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Acute Interactive Pharmacologic Effects of Inhaled Toluene and Dichloromethane on Rat Brain Electrophysiology

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REBERT, C. S., M. J. MATTEUCCI AND G. T. PRYOR. *Acute interactive pharmacologic effects of inhaled toluene and dichloromethane on rat brain electrophysiology*. PHARMACOL BIOCHEM BEHAV **36**(2) 351–365, 1990.—Toluene (TOL) and dichloromethane (DCM) are widely used industrial solvents and are common components of solvent mixtures that are voluntarily inhaled to produce altered states of consciousness. In previous studies we characterized some of the acute electrophysiologic effects of these solvents. Opposite effects were noted for some measures, suggesting that they might be antagonistic when combined. In this study we examined the solvents again singly (10,700 and 16,000 ppm) and also in combination (16,000 ppm: 33/67 and 67/33% TOL/DCM ratios). The single gases caused effects similar to those observed previously. Combined effects varied, dependent upon the particular variable examined and the major gas in the mixture. In some respects the solvents were concordant, exerting similar effects on a variable, e.g., both solvents prolonged the latencies of components of the brainstem auditory-evoked response. In other respects they were discordant, e.g., whereas toluene caused mean EEG frequency to increase, dichloromethane had the opposite effect. Sometimes the solvents had similar effects alone, but acted independently in combination. Nonindependent interactions were also observed—both additive/subtractive or positively or negatively synergistic. The results further demonstrate and emphasize the unique patterns of acute central nervous system effects that can be effected by solvents that might have a common behavioral endpoint such as anesthesia, and the results characterize a variety of electrophysiologic interactions between these two solvents. Although there were several variables exhibiting synergistic relationships, independent or additive interactions were the most common.

Toluene Dichloromethane Solvent abuse EEG Sensory-evoked potentials Rats Interactions

BECAUSE toluene (TOL) and dichloromethane (DCM) are common components of solvent mixtures that are voluntarily inhaled to produce euphoria (11,26), they were recently examined separately to determine how they might acutely affect the electroencephalogram (EEG) and sensory-evoked potentials (EPs) of rats (21–23). The two solvents produced quite different patterns of effects. TOL increased the amplitudes of early flash EP (FEP) components, eliminated late components, induced oscillations in visual cortex, and had no discernible effects on FEP component latencies. In contrast, DCM eliminated the FEP N1 component, had little or no effects on amplitudes of late components at moderate concentrations, did not induce oscillations, and affected some latencies. Whereas TOL dramatically increased most somatosensory EP (SEP) components at moderate concentrations with diminishing effect at higher concentrations and exposure times, DCM rather uniformly decreased SEP components in a simple concentration-related way. While TOL and DCM had similar effects on brainstem auditory-evoked response (BAER) component latencies, they had opposite effects on the profile of changes in component amplitudes.

These results highlighted the acute pharmacologic specificity of these solvents and indicated that they acted oppositely in several respects. Because exposures to solvent mixtures is common when solvents are used to induce altered states of consciousness, and toluene and DCM are common components of such mixtures, we examined combinations of TOL and DCM. Because the cellular mechanisms by which these solvents affect nervous system function are generally unknown, there is little independent basis upon which to make specific predictions about how these two solvents might interact. From our electrophysiologic studies it could be suggested that at some proportions of mixing the effects might tend to cancel each other (e.g., the SEP), whereas other effects (e.g., BAER component latencies) would be exacerbated (i.e., the effects might add); since TOL alone affected mostly late components of the FEP and DCM early components, the combination would be expected to severely depress FEP component amplitudes in general.

Several reports indicate that these solvents affect some neurotransmitter systems. Toluene, for example, appears to increase cortical norepinephrine (NE) in awake rats (2), decrease α-adrenergic receptor binding in hypothalamus (16), and increase medullary and midbrain NE and cerebellar, medullary, and striatal

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serotonin (17). Dichloromethane caused a decrease in brain dopamine and increased NE turnover (10), and decreased glutamate and GABA in frontal cortex while increasing glutamine and GABA in posterior cerebellar vermis (4). The endpoints and locations examined in these studies were not comparable for the two solvents, nor very relevant to the pathways associated with sensory processing; we are not aware of any systematic studies of their combined effects on any neurochemical parameters. Thus, we carried out essentially a descriptive and exploratory experiment to characterize how the solvents might combine in their effects on EEG and sensory electrophysiology.

