

Neurotoxicity and Mechanism of Toluene Abuse

Daniel P. Eisenberg

Albert Einstein College of Medicine
Belfer Educational Center
Bronx, New York 10461

ABSTRACT

Toluene is one of the most produced and widely used industrial solvents worldwide. It is a key ingredient in many products used by inhalant abusers. Toluene is responsible not only for instant inebriation but also for devastating neurotoxicity and death in the chronic abusers. Despite its prevalence and associated morbidity and mortality, very little is known about how this volatile solvent enacts its neurotoxic actions. Recent experimental evidence suggests that toluene does not act solely through the non-specific disruption of cell membranes as previously hypothesized. Instead, investigators have begun to describe a diverse and unique profile of neurotransmitter interactions that have brought science closer to understanding the behavioral, cognitive, and psychiatric effects of toluene.

BACKGROUND

Toluene, or methylbenzene, a colorless, sweet, and pungent-smelling liquid naturally present in crude oil, is produced in enormous amounts worldwide (ten million tons in 1986) (Schaumburg, 2000). Few other industrial compounds can rival the reported 112,777,568 pounds released annually into United States atmosphere (United States Environmental Protection Agency, 1997). While some toluene remains in gasoline and motor fuel products, much of the toluene content of crude oil (approximately 800 million gallons in 1991) is extracted in refineries. This toluene is then sold in increasingly greater demand for use in the production of chemicals, like benzene, and in the formulation of paints, graphic pigments, adhesives, lacquers, paint strippers, printing ink, spot removers, cosmetics, perfumes, and antifreeze (United States Environmental Protection Agency, 1994).

In addition to these traditional uses, toluene may be inhaled to achieve instantaneous intoxication. This is done by "sniffing" (i.e., inhaling vapors from an open container), "bagging" (i.e., inhaling more concentrated vapors from a closed container), or "huffing" (i.e. breathing through a solvent-soaked cloth) (Kurtzman et al., 2001). Cheap, legal, and readily available, products with substantial toluene content, typically glues or paint thinners, offer optimal substances of abuse for those without access to other illicit drugs. Consequently, toluene abuse is most prevalent in adolescents, particularly those of lower socioeconomic backgrounds (Schaumburg, 2000). Lifetime prevalence rates of

inhalant abuse ranging from 8% to 25% among high school students (Kurtzman et al., 2001). In addition, more than half of those reporting a history of inhalant use have done so multiple times, and 77% state they have abused inhalants for more than one year (Newmark et al., 1998).

The tremendous morbidity and mortality associated with this behavior are often under-appreciated. From 1987 to 1996 in Virginia alone, there were 39 inhalant-related deaths (2 died abusing pure toluene or products containing mostly toluene and several more died abusing products that contained toluene as a key ingredient) (Bowen et al., 1999). Chronic toluene abuse can cause irreversible renal, hepatic, cardiac, and pulmonary toxicity (Marjot and McLeod, 1989; Schaumburg, 2000). More recent data suggest reproductive effects, since rats exposed to toluene were found to have spermatic dysfunction (Ono et al., 1999), and humans with occupational exposure to toluene, benzene, and xylene had poor semen quality compared with controls (Xiao et al., 1999). Additionally, toluene abuse during pregnancy is associated with renal tubular acidosis in the mother as well as preterm delivery, perinatal death of the newborn, intrauterine growth retardation, and subsequent mental retardation (Wilkins-Haug and Gabow, 1991). Toluene and its metabolites are carcinogenic in rats, yet an actual cancer risk in humans has yet to be elucidated (Murata et al., 1999; Wiebelt and Becker, 1999). However, the magnitude and range of toluene's central nervous system (CNS) effects eclipse its peripheral toxicity profile.

NEUROTOXIC EFFECTS

Behavioral Data

In animal studies of acute toluene exposure, general locomotor activity follows a biphasic dose-effect curve (Bowen et al., 1996). Toluene induces an excitatory effect at very low doses but causes a progressively more depressive effect as the dose increases. At high doses of toluene, an animal's locomotor activity declines well below pre-exposure levels (Benignus et al., 1998; Riegel and French, 1999a). This biphasic pattern also appears during the early and later phases of acute high-level toluene exposure, since animal electroencephalographic studies that show early neocortical arousal that degrades into diffuse slowing after a brief high dose toluene exposure (Benignus, 1981). Behavioral experi-

Neurotoxicity and Mechanism of Toluene Abuse

ments using human subjects are difficult to compare, since they are often methodologically distinct and limited by ethical and safety concerns. In addition, this excitatory-depressive duality correlates to some degree with clinical experience and reports from inhalant abusers (Schaumburg, 2000). Descriptions of acute effects in toluene abusers include initial "hilarity," exhilaration, and irritability followed by increasing relaxation and lethargy that may progress to coma and death. However, during prolonged exposure to low levels of toluene (less than 600 ppm) depressive symptoms, fatigue and lassitude with depressed reflexes predominate, whereas exposure to higher levels of toluene (600 to 800 ppm) can elicit excitatory symptoms, such as feelings of exhilaration (Benignus, 1981). Additionally, residual activating symptoms of nervousness, insomnia, and agitation have been described during days following prolonged toluene exposure of 600 ppm suggesting a possible withdrawal effect (Schaumburg, 2000). Thus, the simple biphasic dose-response curve appears to be more complicated than the animal models suggest. Perhaps, as Benignus et al. (1998) suggests, it may be that the concentration of toluene in the tissue (in lieu of current measures of exposure concentration and duration) would yield a more straightforward relationship between toluene "dose" and the human behavioral response.

Consistent with its central depressant effects on activity and level of consciousness as described above, there is increasing behavioral evidence documenting toluene's anxiolytic effects. For instance, exposure to toluene interferes with animals' ability to learn to avoid behaviors with results that are fear provoking under normal circumstances. In one particularly instructive study, Bowen et al. (1996) exposed mice to 30 minutes of very high concentrations of toluene vapor (up to 6000 ppm) and then measured their performance in an elevated plus-shaped maze that contained both closed and open arms for the mice to enter. Under normal conditions, mice learn to avoid the more dangerous open arms of the maze from which they might fall. Toluene-exposed mice (at concentrations of 2000 ppm or greater), however, entered the open arms more often and stayed in them longer than controls. Total arm entries were minimally and insignificantly elevated and did not account for the findings. Additionally, the results for the toluene-treated group were nearly identical to a diazepam-treated group. The fact that toluene diminished avoidant behavior in this way coupled with its CNS depressant effects may suggest, as Bowen et al. (1996) propose, a pharmacological profile similar to known anxiolytics. Indeed, clinical experience in humans does support a relaxing or sedating effect of toluene (Schaumburg, 2000; Benignus, 1981). Though toluene affects a range of perceptual and cognitive indices that may interfere with performance in the plus-maze or similar tasks, analogies to benzodiazepines, barbiturates, and ethanol have fueled much of the successful

research into the actions of toluene (Schaumburg, 2000; Evans and Balster, 1991; Benignus, 1981).

