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Evoked Potential Changes From 13 Weeks of Simulated Toluene Abuse in Rats[1]

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MATTSSON, J. L., S. J. GORZINSKI, R. R. ALBEE AND M. A. ZIMMER. *Evoked potential changes from 13 weeks of simulated toluene abuse in rats*. PHARMACOL BIOCHEM BEHAV **36**(3) 683–689, 1990.—Fischer 344 rats were exposed to 8000 ppm toluene vapor in an 'abuse' paradigm for 13 weeks to develop an animal model for 'solvent neurotoxicity.' Exposures to toluene were multiple and short (15 to 35 min), adjusted according to tolerance. Although body weight was reduced 23% from controls, the toluene-exposed rats appeared healthy. Evoked potentials taken postexposure were, however, mildly to severely affected. Flash-evoked potentials were slow and topographically disorganized; 10 kHz tone-pip auditory brainstem responses (ABRs) had severe loss of power and loss of detail. Click and 30 kHz ABRs, somatosensory-evoked potentials, and caudal nerve action potentials were less affected. No neuropathologic changes were detected by light microscopy (perfusion fixation, special stains). Thus, postexposure multimodal functional effects were readily detected after subchronic, severe episodic exposures to toluene.

Fischer 344 rats Toluene (methylbenzene) Simulated toluene abuse Inhalation exposures
Flash-evoked potentials Click auditory brainstem responses Tone-pip auditory brainstem responses
Somatosensory-evoked responses Caudal nerve potentials Perfusion fixation Neuropathology

AN experimental model for 'solvent neurotoxicity' requires an appropriate animal species as well as reliable techniques to detect neurotoxic effects. Our model, which uses rats and neurophysiologic techniques, was evaluated by causing neurotoxicity with toluene. Toluene was selected as the test solvent because, although the issue of solvent neurotoxicity in humans is controversial, it is perhaps least controversial for toluene abuse.

Toluene commonly is cited as a cause of neurotoxicity in people who intentionally and repetitively breathe high concentrations over long periods of time (3,10). The human toluene abuse syndrome can be severe. There are reports of hearing loss (6, 10, 12, 15), visual defects (6), poor mental performance (2, 6, 7, 10), EEG changes (7,10), peripheral neuropathy (16), cortical atrophy (6, 7, 10, 12), and diffuse central nervous system white matter changes (21).

Except for ototoxicity (19), standard experimental designs in rodents have revealed only mild alterations in CNS function, and not the severe neurotoxic effects seen in humans who abuse toluene (5, 9, 17). The lack of severe changes in rats may be due to many factors; e.g., perhaps the rat is an inappropriate species, or perhaps standard constant-level (6 hr/day) exposures are not as effective as repetitive, short-duration high-level exposures. For example, Himnan (8) demonstrated selective behavioral tolerance and reverse tolerance to toluene in rats exposed to an 'abuse' paradigm, which involved twice daily 15-min exposures to 10,000 ppm toluene for six weeks. We elected to use an exposure design similar to Himnan (8), to evaluate postexposure toluene effects in rodents exposed for 13 weeks to a simulated abuse paradigm which consisted of multiple, high-level and short-duration exposures.

METHOD

Test Species and Husbandry

Twelve male Fischer 344 rats, approximately 14 weeks old, were used. The rats were purchased from the Charles River Breeding Laboratory, Kingston, NY. Rats were housed one per cage in suspended stainless steel cages which have wire-mesh floors. The holding room was maintained at approximately 22°C, 50% humidity, and a 12-hour light-dark cycle. Purina Certified Rodent Chow (No. 5002) and municipal drinking water were available ad lib throughout the study except during exposure.

Test Materials

Toluene, certified A.C.S. grade (T324) of 99.9% purity, was

[1]A poster (No. 901) on the toluene 'abuse' study was presented at the 27th Annual Meeting of the Society of Toxicology, Dallas, TX, February 15–19, 1988.

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obtained from the Fisher Scientific Company, Fair Lawn, NJ. The identity and purity of the test material was confirmed by infrared spectroscopy, and gas chromatography/mass spectrometry.