METHOD

Subjects and Surgical Preparation

Twelve male Fischer-344 rats, with average weight at the time of electrode implant of 304 g, were used as subjects. They were kept in 23 × 14 × 45 cm plastic cages (3/cage) housed in laminar flow racks. Food and water were available ad lib. Lights were on from 7 a.m. to 7 p.m. Surgical procedures were like those described before (18). Epidural stainless steel bolts (0-80 × ⅛ in) were placed in the cranium over visual (6.0 mm posterior to bregma and 3.5 mm to the right of midline) and somatosensory (2.0 mm posterior to bregma and 2.0 mm left of midline) cortices and in midline frontal bone over the olfactory bulbs (reference electrode). Wires were soldered to the bolts prior to implant to preclude heat damage to the cortex (6). Hooks embedded in the acrylic headplug were used during testing to hold the head in a frame with rubber bands to maintain proper orientation of the rat to the sources of stimuli (19).

Exposure to Solvents

Mallinckrodt reagent grade solvents were used (TOL 99.8% pure, DCM 99.9% pure), and two of three channels of a mass-flow controlled gas blending system were adjusted to deliver the desired concentrations. One channel gated compressed air through separate 4-l containers of TOL and/or DCM, the outflow of which was mixed with air from another channel in a 500-ml flask. Outflow from the flask was routed through a 190-ml sampling bulb in the line just before entry into the rat exposure chamber. The rat was restrained in a plastic holder with an enclosed front that served as a head-only exposure chamber (19).

Gas flow was continuous throughout a 1-hr exposure at 1,000 to 1,800 ml/min; at this flow rate ambient noise was not loud enough to mask experimental auditory stimuli. Gas concentrations were sampled from a needle in front of the rat's nose. Exposures were calibrated, for each gas separately and combined, against standards by gas chromatography. Each rat was exposed to each gas alone at 10,700 and 16,000 ppm and to combinations of the gases (total = 16,000 ppm). Because asymmetric interactions are possible (9), we used gas proportions of 0.33 and 0.67 of each gas, rather than a single proportion of 0.5, in order to detect any such asymmetrical relationships. The 10,700 ppm exposure to each gas alone was equivalent to the level of the gas during the 67% condition during combined exposures. Exposures for each rat were separated by ten days to three weeks. After EEG/EP recordings were obtained during exposure to air only, the rats were exposed to a gas or gas mixture for 60 min. Recordings were obtained after 25 and 55 min of exposure, and 5, 30, and 60 min after the exposures ended. During the baseline and recovery phases a flow of air was delivered at the same rate as during exposure to the gases.

The same sequence of tests was also obtained in a pseudoexposure session during which the rats were exposed only to air throughout. The order of exposure to the various exposure conditions was counterbalanced across rats.

Electrophysiologic Tests

A battery of electrophysiologic tests (TSTBAT) was used. TSTBAT consisted of samples of the spontaneous EEG and sensory-evoked potentials elicited by tone pips, light flashes, and brief electric shocks to the tail (described below). All recordings were in reference to the anterior electrode. The spontaneous EEG and SEP were recorded simultaneously from the somatosensory and visual cortices. FEPs and BAERs were recorded from visual cortex.

Spontaneous EEG. Four consecutive 5-sec samples of EEG (500 data points) were obtained with a recording bandpass of 1 to 40 Hz.

Pip-evoked brainstem auditory-evoked response (PBAER). This response was elicited by 1.4-msec duration, 16-kHz tone pips (0.2 msec rise and fall), with alternating polarity, delivered through a tweeter (1.5 to 20 kHz) suspended 24 cm directly above the rat's head (adding 0.8 msec to component latencies). Intensity was about 70 dB above the level at which PBAERs are just discernible in rats (20,24). PBAER averages (8-msec epoch) were based on 1,000 pips presented at 18.8/sec, using a recording bandpass of 400 Hz to 6 kHz. This relatively high highpass setting improves the signal-to-noise ratio and the definition of early peaks.

Flash-evoked potential (FEP). FEPs were elicited by a Grass PS-2 strobe lamp (intensity setting = 8) centered 20 cm above and 7 cm in front of the rat, angled toward the rat's face. FEP averages (500-msec epoch) were based on 50 stimuli presented at 0.37/sec, using a recording bandpass of 1 to 55 Hz. This low-pass filter setting reduces 60 Hz interference without distorting the waveform. During testing the chamber was dimly lit (14 FL).