As a substance of abuse, toluene's reinforcing and addictive effects are evident clinically. Only recently have researchers begun to examine this property in the laboratory. Funada et al. (2002) used a two-compartment chamber to condition mice (20 minute sessions repeated twice for 5 consecutive days) to associate toluene exposure with one compartment and drug-free air with the other. Concordant with pretest hypotheses, after conditioning, the mice showed a significant place preference for the toluene-associated chamber in the absence of toluene when entry between the two chambers was permitted. Interestingly, only toluene levels equal to or greater than 700 ppm during conditioning significantly produced this effect. This study supports previous work showing that animals will self-administer toluene by lever press when available (Weiss et al., 1979).

Neurological Data

Coordination and gait impairment have been documented in animal experiments and toluene abusers (Schaumburg, 2000). Even when rats are exposed to prolonged low level toluene (80 ppm), they demonstrate poor beam-walking performance 1 month post-exposure (von Euler et al., 2000). These findings are consistent with reports of nystagmus, ataxic gait, slurred speech, and intention tremors in chronic toluene abusers. Correspondingly, cerebellar atrophy and cerebellar tract damage has been documented by magnetic resonance imaging (MRI) in these patients (Aydin et al., 2002; Maas et al., 1991). Other neurological signs in chronic toluene abusers include rigidity, spasticity, hyperreflexia, and Babinski's sign (Aydin et al., 2002).

Additionally, an association with temporal lobe epilepsy has been reported by clinicians caring for chronic toluene abusers (Byrne et al., 1991). This observation is in direct contrast to data suggesting that toluene increases the seizure threshold (Evans and Balster, 1991; Bowen et al., 1996; Cruz et al., 2003). This discrepancy is likely a reflection of epileptogenic foci created during chronic toluene-induced cortical damage in spite of toluene's acute action as a CNS depressant.

Cognitive and Psychiatric Data

Subjects under the influence of toluene respond to stimuli slower than controls. Although most studies examined various length exposures at occupational levels of toluene, when estimating arterial blood toluene by a logistical-modeled meta-analysis of five such human studies, a clear linear correlation with poor performance in choice reaction time tasks emerges (Benignus et al., 1998). Such tasks present subjects with two or more options from which to choose and are

Neurotoxicity and Mechanism of Toluene Abuse

measured by the length of time between presentation of the stimulus and selection of an option. These conclusions support one of the earliest studies on the CNS effects of toluene in which 12 men were exposed to increasing concentrations of toluene (beginning at 300 ppm) while performing various tasks. Their reaction times increased and their perceptual speed decreased with the concentration of vapor (Gamberale and Hultengren, 1972; Benignus, 1981). Even after one acute intoxication, the residual deficits in processing speed and choice reaction time may persist, even several years later (Stollery, 1996).

Toluene interferes with a number of cognitive and perceptual functions both during the acute intoxication and as residual sequelae of high-level exposure. During intoxication, toluene abusers are prone to confusion, altered level of consciousness, and perceptual distortions, including paranoid auditory and visual hallucinations. In the long-term, a decline in intelligence quotients, residual dementia, and persistent psychosis featuring prominent visual hallucinations are observed (Byrne et al., 1991). Animal studies of toluene's impact on learning and memory have been inconsistent in methodologies and findings, especially at low concentrations (Benignus, 1981). Rodent studies using radial arm and water maze tasks after prolonged low-level toluene exposure failed to show reliable impairments in working or reference memory. This study only weakly suggests a possible interference with spatial memory (Miyagawa et al., 1995; von Euler et al., 2000). However, monkey and pigeon performances in delayed matching to sample tasks are consistently impaired with acute toluene exposure, supporting toluene-induced short-term memory loss at least in the acute setting (Evans et al., 1985).

Radiologic Data

Radiologic data of chronic toluene abusers reveals a predominant toxic leukoencephalopathy and atrophy that correlate with clinical observations, including dementia and cerebellar-tract dysfunction (Filley and Kleinschmidt-DeMasters, 2001). Robust findings on MRI of chronic toluene abusers include atrophy (when advanced, including the hippocampus), loss of gray/white differentiation, hyperintensities in the white matter, and hypointensities in the thalamus. These observations have led some to hypothesize about mechanisms of toluene-induced demyelination and/or gliosis. Interestingly, Yamanouchi et al. (1995) have characterized two patterns of white matter changes, restricted and diffuse, among toluene abusers. The restricted pattern appeared mainly in abusers of high-purity industrial toluene and abusers with fewer years of inhalant abuse. The features of restricted white matter changes are hyperintensities in the middle cerebellar peduncles and the cerebellar white matter surrounding the dentate nuclei in the T2-weighted MRI with occasional corre-

sponding hypointensities on T1. The diffuse pattern appeared mainly in abusers of commercial toluene-containing products, such as lacquer thinner that may contain other active ingredients, and abusers with longer histories of inhalant abuse. The features of diffuse white matter changes are marked atrophy of the brain with involvement of the hippocampus and corpus callosum. Recently, this dichotomy has been further refined by Aydin et al. (2002). They found that these two patterns correlate best with the duration of toluene abuse rather than the formulation of the toluene product. In addition, they demonstrated that white matter changes begin periventricularly and become more diffuse with increasing atrophy and enlargement of the ventricles as the individual continues to abuse toluene.

As imaging technology advances, so too does the opportunity to obtain greater functional and anatomic information about toluene's action in the brain. Gerasimov et al. (2002a) administered radiolabeled toluene to baboons during positron emission tomography (PET) scanning to image toluene's regional neural pharmacokinetics. They discovered rapid uptake into the striatum, frontal cortex, and the cerebellum (at lower levels). Toluene's rapid uptake and clearance (i.e., half-life approximately 20 minutes) are properties typical of most abused drugs (e.g., cocaine, amphetamines, short-acting benzodiazepines). Likewise, toluene-induced cerebellar activity helps explain the resulting ataxia, nystagmus, scanning speech, and loss of coordination.