Dose Selection

A toluene concentration of 8000 ppm was selected to avoid by a comfortable margin the lower flammability limit of 14,000 ppm, and still have a high level of exposure. Solvent abuse was simulated by intermittent exposure of rats to 8000 ppm several times a day, 5 days/week. The number and durations of the exposure repetitions per day were varied for the first nine days based on clinical observations of excessive toxicity. The exposure pattern for four of the first five days was 9 repetitions per day of 15 min exposure/45 min of no exposure (one repetition = 15 min on/45 min off). Rats were not exposed on the weekends. On Monday and Wednesday of the second week, there were four repetitions per day of 30 min on and 90 min off. Beginning on Friday of week two, the animals were exposed on Mondays, Wednesdays, and Fridays. There were 4 exposure repetitions/day, with 35 min of 8000 ppm exposure separated by 85-min intervals of 0 ppm exposure.

Experimental Design

The number of rats on the toluene study was limited by chamber size. Small chambers allowed rapid changes in toluene concentration and conservation of toluene (see the Exposure Conditions section below). Six male rats were exposed to toluene, and six male rats were controls. These rats were implanted with epidural electrodes before exposure. Rats are physically restrained during electrophysiological testing, and evoked potential data were collected after 3 months of exposure. Flash-evoked potential (FEP) data also were collected at 2.5 months. At least 65 hr lapsed between the last exposure and testing. After all exposures and tests were accomplished, all rats were perfusion-fixed, necropsied, and nervous tissues were examined by light microscopy.

Exposure Conditions

Toluene exposures were conducted in 0.156 m^3 stainless steel and glass, Rochester-type inhalation chambers (square with pyramidal top and bottom). Total chamber airflow was maintained at approximately 60 liters per minute to attain 95% of target concentration (8000 ppm) in less than eight minutes. Control rats were sham-exposed similarly.

The analytical concentration of toluene in the chamber was measured continuously throughout the exposure intervals by a Miran I infrared spectrophotometer (Foxboro Co., Norwalk, CT) equipped with a variable pathlength gas cell. The wavelength for the analysis was 3.4 microns. The mean ($\pm$ SD) analytical concentration of toluene in the chambers during exposures was 8061 ± 144 ppm. Mean temperature and relative humidity values during toluene exposures ranged from 25 to 29°C and 30 to 40%, respectively.

Surgery

Epidural electrodes were implanted surgically 1 to 2 weeks before exposure. Anesthesia was by intramuscular injection (posterior-lateral thigh) of a combination of xylazine (13 mg/kg) and ketamine (87 mg/kg). Epidural electrodes (7 mm long, No. 0-80, stainless-steel set screws) were inserted into the skull and supported with dental acrylic. The outer 3 mm of the set screws were exposed above the acrylic so recording leads could be attached directly to the screws (an electrical plug was not used). The somatosensory electrode was placed 1.5 mm posterior and 3.0 mm lateral left of bregma, the visual cortex electrode was placed 6.8 mm posterior and 3.0 mm lateral right of bregma, and the cerebellar electrode was located 12.0 mm posterior and 0.0 mm lateral of bregma. A reference electrode was placed 7.0 mm anterior and 1.0 mm lateral left of bregma.

Body Weights

Body weights were measured prior to initiation of the study and weekly thereafter.

Functional Tests

The following data were collected beginning 65 or more hours after 13 weeks of exposure: flash-evoked potential (FEP), cortical flicker fusion (CFF), auditory brainstem response to clicks (ABR_c), auditory brainstem response to tone-pips at 10 kHz (ABR_{10}) and 30 kHz (ABR_{30}), somatosensory-evoked potentials (SEP), and caudal nerve action potentials to single stimuli ($CNAP_1$) and to paired stimuli ($CNAP_2$).

Electrophysiological system. The electrophysiological system was a Nicolet Pathfinder II (Nicolet Biomedical Instruments, Madison, WI). Data sweeps (msec segments of EEG) were digitally sampled 512 times and averaged by an online computer. Rectal or tail temperature was recorded prior to each electrophysiological test.