Somatosensory-evoked potential (SEP). SEPs were elicited by 50-μsec duration, 3 mA, cathodal constant-current square waves applied at a rate of 1.3/sec via needles inserted into the mid-ventral aspect of the tail (cathode proximal). The recording bandpass was 5 to 250 Hz (200-msec epoch), and each average comprised 50 samples. Because the SEP recorded from visual cortex was found to be more susceptible to ischemia than that in somatosensory cortex (unpublished observations), recordings were obtained from both cortices in this investigation to determine if there might also be a differential sensitivity to the effects of solvents.

Data Quantification and Analysis

The four individual EEG samples obtained at each test were converted to frequency spectra (0 to 25 Hz) and the spectra were averaged. Mean frequency and integrated power in 4-Hz bands and total power were obtained from the averaged spectra. Evoked potentials were quantified by measuring peak latencies, peak-to-peak amplitudes, and sometimes integrated amplitude.

Statisical analyses were carried out in several steps, as follows:

1. A two-factor repeated-measures ANOVA was carried out, using the raw data, for each EEG and EP measurement. The two factors were: 1) "Conditions," i.e., pseudoexposure, single gas conditions at 16,000 ppm, and the mixtures (also totaling 16,000 ppm), and 2) "Tests" within sessions, i.e., measurements obtained during Baseline (one test), Exposure (two tests at 25 and 55 min), and Recovery (three tests). Since each rat was tested in all conditions, both factors were repeated measures. Only if this ANOVA exhibited a significant ($p<0.05$) Condition × Test interaction were additional analyses carried out; this interaction indicated whether or not there was any significant difference in

profiles across Tests among the five conditions (pseudoexposure, two mixtures, and two single gases at 16,000 ppm).

2. To determine if any of the exposures totaling 16,000 ppm taken alone caused a change from Baseline to Exposure different from any such change in the pseudoexposure control condition, two-factor ANOVAs were carried out on raw data from the preexposure Baseline run and the 55-min exposure run for each 16,000 ppm exposure Condition by itself, as shown below:

Condition	Pseudoexposure		Exposure (55 min)	
Test	Baseline (T1)	"Exposure" (T3)	Baseline (T1)	Exposure (T3)
Rat R_1				
R_2				
R_n				

Four of these were done for each variable (two single gases and two mixtures). We refer to the Baseline test as T1 (the first test of a session) and the 55-min Exposure test (the third test) as T3. Only the Condition × Test interaction was of interest, referred to hereafter as the T1/T3 Condition × Test interaction. In general, we expected no differences between Conditions in the Baseline test, but significant deviation from the control session during Exposure, giving rise to significant T1/T3 Condition × Test statistical interactions.

3. Combined effects were evaluated as follows: To simplify matters, each rat's baseline data were subtracted from that obtained at the 55-min exposure or pseudoexposure condition; this allowed comparison of mixtures, single gases, and predicted effects by paired-comparison *t*-tests rather than ANOVAs. *t*-Tests involved comparing the 67% mixes with scores from the equivalent (10,700 ppm) condition for each gas alone (e.g., 67/33 TOL/DCM vs. 10,700 TOL), with a predicted value assuming additivity of the effects of the single gases. The predicted values (PRE) of a measured variable due to the mixture was taken to be the sum of the effect of the major gas alone at the level in the mixture plus the effect of the minor gas alone at its level in the mixture, i.e., in this case

$$\text{PRE TOL} = (\text{TOL}_{0.67}) + (\Delta\text{DCM}_{0.33})$$
$$\text{PRE DCM} = (\text{DCM}_{0.67}) + (\Delta\text{TOL}_{0.33})$$

Since data were not obtained for 5,300 ppm exposures alone (equivalent to the 33% level in the mixtures), an estimate for this condition was obtained by regression analysis from the 0-to-10,700 mean concentration-response function. The estimated effects at 5,300 ppm appear as solid triangles in the concentration-response figures. This information was used to obtain PRE. Predicted values are shown on the concentration-response functions as open squares.