NEURAL MECHANISMS OF CENTRAL NERVOUS SYSTEM EFFECTS**Dopamine**

Rea et al. (1983) at Purdue University sacrificed rats after 8 hours of toluene vapor exposure at 0, 100, 300, or 1000 ppm and analyzed their brain tissues for dopamine, norepinephrine, and serotonin concentrations by spectrofluorometry. They found a statistically significant increase of dopamine in both the whole brain and the striatum of the 1000 ppm toluene-exposed rats. While no effect was found when assessing whole-brain norepinephrine and serotonin levels, these neurotransmitters were altered regionally. Norepinephrine was increased in the medulla and midbrain while serotonin was increased in the cerebellum, medulla, and striatum. Importantly, regional brain analysis was conducted grossly in seven dissected structures (cerebellum, medulla, midbrain, striatum, hypothalamus, cortex, and hippocampus), so any changes in smaller, sub-structural areas may lack the neural density to impact the results. Equivalently, diverging levels of amines in discreet areas within the same structure would also be silent. A further caution in interpreting these results arises from the indeterminate significance

Neurotoxicity and Mechanism of Toluene Abuse

of post-mortem tissue neurotransmitter levels. The finding of elevated striatal dopamine, for instance, may reflect actual increased transmission (i.e., if toluene stimulated dopaminergic efferents from the substantia nigra/pars compacta or blocked the dopamine transporter), decreased transmission (i.e., if toluene caused postsynaptic blockade of the dopamine receptor), or any mechanism involving altered synthetic or metabolic pathways. Unfortunately, the toluene exposure conditions failed to resemble those commensurate with acute or chronic toluene abuse (the 100 ppm condition alone may be considered an occupational-type exposure, though no significant results were seen at this dose), hence its applicability to mechanisms of toxicity in humans may be limited. This is especially the case when one considers the diverse dose-dependent behavioral data described earlier. Additionally, the authors failed to remark on activity exhibited by the toluene-exposed rats, and so even these basic cues as to the extent of intoxication (and thus, confirmation of actual physiologically significant exposure) are unavailable to make any correlation with other studies. This may have been particularly beneficial given the authors' brief remark that the rats tended to stay near the exit port of the study chamber—it could be that this area had a different concentration of toluene vapor. Assays of toluene in the brain tissue following sacrifice would have provided an alternative and possibly useful correlative measurement. A further problem not addressed by the study was the possibility of residual toluene enacting post-mortem effects. Though toluene demonstrates rapid clearance from the CNS, an eight hour exposure followed by immediate sacrifice and refrigeration with only a saline rinse (toluene is lipophilic) as described in the study leaves this a possibility however unlikely. Nonetheless, this study was an important early step towards a neurochemical understanding of toluene toxicity and away from hypotheses that all volatile lipophilic substances function via nonspecific cell-membrane interactions. Its suggestion that regional changes in dopamine activity, as well as norepinephrine and serotonin activity, occur in response to toluene exposure proves to be an invaluable concept in decoding toluene's CNS effects.

Since the Purdue study (Rea et al., 1984), a number of laboratories have turned their attention to dopamine transmission during toluene abuse. Dopamine-associated neurological and psychiatric findings in chronic toluene abusers (i.e., Parkinson- and schizophreniform-like manifestations) as well as dopamine-associated behavioral stereotypies in rats (i.e., head-rearing) support this avenue of research. Kondo et al. (1995) subsequently studied striatal extracellular dopamine and its metabolites in live rats treated with toluene, methamphetamine, or no drug in a well-designed microdialysis experiment. They were able to monitor dopamine levels by inserting an intracranial probe into the left striatum via a surgically placed guide cannula,

allowing in vivo recording during simultaneous observation of the rats' behavior. After administering 1 dose of toluene (800 mg/kg by intraperitoneal injection), though rats demonstrated the characteristic activity response to toluene intoxication (i.e., immediate hyperactivity with ataxia followed by stereotyped movements and progressively diminished activity), surprisingly no significant change in dopamine or dopamine metabolites was found. Methamphetamine administration, the positive control, induced an immediate increase in striatal dopamine and decrease in its metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) with similar behavioral activity as the toluene exposed rats (though methamphetamine increased stereotyped movements without ataxia). Proper cannula placement was verified by dissection after the monitoring, though no precise information regarding their ultimate locations or sensitivities was presented. The investigators concluded that unlike other drugs of abuse, toluene's behavioral effects are not related to striatal dopamine release. One explanation may be that the toluene's acute behavioral effects are due to altered dopaminergic transmission primarily in related areas not accessible to the cannulae such as mesocorticolimbic areas. Another may be that dopaminergic activity in the striatum is influenced by toluene, but at a post-synaptic site of action that does not rely on increasing extracellular dopamine.

The results of Kondo et al. (1995) are weakened less by methodology than by the fact that Stengard et al. (1994) published a study that used the same microdialysis protocol as Kondo et al. (1995) but found a 47% increase in striatal dopamine with no change in homovanillic acid with acute high-level toluene exposure. One notable methodological difference between the two experiments is that Stengard et al. (1994) utilized inhaled toluene during the exposure conditions. Importantly, it was only at higher concentrations (1000 to 2000 ppm) that they were able to demonstrate a significant dopamine increase. This suggests the possibility that measurable changes in extracellular striatal dopamine do occur in response to toluene in a dose-dependent manner. It may be that the intraperitoneal toluene administered in Kondo et al. (1995) was insufficient to reach the threshold toluene concentration required to demonstrate these dopaminergic changes. Behavioral data described in Kondo et al. (1995) (i.e., predominantly increased activity, stereotyped movements, and some ataxia) appear to correlate with a toluene dose burden less than that experienced by rats in Stengard et al. (1994) (i.e., demonstrated eventual immobility of subjects after 30 minutes of exposure). Stengard et al. (1994) suggested that the lack of changing homovanillic acid is more consistent with a toluene-induced post-synaptic blockade of dopamine receptors rather than an increase in dopamine synthesis and release. Nonetheless, the contradictory nature of these two studies cannot be avoided, and further confir-

Neurotoxicity and Mechanism of Toluene Abuse

matory experiments must be done to conclusively decide the role of toluene in the striatum.

Interestingly, chronic low-level exposure to toluene (80 to 320 ppm for 1 month, 5 days per week, 6 hours per day) has been reported to increase dopamine D₂ agonist binding in the caudate-putamen without changing the number of D₂ receptors after a one-month postexposure period (Hillefors-Berglund et al., 1995). Taken together with Stengard et al. (1994), Hillefors-Berglund et al. (1995) may represent either a long-term reactive increase of D₂ receptor affinity or distinct mechanisms of action in the nigrostriatal system for acute moderate-high-level exposure and chronic low-level exposure. Whether repeated or prolonged toluene exposure affects neurotensin receptors that are both resident on dopaminergic neurons and able to enact phosphorylation-mediated alterations in D₂ autoreceptor affinity (Kalivas, 1993) has not been thoroughly studied and may prove a useful new direction in toluene research.