Flash-evoked potential (FEP). Rats, in a restrainer, were placed in an isolation cubicle that has white plastic walls. Rats face the wall opposite the strobe. The visual system was stimulated with a low intensity flash (approx. 0.3 cd-sec/m^2) at a rate of 1.1 flashes/sec. Calibration was with a United Detector Technology 350 photodetector (plus 111 filter and lumilens 1153) placed in the same position as the rat, facing the wall opposite the strobe. The amplifier filters were set to pass EEG between 0.5 and 1500 Hz. Two simultaneous FEPs were collected on dual digitizers. One FEP sweep duration was 150 msec and the other was 750 msec. The final FEP was an average of 200 sweeps for each duration. FEPs at medium intensity also were collected at 2.5 and 3 months.

Cortical flicker fusion (CFF). The maximum rate of flash that elicited a synchronized cortical response was determined by increasing or decreasing the flash rate, in 2 Hz or larger steps, from 48 Hz. Amplifier filter settings were the same as for the FEP, and a CFF response was an average of 200 sweeps.

Auditory brainstem response to clicks (ABR_c). Rats, in restrainers, were placed in a cubicle specially designed for acoustic isolation and to minimize sound reflections. The distance from speaker to ears was about 11.5 cm. Sound pressure level calibration indicated 80 dB linear scale (rat removed, Bruel & Kjaer Model 2230 with ¼ inch condenser microphone model 4135 placed in the restrainer at the location of the ears). The click rate was 19.1 clicks/sec. Sweep duration was 10 msec and 2000 sweeps were averaged. Bandpass filters were set at 150–3000 Hz.

Auditory brainstem response to tone-pips (ABR_{10} and ABR_{30}). Auditory brainstem responses to tone-pips were tested at middle and high frequencies (10 kHz at approximately 55 dB, and 30 kHz at 82 dB). Each tone-pip had a 2.25 msec rise/fall ramp and no plateau (4.5 msec total duration). Tone-pips were presented at a rate of 19.1 pips/sec. Bandpass filters were set at 150–3000 Hz. Data sweep duration was 10 msec and 4000 sweeps were averaged.

Somatosensory-evoked potentials (SEP). Ventrolateral caudal nerves were stimulated at the base of the tail and a response was recorded at the somatosensory cortex (SEP). The stimulating electrodes were small needles set into the bottom of a plastic tray

that fit the tail (18). A 3-mA, 50-μsec electric pulse was presented at 1.1 pulses/sec. The amplifier bandpass settings were 1–1500 Hz. Simultaneous recordings were made with sweep durations of 35 and 150 msec each. The final SEP was an average of 100 sweeps.

Caudal nerve action potentials (CNAP). Ventrolateral caudal nerves were stimulated near the tip of the tail and mixed nerve action potentials from single stimuli ($CNAP_1$) were recorded at the base of the tail (18). The stimulating and recording electrodes (separated by 9 cm) were mounted in a plastic tray. A single 3-mA, 20-μsec electric pulse was presented at a rate of 10.1 pulses/sec. Subsequently, the nerves were stimulated with a pair of pulses ($CNAP_2$ with interstimulus interval of 3 msec) at 10.1 paired pulses/sec. The amplifier bandpass settings were 1–3000 Hz. Data sweep duration was 20 msec and the final CNAP was an average of 200 sweeps.

Digital Filtering

A computer routine (Nicolet Biomedical Instruments, Madison, WI) digitally filtered each of the waveforms that were collected with a broad-band analog filter. Digital filter settings were as follows: FEP 1–250 Hz; ABR 150–3000 Hz; SEP with a 35-msec data sweep filtered at 1–750 Hz; SEP with a 150-msec data sweep filtered at 1–500 Hz.

Waveform Analyses

All waveforms were evaluated by computer and by visual examination. The visual examination had two purposes; to verify that the computer technique processed each waveform in a logical manner, and for possible treatment related differences not detected by computer analysis. The automated computer technique analyzed the FEP, SEP, ABR, and CNAP data by quantification of differences from a template in waveform shape, latency and power [for more details, see (13)]. A template for each type of response was created by making a 'grand average' of all the control records. A window (starting and ending point in msec) was established for each type of response. The window widths for automated computer scoring were: FEP early components = 20 to 116 msec; FEP late components = 116 to 260 msec; ABR_c = 0.8 to 5 msec; ABR_{10} = 1 to 7 msec; ABR_{30} = 1.2 to 5.2 msec; SEP early components = 4.5 to 19 msec; SEP late components = 19 to 80 msec; CNAP single pulse = 2.8 to 7.4 msec; and CNAP paired pulse = 6.0 to 10.0 msec.