If the concentration-response curves were linear and the two gases were equipotent and additive, the predicted value for a parameter due to exposure to the mixture would be the same as that produced by the highest concentration of either gas alone. If the gases were not equipotent, however, the predicted value would be different from that produced by the highest concentration. In general, significant deviation of the observed effect of the mix from PRE would indicate nonadditivity. Nonadditive results might reflect either independent or synergistic (neither additive or independent) actions (27). Independent effects would be indicated by similarity of the mixture's effect to that of the major gas alone at the concentration in the mix (EQuiValent concentration). In short, Independence: MIX = EQV; Additivity: MIX = PRE; Synergism: EQV ≠ MIX ≠ PRE. Effects could be in either direction from the baseline, i.e., suppressant or facilitatory of the measured parameter.

Additional analyses were done to examine the statistical characteristics of EEG/EP data when multiple variables were examined. That is, to obtain an empirical estimate of Type I errors or real effect of repeated testing, ANOVAs were carried out across

TABLE 1

AVERAGE (SE) GAS CONCENTRATIONS DURING SIXTY-MINUTE EXPOSURE TO TOLUENE AND DICHLOROMETHANE

	Mixture: Percent Toluene (16,000 ppm Total)						
Gas	0	33	% (actual)	67	% (actual)	100	Alone 10,720
TOL		*6,060	36.6	11,770	68.9	16,560	10,470
		(0.19)		(0.33)		(0.13)	(0.11)
Target ppm		5,280		10,720		16,000	10,720
% Target		114.8		109.8		103.5	97.7
DCM	15,370	10,490	63.4	5,300	31.1		10,570
	(0.17)	(0.27)		(0.30)			(0.08)
Target ppm	16,000	10,720		5,280			10,720
% Target	96.1	97.8		100.4			98.6
Total	15,370	16,550		17,070		16,560	
Target ppm	16,000	16,000		16,000		16,000	
% Target	96.1	103.4		106.7		103.5	

*Entries are mean concentrations. Actual measurement precision was about 200 ppm.

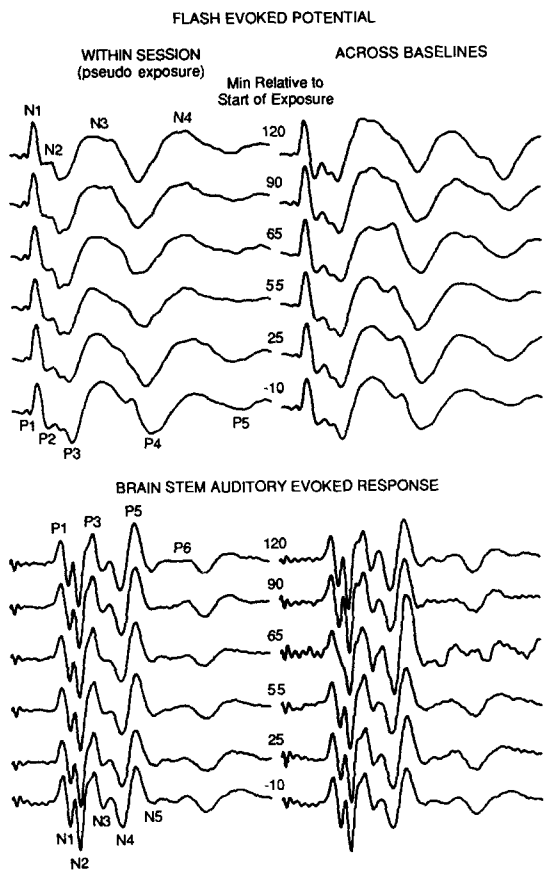

FIG. 1. Group-averaged flash-evoked potentials (upper) and brainstem auditory-evoked responses elicited by tone pips during pseudoexposure sessions (left column) and in several independent baseline tests. In this and subsequent figures, numbers on the right of the waveforms for the within-session records are the times with respect to the start of the 60-min exposure (during pseudoexposures air was delivered throughout the session).