Though toluene's effect on striatal dopamine remains unclear, toluene does impact behaviorally significant dopaminergic transmission in the brain. Riegel and French (1999a) pretreated rats with remoxipride, a D₂ receptor selective dopamine antagonist that has been shown to have greater activity in the nucleus accumbens and mesocortical areas than in the striatum, then administered either 900 mg/kg toluene or scopolamine by intraperitoneal injection. Because scopolamine produces hyperactivity via cholinergic pathways unrelated to dopamine, no changes were seen when scopolamine-treated rats were pretreated with remoxipride. The biphasic, dose-dependent locomotor hyperactivity induced by toluene, however, was significantly decreased (by greater than 60%) when rats were pretreated. Unfortunately, how this reduction related to the rats' baseline activity was not presented. Thus, it is uncertain whether remoxipride suppressed the entire locomotor effect or not. Regardless, these findings led the authors to postulate that toluene induces a dopamine-mediated hyperactivity via mesocorticolimbic systems. This hypothesis fits well with the extensive body of literature examining these systems' vital role in reinforcement and addictive behavior (Koob et al., 1998).

Riegel and French (1999b) also published an electrophysiological study of ventral tegmental dopaminergic neurons in anesthetized rats subjected to abuse-like toluene exposure (11,500 ppm from 1 to 15 minutes) and found distinct patterns of firing as a result of toluene exposure. Interestingly, toluene had a divergent effect on these neurons that occurred at all doses, causing half of the cells to dramatically increase their firing rate (average maximum increase of 221%±72%) that attenuated with exposure length and half to decrease their firing rate (average maximum decrease of 96%±6.8%). Rates of firing were associated with

changes in the proportion of bursts (trains of spikes). Perhaps the most problematic aspect of the study lies in the fact that the rats under examination were anesthetized with ketamine, an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist that also has abuse liability. Importantly, there are NMDA binding sites in the nucleus interfascicularis and the nucleus linearis, two nuclei adjacent to the ventral tegmental area that also comprise part of the A10 region of dopaminergic neurons. Administration of NMDA to the ventral tegmental area has been shown to induce increased burst firing (Kalivas, 1993). Thus, the effect of ketamine on the results of the experiment cannot be discounted and hinder clear interpretation. Nonetheless, the experiment's findings do suggest a complex effect of high-dose toluene exposure on the dopaminergic neurons of the ventral tegmental area. However, there is insufficient information to explain the divergent pattern of firing noted above. The authors of the study suggest that toluene's NMDA effects previously documented by Cruz et al. (1998, 2003) may be responsible for complicating the results. However, it seems unlikely that any significant NMDA effects would be observable in the presence of ketamine. Alternatively, toluene-influenced GABAergic (neurons using gamma-aminobutyric acid (GABA) as a ligand) efferent transmission to the ventral tegmentum could possibly contribute to the findings by Riegel and French (1999b). GABAergic neurons projecting from the nucleus accumbens and pallidum to the ventral tegmentum as well as those intrinsic to the tegmentum synapse on both dopaminergic and non-dopaminergic neurons and have been shown to modulate their firing (Kalivas, 1993). Toluene research has implicated GABA systems in its actions (see below section on GABA and glycine), making this a possibility. Additional studies are required to both confirm and clarify this compound effect of toluene on ventral tegmental dopaminergic neurons. Because the mesocorticolimbic system (of which the ventral tegmentum is a key structure) provides significant output to the striatal system, such future studies may eventually offer insight into some of the inconsistencies and biphasic behavioral effects discussed above in relation to toluene's striatal effects.

One recent microdialysis study out of Brookhaven National Laboratory in New York investigated toluene's effects on extracellular dopamine in two other areas implicated in the central reward pathway: the prefrontal cortex and nucleus accumbens. A 40 minute 3000 ppm toluene exposure significantly increased dopamine levels (by nearly 100%) above those induced by an odorant or air alone in the prefrontal cortex but demonstrated no such increase in the nucleus accumbens. Co-administration with cocaine, however, caused a significant, synergistic increase in nucleus accumbens dopamine greater than found with cocaine alone (Gerasimov et al., 2002b). This discrepancy is difficult to explain and not likely due to dopamine receptor subtype variability or other intrinsic dopaminergic

Neurotoxicity and Mechanism of Toluene Abuse

quality, rather it may be due to indirect influence by other types of modulating neurons present in these areas, such as GABA_A interneurons.

Acetylcholine

The striatum, in addition to its dopaminergic synapses, contains cholinergic interneuron networks that are proposed to modulate this structure's activity. Given toluene's locomotor and stereotypic effects in rats as well as rigidity in humans the nigrostriatal system has been implicated as one of toluene's possible sites of action. Though there is conflicting data regarding striatal dopamine activity, an early study in rats had documented increased striatal and whole brain tissue acetylcholine after 30 days of 200 to 800 ppm toluene exposure (Honma et al., 1983). These considerations lead Stengard (1994), soon after studying dopaminergic striatal activity, to investigate the effect of acute toluene inhalation on extracellular acetylcholine in the rat striatum using a similar microdialysis protocol. He found that a 2 hour 2000 ppm toluene exposure gradually decreased striatal acetylcholine (20% during and 60% post-exposure). Administration of a dopamine uptake inhibitor (not in the presence of toluene) caused a rapid marked increase in extracellular dopamine and a concurrent increase in acetylcholine (145%). Based on the opposite actions of toluene and dopamine uptake inhibitor on acetylcholine and also the unique time course of toluene's alteration of acetylcholine levels, the author asserted that toluene's striatal cholinergic effects are not due to the increased extracellular striatal dopamine which was seen in his prior experiment (Stengard et al., 1994). Toluene's effect on extracellular striatal acetylcholine levels has been replicated by other investigators (Tsuga and Honma, 2000 citing Honma, 1993).

Another microdialysis study by the same investigator evaluated the possibility that this cholinergic effect of toluene may be behaviorally induced by the stress of the exposure conditions. However, he found that stressful stimuli (tail pinch) actually increase extracellular acetylcholine, a response that is unaffected by concurrent toluene exposure (Stengard, 1995). Thus, he concluded that toluene's action on cholinergic striatal neurons is not stress-induced, and further, that it is not related to changes in dopamine neurotransmission. However, this latter conclusion relies on the supposition that the only ways in which toluene might cause dopamine-induced changes in striatal acetylcholine levels is by a stress response or by increasing extracellular dopamine in the same way a dopamine uptake inhibitor might. Importantly, toluene's effects at the striatal D₂ receptor have not been fully characterized, especially in the acute exposure condition, and any increase in D₂ receptor activity (perhaps an early step towards the findings of Hillefors-Berglund et al. (1995) that increased D₂ receptor agonist affinity after chronic

toluene exposure) would inhibit acetylcholine release (Ajima et al., 1990) and possibly explain the findings of Stengard et al. (1994). Inhibition of striatal D₁ receptors would also prevent acetylcholine release (Ajima et al., 1990). Furthermore, toluene's reported anxiolytic effects are consistent with a behaviorally-mediated decline in striatal acetylcholine, given stress-induced increase in acetylcholine. Admittedly, if this last hypothesis were true, one would expect some modulation of the tail pinch effect when toluene was coadministered. The conclusion of Stengard (1995) that striatal cholinergic activity is not dopaminergically influenced is therefore probable, but not absolute.