The analyses included optimal correlation of an individual waveform to the template waveform, the latency difference (phase shift in msec required to reach optimal correlation), and total power in the window (RMS volts).

Statistical Analyses

Each variable was tested for homogeneity of variance by the F-max test, alpha = 0.01 (4). When heterogeneity occurred, and was attributed to one or two outlying data points, these data were removed from parametric analysis and the data were reported separately.

Subsequent parametric analyses were conducted after homogeneity of variance was assured. Electrophysiology data (optimal correlation, latency difference and power) were analyzed by a multivariate analysis of variance (MANOVA), using the GLM procedure SAS (SAS Institute Incorporated, Cary, NC). Probabilities were calculated by an F-test based on Hotelling-Lawley trace statistics. The statistical procedure also provided analogous univariate analyses (ANOVA) for the 3 dependent variables of the MANOVA: optimal correlation, latency difference and power.

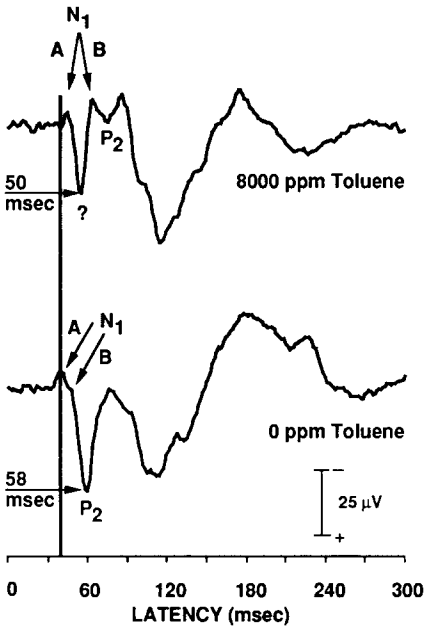

FIG. 1. Group composite flash-evoked potentials (FEPs, low intensity-flash) after three months of treatment. Prompt examination of Fig. 2, medium intensity FEP, will aid considerably in understanding the FEP changes seen in Fig. 1. As a point of reference, a vertical line was drawn through the N_1. Toluene 'abuse' slowed and extremely disorganized the early FEP components (latency ANOVA $p = 0.0002$; correlation ANOVA $p = 0.0001$). The toluene N_1 was slowed and split into two peaks (labeled A and B), and a large positive peak (labeled "?") intruded between N_{1A} and N_{1B}. Peak P_2 was greatly diminished in amplitude.

Temperatures, taken before each electrophysiological test, were analyzed by MANOVA, with analogous step-down ANOVAs for temperature for each test. Single variable measures (body weight, CFF rate) were tested with univariate analysis of variance (ANOVA).

This study was exploratory and numerous measurements were statistically compared in the same group of animals. For this reason, the overall false positive rate (Type I error) was greater than the cited alpha would suggest and a firm statement of statistical probability cannot be made. Because of the small sample size in this study (due to use of small chambers), and to preserve statistical power, no alpha corrections were made for repetitive use of statistics and alpha = 0.05.

Necropsy

Necropsies were performed after completion of 13 weeks of exposure and after neurologic testing on all rats. Each rat was heparinized and anesthetized with methoxyflurane. Tissues were preserved by whole-body perfusion with phosphate-buffered 1.5% glutaraldehyde/4% formaldehyde solution (pH 7.4, 540 mOsM). Gross pathological examination by a veterinary pathologist was as thorough as possible for perfused tissues.