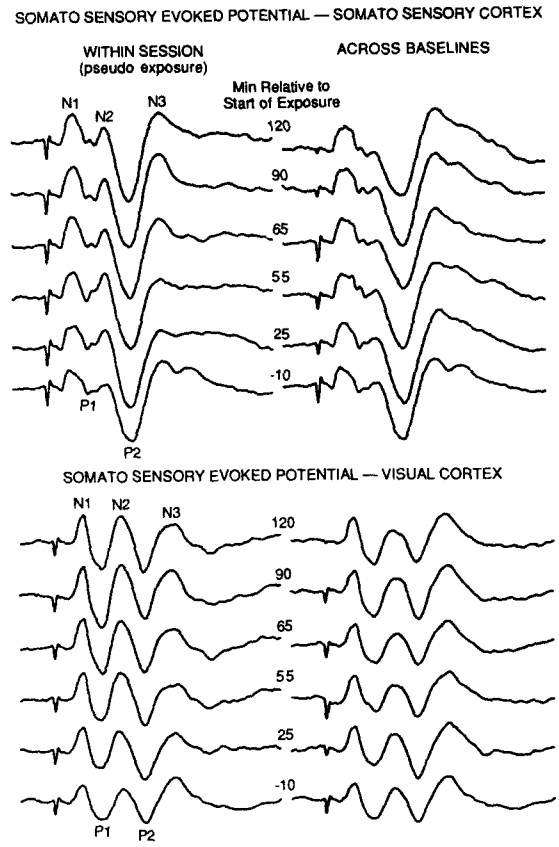

FIG. 2. Group-averaged somatosensory-evoked potentials in somatosensory (upper) and visual cortices during pseudoexposure sessions (left column) and in several independent baseline tests.

all Tests for the pseudoexposure condition alone, and across all Conditions for the preexposure baseline test alone.

RESULTS

Exposure Levels and General Observations

Gas concentrations were measured every ten min during exposures, starting 5 min after the beginning of exposure, and at 5, 15, and 25 min postexposure. Average concentrations (SEs) for the 60-min exposure periods are shown in Table 1. Total exposures ranged from 96 to 107% of the target concentration of 16,000 ppm and exposures to the mixed gases ranged from 98 to 115% of target concentrations (5,280 and 10,720 for the 33 and 67% conditions, respectively). The average ppm for the three measurements during the recovery phase for TOL and DCM postexposure were 244 and 75 ppm, respectively.

Although exposed to the gases several times, the exposures were separated sufficiently in time to preclude any deleterious effects on the rats. There were no indications of ill health and at the end of the experiment the rats weighed, on the average, 106 g more than they did at the time of electrode implants.

Mean colonic temperatures at the 55-min exposure test were always within ±0.1°C of the baseline temperature, a change insufficient to have any significant effect on electrophysiologic measures.

Baseline Comparisons of Electrophysiologic Parameters

Figure 1 shows group-averaged FEPs and PBAERs obtained during the pseudoexposure sessions (left column) and during the baseline run preceding the several exposures. Somatosensory EPs recorded from somatosensory and visual cortices are shown in Fig. 2. It is clear that the general configuration of PBAERs and SEPs remained remarkably stable both within sessions when the rats were exposed only to air, and across the several baseline tests. Cross-correlations of the first baseline waveform (the lowest waveform in each column) with subsequent EPs obtained during the pseudoexposure session averaged (±SD) .97 (.01), .92 (.02), .84 (.02) for the PBAER and somatosensory (Som, Vis Cortex) responses, respectively. Cross-correlations over the baseline tests preceding each exposure were .94 (.07), .97 (.02), .96 (.02), for these same EPs. Despite these consistencies in shape, most SEP component amplitudes varied significantly across tests within the pseudoexposure, but the pattern of change across tests was different for each component (see Fig. 16).

As we suggested before (23), FEP component latencies tend to lessen during repeated testing, and several highly significant changes were observed in this experiment (the latencies of components N1, P3, N3, P4, and N4 decreased, *p*s ranging from 0.001 to 0.004). This effect was evident primarily during the pseudoexposure sessions, but there was also a marginally signif-