Though the dopaminergic system appears to be a natural site of action for toluene, given its abuse potential, reported psychotic symptomatology, and parkinsonian symptoms in chronic abusers, a number of features suggest that independent cholinergic pathways may also be important in understanding the neural mechanisms of toluene's CNS effects. The cholinergic neurons of the basal forebrain complex have been implicated in memory and cognitive functioning. They are some of the first neuron populations to suffer damage in Alzheimer's disease and centrally acting anticholinergic drugs have detrimental effects on mental status. In light of the dementia and cognitive sequelae of toluene abuse, studying whether toluene modulates cholinergic activity in the brain may yield valuable insight into this issue.

For these reasons and the fact that other CNS depressants, such as ethanol and general anesthetics, alter the function of nicotinic acetylcholine receptors, Bale et al. (2002) recently performed a series of voltage-clamp and patch-clamp experiments in both *Xenopus* oocytes and plated rat hippocampal neurons to evaluate toluene's action at this receptor. The oocytes were prepared to express various nicotinic receptor subtypes. The investigators found that toluene inhibited acetylcholine-induced currents in a dose-dependent, non-competitive, and reversible manner. This effect was seen more profoundly with receptors bearing $\beta 2$ subunits than those bearing $\beta 4$ subunits. Mutating these subunits weakened this association. Such an effect had been previously demonstrated with nitrous oxide, pentobarbital, and hexanol (Yamakura et al., 2000). Toluene demonstrated no independent effect on resting membrane potential, and the toluene concentrations used are behaviorally significant based on previous studies (Benignus et al., 1981). This study provides additional evidence that toluene's CNS activity is not mediated solely by nonspecific lipophilic membrane interactions as previously thought. Experiments are needed to assess not only whether this effect is seen in the living brain after toluene inhalation, but also to determine what structures are most sensitive to nicotinic modulation and which behavioral, neurologic, and cognitive sequelae may be associated with this activity.

Neurotoxicity and Mechanism of Toluene Abuse

Tsuga and Honma (2000) examined ligand binding to muscarinic acetylcholine receptors in the rat frontal cortex and hippocampus after toluene exposure of 500 to 2000 ppm for 6 hours. In the hippocampus, toluene dose-dependently increased agonist binding at high-affinity receptor sites (i.e., sites associated with receptors that are coupled to their G-protein are high-affinity sites, while uncoupled receptors are low-affinity). The investigators postulated that toluene (at concentrations of 1000 ppm or greater) increases G-protein-receptor complex coupling in the hippocampus. Toluene caused the opposite effect in the frontal cortex, shifting the dose response curve for high-affinity agonist binding to the right. Perhaps this variable anatomic effect is a result of different receptor subtypes present in the two regions. This delineation would require additional study. Nonetheless, the biochemical action of toluene in both the frontal cortex and hippocampus correlates well with the known radiological data noting both rapid frontal uptake with acute toluene exposure (Gerasimov et al., 2002a) and hippocampal atrophy with chronic toluene abuse (Aydin et al., 2002). Unfortunately, since Tsuga and Honma (2000) evaluated the entire frontal cortex at once and the rat frontal cortex is less developed than in humans, the functional correlation of their findings in the frontal cortex remain difficult to assess.

GABA and Glycine

Early in 1988, Steinar Bjornaes and Liv Unni Naalsund published a report documenting alterations in GABA binding in the brains of rats subjected to subchronic (1 month, 16 hours per day of 50, 250, or 1000 ppm) or chronic (3 months, 16 hours per day of 500 ppm) toluene exposure. GABA binding increased in the brainstem and frontal cortex under the subchronic condition, increased in the hippocampus and frontal cortex under the chronic condition, and decreased in the cerebellar hemispheres under the chronic condition. The authors posited that these changes might be a reaction to prolonged alterations in GABAergic transmission (Bjornaes and Naalsund, 1988). Though this is certainly not the only possible explanation and the inconsistencies between the subchronic and chronic conditions weaken either the results or this hypothesis, the fact that GABA-related changes take place in the brain in response to toluene is an important piece of data. Furthermore, though the exposure protocol varied greatly from that which solvent abusers experience, this study opened the door for more recent, well-designed investigations into the effects of toluene on GABA.

GABA transmitter systems present a particularly logical target for toluene. Other central depressants, such as ethanol and volatile anesthetics, have sites of action at the GABA receptor. GABAergic neurons include the Purkinje efferents, basket cells, stellate cells, and Golgi interneurons of the cerebellum, a structure that by

imaging and clinical evidence suffers greatly during toluene-induced toxic effects (Stengard et al., 1993). Many of the central reward pathways implicated in toluene's addictive potential, which involve the ventral tegmental area and related mesocorticolimbic areas, are reliant on GABAergic neurons, such as those arising in the nucleus accumbens and ventral pallidum. Even the nigrostriatal circuits include a tremendous amount of GABA-based synapses (Kalivas, 1993). Ventral tegmental area GABAergic neurons have been shown to discharge immediately prior to intracranial self-stimulation (Steffensen et al., 2001).

One microdialysis study found that 2 hours of 2000 ppm toluene, but not halothane, increased cerebellar extracellular GABA (167% during and 60 minutes after exposure) (Stengard et al., 1993). Because this effect was completely blocked by tetrodotoxin, a sodium channel blocker, the increase in extracellular GABA is most likely neuronal in origin. Though the authors suggested that the results might be attributable to cerebellar input from the spinal cord and brainstem nuclei, there is some evidence to indicate that toluene has a more direct effect on the cerebellar GABAergic neurons themselves. Chronic toluene abuse causes cerebellar degeneration, and rapid uptake of toluene by the cerebellum is seen radiologically in toluene exposure. Additionally, a more recent investigation found that toluene exposure, similar to ethanol, reversibly increases GABA_A receptor-mediated currents in rat hippocampal slices and in *Xenopus* oocytes. Toluene did not alter the resting membrane potential in the absence of agonist. Though this experiment was not performed in the context of the cerebellum, it is notable that GABA_A neurons are a fundamental element of the granular layer of the cerebellum (Beckstead et al., 2000).

In the same experiment, Beckstead et al. (2000) evaluated toluene's effect on glycine, another inhibitory ligand-gated ion channel receptor type shown to be modulated by exposure to ethanol and volatile anesthetics. Again, this analogy with other CNS depressants produces valuable results: toluene enhances $\alpha 1$ glycine receptor-mediated current in the same way as it did with GABA_A receptor-mediated current. Even more fascinating, this effect was significantly impaired by a single mutation from serine to phenylalanine at position 267 of the $\alpha 1$ subunit. While different amino acid substitutions at the same location altered the effects of other glycine-modulating solvents, volatile anesthetics, and ethanol, this particular property of toluene was unique. This suggests that toluene, though unique in its biochemistry, may share a similar binding site on the glycine and related GABA receptor as these other depressants.

