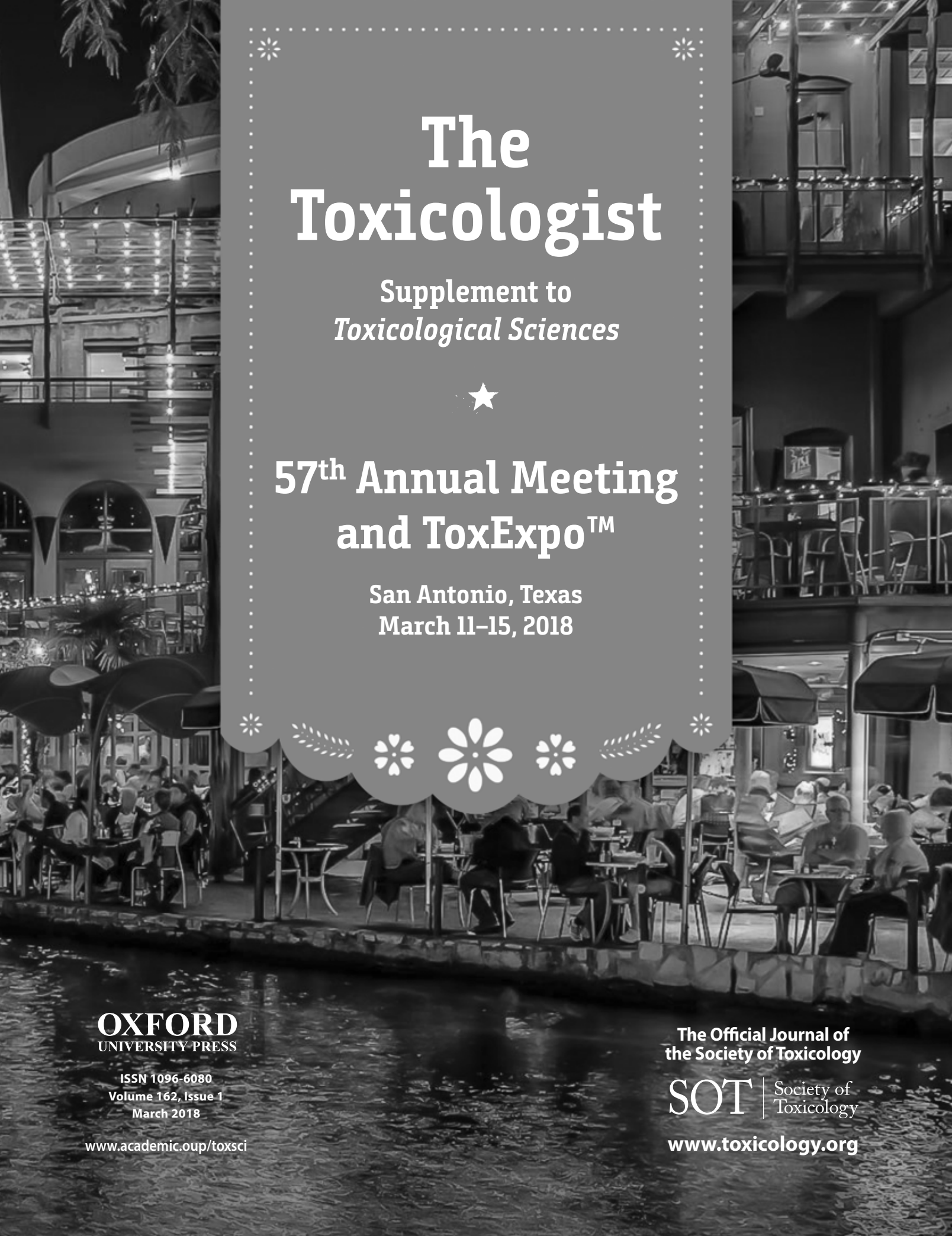

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