Microscopic Pathology

Neural tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, luxol fast blue/periodic acid-Schiff-hematoxylin, and Sevier-Munger silver. Tissues ex-

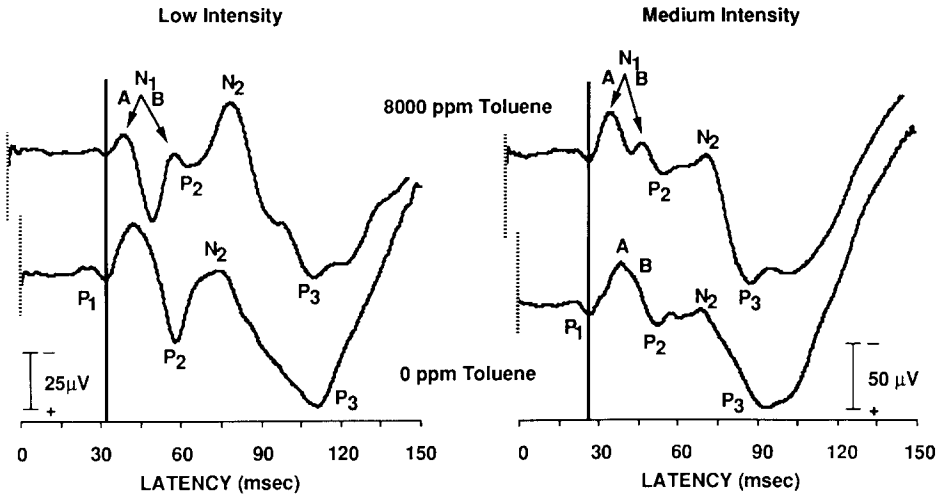

FIG. 2. Group composite flash-evoked potentials after 2.5 months of toluene 'abuse.' As a point of reference, a vertical line was drawn through P_1. The upper waveforms, from treated rats, were slow and were computer-shifted to the left on the time base to facilitate recognition of the various peaks. To line up the P_1 peaks, the toluene low intensity FEP was shifted 6.3 msec to the left of control, and the toluene medium intensity FEP was shifted 7.2 msec. The shape of the medium intensity FEP was less affected than the shape of the low intensity FEP, and shows clearly the separation of N_1 into two peaks, A and B, and shows a normal relationship between P_2 and N_2. The low intensity FEP was affected more severely, and had a large separation of N_{1A} and N_{1B}, with a large intervening cleft. The P_2 was reduced in amplitude.

amined by light microscopy were forebrain, midbrain, cerebellum and pons, medulla oblongata, optic nerves and eyes.

RESULTS

Overall Perspective

Rats were sedated to the point of immobility within 5 minutes of the beginning of each exposure cycle. Small jerking movements of the limbs commonly were seen during the peak of exposure. Mobility usually returned about 5 min after each exposure cycle was terminated.

Rats exposed to toluene weighed 16% less than controls after four weeks on test, and weighed 23% less after 13 weeks of treatment. In spite of the severely diminished weight gain, all treated animals appeared clinically normal and did not exhibit an unkempt appearance, staining of the haircoat or impaired movement.

Evoked potentials were recorded 65 or more hours postexposure. All of the evoked potentials were affected by treatment, with effects most apparent in flash-evoked potentials and 10 kHz auditory brainstem responses.

Postexposure Body Weights

Control rats weighed 331 ± 29 g, and toluene-exposed rats weighed 255 ± 6 g (mean $\pm$ SD). This difference is statistically significant, $p<0.01$.

Body and Tail Temperatures

Temperatures, taken during the different electrophysiological tests, were unaffected by treatment.

Flash-Evoked Potentials (FEPs)

After 13 weeks of exposure, the early components of FEPs of toluene-exposed rats (Fig. 1) were significantly altered in shape and were very slow (early component MANOVA $p=0.0026$; correlation ANOVA $p=0.0001$; latency ANOVA $p=0.0002$). The mean ($\pm$SD) correlations to the template were (control vs. toluene) $r=.76 \pm .14$ vs. $r=.35 \pm .07$. Mean ($\pm$SD) latency differences for control vs. toluene were 0.25 ± 2.84 msec vs. 10.75 ± 3.42 msec.