NMDA Receptor

NMDA glutamate receptors are implicated in contrib-

Neurotoxicity and Mechanism of Toluene Abuse

uting to the pathogenesis of epilepsy. NMDA, when administered centrally to mice, causes convulsions (Cruz et al., 2003). Toluene, like other CNS depressants, has anticonvulsant properties (Evans and Balster, 1991). Therefore, the NMDA receptor is a likely target for toluene action. In fact, Cruz et al. (1998), in an electrophysiological experiment investigating the effects of toluene on recombinant NMDA receptors, demonstrated that toluene acts as a noncompetitive antagonist at this receptor. Though their *in vitro* study design was only able to speculate on any functional correlation, they recently published a report that toluene exposure was protective against NMDA-induced seizures and lethality in mice (Cruz et al., 2003). Toluene increased latency to seizures, decreased the percent of convulsing animals, and reduced the amount of behaviors associated with NMDA toxicity. This was a dose-dependent effect (1000 to 6000 ppm) and most robust when toluene was given both before and after the NMDA injection. This effect was not attenuated with a prior week of bidaily high-level (4000 ppm) 30 minute toluene exposure. The anticonvulsant properties of toluene in the protocol used by Cruz et al. (2003) are not necessarily the result of NMDA receptor blockade. Toluene's enhancement of GABA and glycine transmission would offer a central inhibitory effect. An additional experiment in which seizures were induced by a non-NMDA convulsant may have clarified this distinction. However, toluene's demonstrated antagonist activity at the NMDA receptor provides an excellent explanation for its strong anticonvulsive action in NMDA toxicity.

Serotonin Receptor

Because of the success of experimentation linking toluene's effects to ligand-gated ion channels (i.e., nicotinic acetylcholine receptors, GABA_A, glycine, and NMDA), Lopreato et al. (2003) studied whether toluene affected the related serotonin-3 receptors as well. By electrophysiological testing of recombinant serotonin-3 receptors expressed in *Xenopus* oocytes, they were able to show that toluene enhanced serotonin-mediated current through these receptors. No effect was seen without agonist administration. Unfortunately, no preparation was done to confirm that this enhancement occurs in neural cells. Though *in vivo* studies involving toluene's action at this receptor have not been done, these results correlate well with two aspects of the central serotonin-3 system. The action of serotonin-3 receptors is associated with nausea and vomiting, especially those receptors located in the area postrema, and serotonin-3 antagonists, like ondansetron, are used for their antiemetic properties. Toluene intoxication can involve significant nausea and vomiting, and this pathway would account for this well. In addition, serotonin-3 receptors are present in great number in the nucleus accumbens and implicated in drug-seeking behavior as part of the central reward pathway.

Glia

Though the literature evaluating toluene's effects on neurotransmitter systems is limited, studies addressing the solvents actions on astrocytes are even more so. Imaging in chronic toluene abusers demonstrates a characteristic leukoencephalopathy with notable atrophy and likely reactive gliosis. The critical role of astrocytes and oligodendroglia in responding to neural damage and maintaining the extracellular milieu via neurotransmitter and neurohormone regulation make these cells particularly relevant to understanding toluene's chronic neurotoxic effects. Fukui et al. (1996) studied hippocampal astrocytes by immunocytochemistry after exposing rats to 2000 ppm of toluene for 1 month, 4 hours per day. They marked cells with glial fibrillary acidic protein and found that toluene exposure caused proliferation of their processes (not numbers) in the dentate gyrus. Though no notable neuronal damage was evidenced, the authors proposed that this was likely due to the protective effects of the hypertrophic astrocytic response to the solvent.

DISCUSSION

The past decade has been an important one in the development of understanding the neural mechanisms of toluene neurotoxicity. Perhaps most notably, science has challenged the view that toluene acts solely via nonspecific cell membrane interactions by virtue of its lipophilic nature. Instead, research, benefiting from modern recombinant and microdialysis techniques and encouraged by analogies to better described central nervous system depressants, has revealed a number of specific effects of toluene on various neurotransmitter systems, often shown to occur at the level of ligand-gated ion channel or G-protein coupled receptor.

Evidence suggests that in spite of important inconsistencies in the literature (i.e., microdialysis studies of toluene's effect on extracellular dopamine in the striatum), toluene exerts an effect on the mesocorticolimbic system that likely involves dopaminergic (especially, D₂), cholinergic, GABAergic, and serotonergic neurons. This best explains toluene's biphasic locomotor effect in animal models, reinforcement and clinical drug-seeking behavior, and some of the neuroimaging data. Alterations of dopaminergic transmission, particularly of the D₂ type, provide a theoretical basis for some of the psychiatric manifestations of toluene abuse. Though modulation of the nigrostriatal circuits in response to toluene exposure would offer a cause for the Parkinsonian-like clinical features of toluene intoxication, the only consistent data is from cholinergic studies documenting a slow decline in striatal extracellular acetylcholine levels. Clearly, more experimentation is needed evaluating toluene's influence on dopaminergic neurons within this area. One valuable future

Neurotoxicity and Mechanism of Toluene Abuse

direction in this regard might be in vitro electrophysiological studies of dopamine receptors to establish whether toluene exerts a direct or indirect effect at these synapses.

Research of cholinergic systems presents the opportunity to possibly address neural mechanisms of cognitive impairment seen in toluene abuse. Though few studies have been performed, toluene's documented actions at the nicotinic and muscarinic receptors of the frontal cortex and hippocampus may begin to provide this opportunity. However, more functional studies linking toluene's cholinergic activity to the clinical effects of toluene are much needed in order to demonstrate the importance of this site of action.

Toluene has shown significant and multifaceted inhibitory effects on the CNS, evidenced best by enhancement of GABA_A and glycine action as well as inhibition of NMDA glutamate receptors. Toluene's anti-convulsant, anxiolytic, and depressant features likely spring from these effects, yet still very little data exists on these topics. Even less information exists that assesses toluene's effects on other neurotransmitters, such as serotonin or neurotensin, or other neural elements such as astrocytes and myelin, which promise to expand understanding of toluene's diverse central toxicity. Examination of these latter elements, for example, may offer insight into the brain's management of a toluene challenge and the subsequent development of toluene-induced leukoencephalopathy in the chronic abuser, manifesting in part as multiple sclerosis-like, cerebellar and upper motor neuron neurological findings.

However, regardless of the neural system being studied, the literature on toluene is fraught with methodological problems and oversights, hindering optimal interpretation of results. Possibly the most obvious and important weakness of the literature on toluene is the repeated discrepancies of dose and chronicity of toluene exposure. Almost none of the studies reviewed utilized exposures consistent with toluene abuse in humans (repetitive exposures in which peak concentrations are estimated to reach 10,000 ppm or greater (Riegel and French, 1999b). Instead, moderate concentrations were often used for numerous hours at a time that reflect neither toluene abuse nor occupational exposure conditions. Furthermore, the diverse chronicity of exposures utilized (from 30 minutes to 3 months with varying frequencies of exposure) limits the generalizations that can be drawn from the literature. Acute, subchronic, and chronic exposures may present varied results secondary to reactive, attenuated, or essentially distinct pathophysiology, especially in the case of toluene that acts at so many different sites. Thus, dosimetric meta-analyses such as those performed by Benignus et al. (1998) may or may not be fully representative of toluene's effects over time. This distinction is not always well represented in the literature.

In conclusion, toluene is one of the least understood drugs of abuse, and, yet, it is also one of the most widely spread and greatly produced solvents known. Its abuse potential continues to assault the health of young lives worldwide. Toluene's acute and subacute neurotoxic effects range from biphasic behavioral patterns to neuropsychiatric complications and are often analogous but not identical to more classical depressants such as ethanol. The underlying neuropathology of these effects is likely equally as diverse, as recent research has identified multiple neurotransmitter systems modulated by toluene exposure.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Herbert Schaumburg (Albert Einstein College of Medicine, Bronx, New York) and Dr. David Kaufman (Montefiore Medical Center, Bronx, New York) for their guidance and review of this manuscript.

REFERENCES

Ajima, A., Yamaguchi, T., and Kato, T. (1990) Modulation of acetylcholine release by D₁, D₂ dopamine receptors in rat striatum under freely moving conditions. *Brain Res.* **518**:193-198.

Aydin, K., Sencer, S., Demir, T., Ogel, K., Tunaci, A., and Minareci, O. (2002) Cranial MR findings in chronic toluene abuse by inhalation. *Am. J. Neurodiol.* **23**:1173-1179.

Bale, A.S., Smothers, C.T., and Woodward, J.J. (2002) Inhibition of neuronal nicotinic acetylcholine receptors by the abused solvent toluene. *Br. J. Pharmacol.* **137**:375-383.

Beckstead, M.J., Weiner, J.L., Eger II, E.L., Gong, D.H., and Mihic, S.J. (2000) Glycine and gamma-aminobutyric acid(A) receptor function is enhanced by inhaled drugs of abuse. *Mol. Pharmacol.* **57**:1199-1205.

Benignus, V.A. (1981) Health effects of toluene. *Neurotoxicol.* **2**:567-588.

Benignus, V.A., Muller, K.E., Barton, C.N., and Bittikofer, J.A. (1981) Toluene levels in blood and brain of rats during and after respiratory exposure. *Toxicol. App. Pharmacol.* **61**:326-334.

Benignus, V.A., Boyes, W.K., and Bushnell, P.J. (1998) A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. *Toxicol. Sci.* **43**:186-195.

Bjornaes, S. and Naalsund, L. (1988) Biochemical changes in different brain areas after toluene inhalation. *Toxicol.* **49**:367-374.

Bowen, S.E., Wiley, J.L., and Balster, R.L. (1996) The effects of abused inhalants on mouse behavior in an elevated plus-maze. *Eur. J. Pharmacol.* **312**:131-136.

Bowen, S.E., Daniel, J., and Balster, R.L. (1999) Deaths associated with inhalant abuse in Virginia from 1987 to 1996. *Drug Alcohol Depend.* **53**:239-245.

Byrne, A., Kirby, B., Zibin, T., and Ensminger, S. (1991) Psychiatric and neurological effects of chronic solvent abuse. *Can. J. Psychiatry.* **36**:735-738.

Cruz, S., Mirshahi, B., Thomas, B., Balster R., and Woodward, J. (1998) Effects of the abused solvent toluene on recombinant N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *J. Pharmacol. Exp. Ther.* **286**:334-340.

Cruz, S., Gauthereau, M., Camacho-Muñoz, C., López-Rubalcava, C., and Balster, R. (2003) Effects of inhaled toluene and 1,1,1-trichloroethane on seizures and death produced by N-methyl-D-aspartic acid in mice. *Behav. Brain Res.* **140**:195-202.

Evans, E.B. and Balster, R.L. (1991) CNS depressant effects of volatile organic solvents. *Neurosci. Biobehav. Rev.* **15**:233-239.