Careful examination of toluene FEPs in Fig. 1 shows that the waveform was disorganized in the area of N_1, P_2, N_2. The onset to N_1 was very slow, and N_1 split into two distinctive components, N_{1A} and N_{1B}, separated by a deep cleft labeled "?." The P_2 valley was quite shallow. This interpretation is better appreciated in FEPs at 2.5 months (Fig. 2). Medium intensity FEPs at 2.5 months were less affected than low intensity FEPs, and the beginning of the split of N_1 into two components is easily seen. In addition, the medium intensity FEPs have readily identifiable P_2 and N_2 peaks. The low intensity FEP at 2.5 months is more severely disorganized, and is very similar to the 3-month low intensity FEP. Although reduced food intake and consequently lower body weights have been associated with changes in FEPs (e.g., normal latency with diminished amplitude of N_1 and N_3), the changes seen in the toluene-treated rats were unlike those associated with dietary restriction (1).

Cortical Flicker Fusion (CFF)

CFF was unaffected by toluene treatment.

Auditory Brainstem Responses

As expected from the work of Pryor *et al.* (17) and Rebert *et al.* (19), ABRs were affected severly by toluene exposure (Figs. 3, 4 and 5). The effects were most apparent at 10 kHz (ABR_{10}, Fig. 4). The ABR_{10} was slowed and much reduced in power (MANOVA $p=0.0211$; latency ANOVA $p=0.0079$; power ANOVA $p=0.0083$). The changes at 30 kHz (Fig. 5; correlation ANOVA $p=0.0204$) and for clicks (Fig. 3; power ANOVA $p=0.0274$) were less impressive.

The two small oscillations that just precede peak I on the click

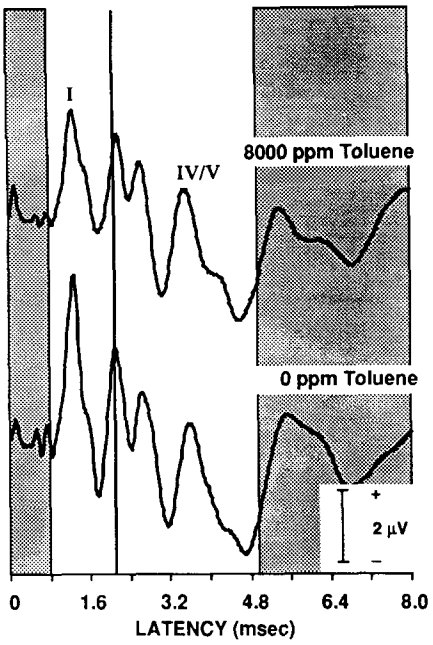

FIG. 3. Group composite click auditory brainstem responses (ABRc): The analysis window is the section of waveform between the shaded areas. As a point of reference, a vertical line was drawn through peak II. Toluene 'abuse' diminished the power of the ABR_c (ANOVA $p=0.0274$), but had little other effect. The power decrease was greatest at peak I. Note the two small waves just before peak I. These are hair-cell potentials (cochlear microphonics). The small microphonic potentials in the toluene-exposed rats are indicative of hair-cell damage (13).

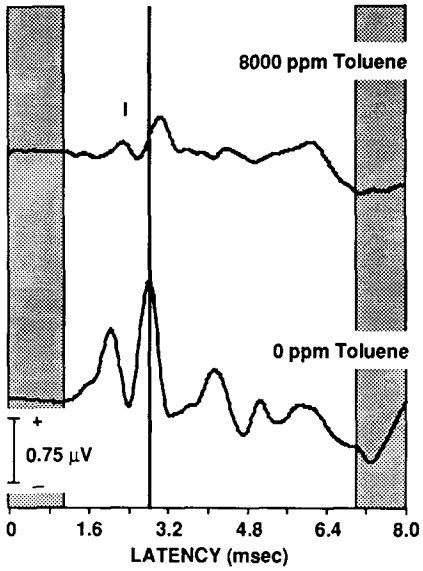

FIG. 4. Group composite 10 kHz auditory brainstem responses (ABR_{10}): The analysis window is the section of waveform between the shaded areas. As a point of reference, a vertical line was drawn through peak II. Toluene 'abuse' severely slowed and flattened the ABR_{10} (latency ANOVA $p=0.0079$; power ANOVA $p=0.0083$). Toluene is known to damage hair-cells (22), and this is the likely cause of the abnormal ABRs.