Neurotoxicity and Mechanism of Toluene Abuse

- Evans, H.L., Bushnell, P.J., Pontecorvo, M.J., and Taylor, J.D. (1985) Animal models of environmentally induced memory impairment. *Ann. N. Y. Acad. Sci.* **444**:513-514.
- Filley, C.M. and Kleinschmidt-DeMasters, B.K. (2001) Toxic leukoencephalopathy. *N. Engl. J. Med.* **345**:425-432.
- Fukui, K., Utsumi, H., Tamada, Y., Nakajima, T., and Iyata, Y. (1996) Selective increase in astrocytic elements in the rat dentate gyrus after chronic toluene exposure studied by GFAP immunocytochemistry and electron microscopy. *Neurosci. Lett.* **203**:85-88.
- Funada, M., Sato, M., Makino, Y., and Wada, K. (2002) Evaluation of rewarding effect of toluene by the conditioned place preference procedure in mice. *Brain Res. Protocols* **10**:47-54.
- Gamberale, F. and Hultengren, M. (1972) Toluene exposure II: psychophysiological functions. *Work-Environ. Health* **9**:131-139.
- Gerasimov, M.R., Ferrieri, R.A., Schiffer, W.K., Logan, J., Gatlley, S.J., Gifford, A.N., Alexoff, D.A., Marsteller, D.A., Shea, C., Garza, V., Carter, P., King, P., Ashby Jr., C.R., Vitkun, S., and Dewey, S.L. (2002a) Study of brain uptake and biodistribution of [¹¹C]toluene in non-human primates and mice. *Life Sci.* **70**:2811-2828.
- Gerasimov, M.R., Schiffer, W.K., Marsteller, D., Ferrieri, R., Alexoff, D., and Dewey, S.L. (2002b) Toluene inhalation produces regionally specific changes in extracellular dopamine. *Drug Alcohol Depend.* **65**:243-251.
- Hillesfors-Berglund, M., Liu, Y., and von Euler, G. (1995) Persistent, specific and dose-dependent effects of toluene exposure on dopamine D₂ agonist binding in the rat caudate-putamen. *Toxicol.* **100**:185-194.
- Honma, T., Miyagawa, M., Sato, M., and Hasegawa, H. (1983) Significant changes in the amounts of neurotransmitter and related substances in the rat brain induced by subacute exposure to low levels of toluene and xylene. *Ind. Health* **21**:143-151.
- Honma, T. (1993) Microdialysis study of effects of organic solvents on biogenic amines in the brain. *Jpn. J. Ind. Health* **35**:390.
- Kalivas, P.W. (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Rev.* **18**:75-113.
- Kondo, H., Huang, J., Ichihara, G., Kamijima, M., Saito, I., Shibata, E., Ono, Y., Hisanaga, N., Takeuchi, Y., and Nakahara, D. (1995) Toluene induces behavioral activation without affecting striatal dopamine metabolism in the rat: behavioral and microdialysis studies. *Pharmacol. Biochem. Behav.* **51**:97-101.
- Koob, G.F., Sanna, P.P., and Bloom, F.E. (1998) Neuroscience of addiction. *Neuron* **21**:467-476.
- Kurtzman, T., Otsuka, K., and Wahl, R. (2001) Inhalant abuse by adolescents. *J. Adolesc. Health* **28**:170-180.
- Lopreato, G.F., Phelan, R., Borghese, C.M., Beckstead, M.J., and Mihie, S.J. (2003) Inhaled drugs of abuse enhance serotonin-3 receptor function. *Drug Alcohol Depend.* **70**:11-15.
- Maas, E.F., Ashe, J., Spiegel, P., Zee, D.S., and Leigh, R.J. (1991) Acquired pendular nystagmus in toluene addiction. *Neurol.* **41**:282-285.
- Marjot, R. and McLeod, A.A. (1989) Chronic non-neurological toxicity from volatile substance abuse. *Hum. Toxicol.* **8**:301-306.
- Miyagawa, M., Hanma, T., and Kawanishi, S. (1995) Effects of subchronic exposure to toluene on working and reference memory in rats. *Neurotoxicol. Teratol.* **17**:657-664.
- Murata, M., Tsujikawa, M., and Kawanishi, S. (1999) Oxidative DNA damage by minor metabolites of toluene may lead to carcinogenesis and reproductive dysfunction. *Biochem. Biophys. Res. Com.* **261**:478-483.
- Newmark, Y.D., Delva, J., and Anthony, J. (1998) The epidemiology of adolescent inhalant drug involvement. *Arch. Ped. Adolesc. Med.* **152**:781-786.
- Ono, A., Kawashima, K., Sekita, K., Hirose, A., Ogawa, Y., Saito, M., Naito, K., Yasuhara, K., Kaneko, T., Furuya, T., Inoue, T., and Kurokawa, Y. (1999) Toluene inhalation induced epididymal sperm dysfunction in rats. *Toxicol.* **139**:193-205.
- Rea, T.M., Nash, J.F., Zabik, J.E., Born, G.S., and Kessler, W.V. (1984) Effects of toluene inhalation on brain biogenic amines in the rat. *Toxicol.* **31**:143-150.
- Riegel, A.C. and French, E.D. (1999a) Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride. *Pharmacol. Biochem. Behav.* **62**:399-402.
- Riegel, A.C. and French, E.D. (1999b) An electrophysiological analysis of rat ventral tegmental dopamine neuronal activity during acute toluene exposure. *Pharmacol. Toxicol.* **85**:37-43.
- Schaumburg, H.H. (2000) Toluene. In: *Experimental and Clinical Neurotoxicology*. Spencer, P.S. and Schaumburg, H.H. (eds.), Oxford University Press, New York. pp. 1183-1189.
- Steffensen, S., Lee, R., Stobbs, S., and Henriksen, S. (2001) Responses of ventral tegmental area GABA neurons to brain stimulation reward. *Brain Res.* **906**:190-197.
- Stengard, K. (1994) Effect of toluene inhalation on extracellular striatal acetylcholine release studied with microdialysis. *Pharmacol. Toxicol.* **75**:115-118.
- Stengard, K. (1995) Tail pinch increases acetylcholine release in rat striatum even after toluene exposure. *Pharmacol. Biochem. Behav.* **52**:261-264.
- Stengard, K., Tham, R., O'Connor, W., Hoglund, G., and Ungerstedt, U. (1993) Acute toluene exposure increases extracellular GABA in the cerebellum of rat: a microdialysis study. *Pharmacol. Toxicol.* **73**:315-318.
- Stengard, K., Hoglund, G., and Ungerstedt, U. (1994) Extracellular dopamine levels within the striatum increase during inhalation exposure to toluene: a microdialysis study in awake, freely moving rats. *Toxicol. Lett.* **71**:245-255.
- Stollery, B. (1996) Long-term cognitive sequelae of solvent intoxication. *Neurotoxicol. Teratol.* **18**:471-476.
- Tsuga, H. and Honma, T. (2000) Effects of short-term toluene exposure on ligand binding to muscarinic acetylcholine receptors in the rat frontal cortex and hippocampus. *Neurotoxicol. Teratol.* **22**:603-606.
- United States Environmental Protection Agency (1994) Chemicals in the environment: toluene (CAS NO. 108-88-3). Retrieved August 2001 from Environmental Protection Agency Office of Pollution Prevention and Toxics Website, http://www.epa.gov/opptintr/chemfact/_toluen.txt
- United States Environmental Protection Agency (1997) The Toxics Release Inventory. Retrieved August 2001 from Environmental Protection Agency Website, <http://www.epa.gov>.
- Von Euler, M., Pham, T.M., Hillefors, M., Bjelke, B., Henriksson, B., and von Euler, G. (2000) Inhalation of low concentrations of toluene induces persistent effects on a learning retention task, beam-walk performance, and cerebrocortical size in the rat. *Exper. Neurol.* **163**:1-8.
- Weiss, B., Wood, R., and Macys, D. (1979) Behavioral toxicology of carbon disulfide and toluene. *Environ. Health Perspectives.* **30**:39-45.
- Wiebelt, H. and Becker, N. (1999) Mortality in a cohort of toluene exposed employees (rotogravure printing plant workers). *J. Occupational Environ. Med.* **41**:1134-1139.
- Wilkins-Huang, L. and Gabow, P.A. (1991) Toluene abuse during pregnancy: obstetric complications and perinatal outcomes. *Obstet. Gynecol.* **77**:504-509.
- Xiao, G., Pan C., Cai Y., Lin H., and Fu, Z. (1999) Effect of benzene, toluene, xylene on the semen quality of exposed workers. *Chin. Med. J.* **112**:709-712.
- Yamakura, T., Borghese, C., and Harris, R. (2000) A transmembrane site determines sensitivity of neuronal nicotinic acetylcholine receptors to general anesthetics. *J. Biol. Chem.* **275**:40879-40886.
- Yamanouchi, M., Okada, S., Kodama, K., Hirai, S., Sekine, H., Murakami, A., Komatsu, N., Sakamoto, T., and Sato, T. (1995) White matter changes caused by chronic solvent abuse. *Am. J. Neuroradiol.* **16**:1643-1649.