ABR (Fig. 3) are cochlear microphonic potentials (13). Note that the microphonics are smaller in the toluene rats. Peak I also is smaller (peak I is associated with acoustic nerve firing). Cochlear microphonics are generated by cochlear hair-cells, and hair-cell damage in rats is a known consequence of toluene exposure (22). Thus, it is likely that the altered ABR_c is a down-stream reflection of hair-cell damage, and interpretations about brainstem function are not possible.

Peak I of the ABR_{10} and ABR_{30} also are small compared to controls (Figs. 4 and 5). Unfortunately, microphonics are not seen on the tone-pip ABRs due to technology limitations at the time of this study. The small peak I's are, however, consistent with diminished hair-cell activity.

Somatosensory-Evoked Potentials (SEPs)

SEPs also were affected by toluene (Fig. 6). Most of the change occurred in the waveform shape of later components. The mean correlation ($\pm$ SD) for controls was $.96 \pm .02$ and for toluene was $.87 \pm .05$, with correlation ANOVA $p=0.0029$. Although neither latency nor power changes were detected statistically, a low p-value for late-component MANOVA ($p=0.0037$) indicated that all dimensions of the SEP were somewhat affected.

Caudal Nerve Action Potentials

Standard analyses (comparisons of individual waveforms to a template) were not conducted on CNAPs because of the small tail sizes in the toluene-exposed rats. Instead, each animal was used as its own control. Small tail size, per se, would not be expected to affect a nerve's responsiveness to a second stimulus (recovery function), so each rat's CNAPs were evaluated for recovery by computing the baseline to negative peak amplitude ratio of $CNAP_2/CNAP_1$. The mean amplitude ratio ($\pm$ SD) for controls was 0.87 ± 0.04, and for treated rats was 0.63 ± 0.19. The differences between these ratios were statistically significant, but not impressively (ANOVA $p=0.0438$).

Outliers

The early components of the SEP of one toluene-exposed rat were too poorly shaped to evaluate. The same rat also had an ABR_{10} that was too flat to evaluate. Data from these specific waveforms for this rat were not included in the statistical analyses.

Pathology

No gross or microscopic pathologic observations were associated with exposure to toluene.

DISCUSSION

The postexposure effects of toluene 'abuse' in rats in our study were somewhat comparable to the human syndrome in that multimodality effects clearly were demonstrable. Rats had altered visual-, auditory-, somatosensory-, and peripheral-nerve-evoked potentials. Neuropathologic changes were not observed by light microscopy, in spite of use of perfusion techniques and special stains. In addition, the rats appeared to be in good health, although much smaller than controls. Thus, the rat syndrome appeared to be somewhat milder than that reported for humans, although rat visual and auditory effects were severe. If exposures had continued for many more weeks, the rat syndrome possibly would have increased in severity. There is no reason to believe that the changes had reached steady-state after three months of exposure.

Rebert *et al.* (20) reported that a 30-min exposure to high concentrations of toluene induces large, transient changes in

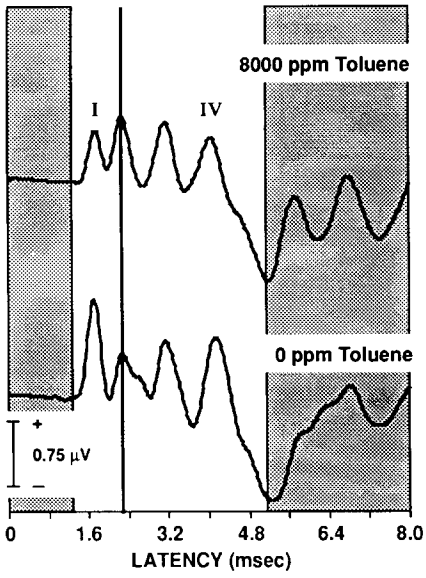

FIG. 5. Group composite 30 kHz auditory brainstem responses (ABR_{30}): The analysis window is the section of waveform between the shaded areas. As a point of reference, a vertical line was drawn through peak II. Toluene 'abuse' altered the shape of the ABR_{30} (correlation ANOVA $p=0.0204$). Note the diminished amplitude of peak I, which is attributed to downstream consequences of hair-cell damage (22).

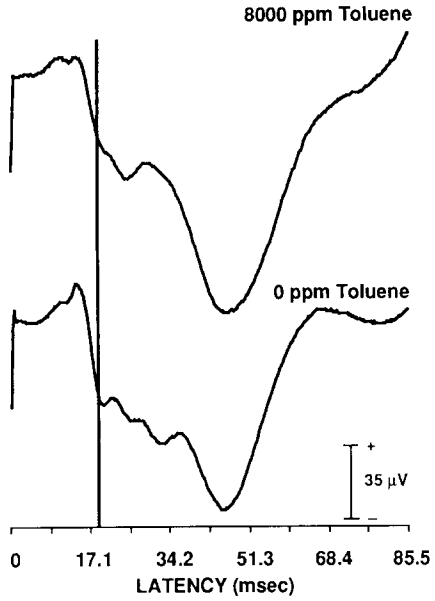

FIG. 6. Somatosensory-evoked potentials-sensory cortex (SEP): A vertical line was drawn at about 19 msec, the dividing line for analyses of early and late SEP components. Toluene 'abuse' significantly altered SEP late components (MANOVA $p=0.0037$ for latency, correlation and power; correlation ANOVA $p=0.0029$).

visual, auditory brainstem, and somatosensory-evoked potentials. Interestingly, acute effects of 8000 ppm and 16000 ppm toluene on the N_1–P_2 area of the FEP [Fig. 11, (20)] resembled the postexposure subchronic effects in our study (Figs. 2 and 3). In their study, acute exposure to 8000 ppm toluene broadened the FEP N_1, and sharpened the N_2. The broadening of the N_1 appeared to be a precursor to the separation of N_1 into two peaks at 16000 ppm. In addition to splitting the N_1 wave into smaller peaks, acute exposure to 16000 ppm decreased the depth of P_2 while retaining the sharpened N_2. This interpretation relies on the identification of N_2 (at about 74 msec) at 8000 ppm, and the likelihood that the 16000 ppm FEP negative wave at about 83 msec also is the N_2.

In our study, ABRs from 10 kHz tone-pips were affected much more severely than either click (3 to 4 kHz stimulus frequency) or 30 kHz tone-pip ABRs. This finding is consistent with both the toluene-induced pattern of hair-cell loss and the tone-pip ABR changes reported in rats by Sullivan *et al.* (22). These investigators (22) reported toluene-induced cochlear hair-cell loss that began in the middle and upper base turns, and progressed towards the apical region. These areas of the cochlea are responsible for midfrequency sound transduction, and ABR thresholds in the midrange were more affected than either high or low frequency ABRs (22).

The selection of ABR stimulus intensities for neurotoxicity screening can be important, however. Rebert *et al.* (19) showed that ABR stimulus intensity has important effects on the magnitude of toluene-induced ABR changes. Toluene affected ABR peak I latencies more at lower stimulus intensities than at high intensities, but stimulus intensity was a less important variable for detecting toluene-induced ABR amplitude decreases.

The mechanism for acute and subchronic toluene effects is not clear. In addition to toluene itself, one must consider the possible role of metabolites of toluene. We have examined the acute excitatory effects of light anesthetic levels of toluene on the somatosensory-evoked potential (SEP), and have identified comparable excitatory SEP effects from IV *o*-cresol (14). Toluene is metabolized principally to hippuric and benzoic acids, and neither of these acids caused SEP excitation upon IV administration. *o*-Cresol is a minor metabolite when toluene exposures are low, but increases disproportionately when toluene exposures are high (11). Therefore, *o*-cresol may have a role in high-exposure toluene phenomenology.

Regardless of the mechanism of neurotoxicity of toluene, Fischer 344 rats, when exposed to a toluene 'abuse' paradigm, develop mild to severe multimodality changes in sensory-evoked potentials. Thus, rats appear to be suitable models for the study of at least some aspects of the 'solvent neurotoxicity syndrome,' and evoked potentials appear to be reasonably sensitive tools for the detection and partial evaluation of solvent neurotoxicity.

ACKNOWLEDGEMENTS

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