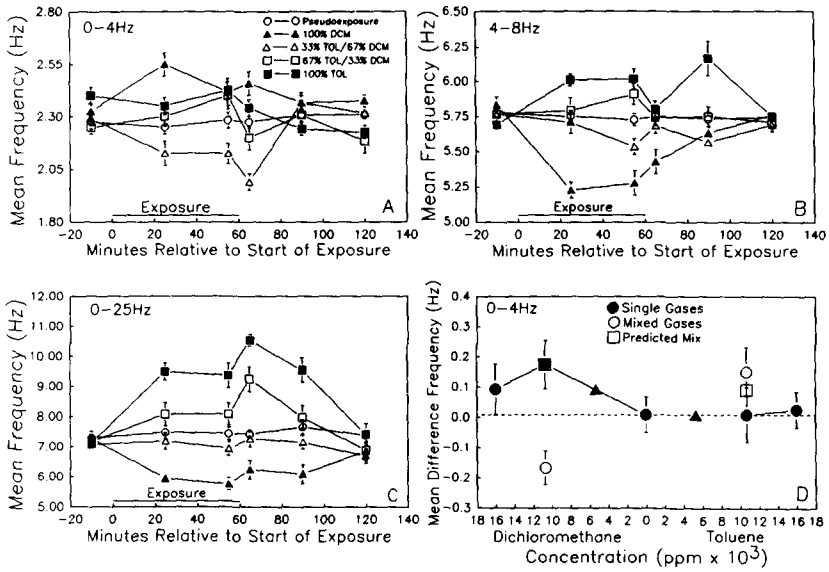

FIG. 3. (A–C) Mean bipolar EEG frequency in several frequency bands as a function of time relative to the start of exposure and the several exposure conditions. (D) Concentration-response function for the 0–4 Hz frequency band measured after 55 min of exposure, showing an antagonistic synergism by toluene of dichloromethane's effect.

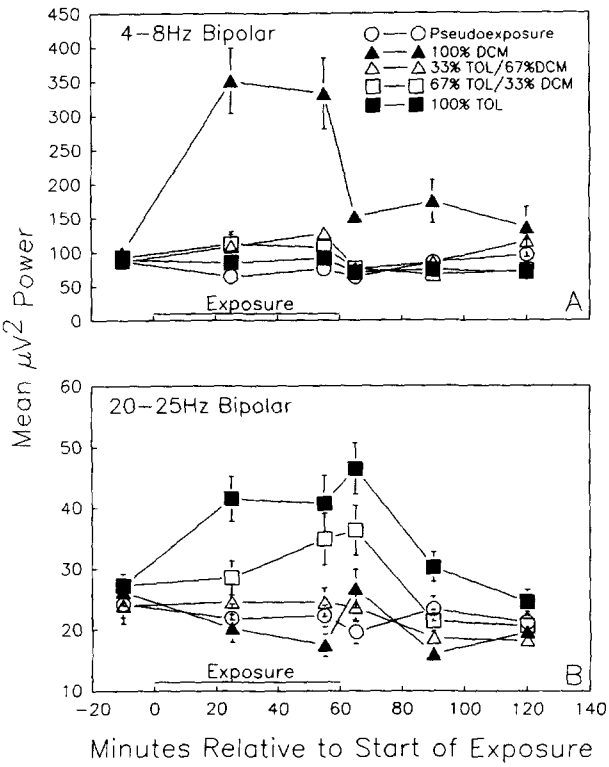

FIG. 4. Mean bipolar EEG power in the 4–8 Hz (A) and 20–25 Hz (B) frequency bands as a function of time relative to the start of exposure and the several exposure conditions.

icant ($p = 0.05$) decrease in N1 latency across the several baselines. The apparent decrease in N4 latency across baselines was not significant. FEP cross-correlations were .80 (.07) and .96 (.08) for within the pseudoexposure session and across multiple baselines, respectively. The first was somewhat less than correlations for the PBAERs, and SEP from somatosensory cortex. SEP correlations from visual cortex also tended to be relatively low. This may be a characteristic of visual cortex. However, the preexposure baseline waveforms were very consistent.

Parameters of the EEG and EPs were, in general, more stable across independent baseline tests than during a single recording session. Only 6% of ANOVAs across baselines were significant, whereas 27% of ANOVAs across tests-within-sessions were significant. Of the latter, a decrease in referentially recorded EEG frequency (related to respiration as recorded from the reference electrode over olfactory bulb) and the decreases in FEP component latencies, because of their temporal patterns, appear to reflect real changes, whereas alterations in SEP amplitudes and the other miscellaneous significant changes probably reflect type I errors, about 11% of the comparisons.

Solvent Effects on Electrophysiologic Parameters

General comparison of DCM and TOL. Because of the extent, and somewhat complex nature, of the results of this experiment, a summary of them is provided here.

EEG. DCM decreased mean frequency, primarily in the 4–8 Hz band, whereas TOL increased it. DCM increased low frequency EEG power and decreased high frequency power; TOL had the opposite effects. TOL synergistically counteracted DCM's effects on low frequency EEG bands.

PBAER. Both DCM and TOL increased component latencies but TOL had the larger effect. TOL, but not DCM, increased the amplitudes of late components. No synergistic interactions were observed for frequency, but TOL synergistically antagonized the effect of DCM on N3P4 amplitude.

FEP. DCM eliminated the N1 component; TOL had no effect.

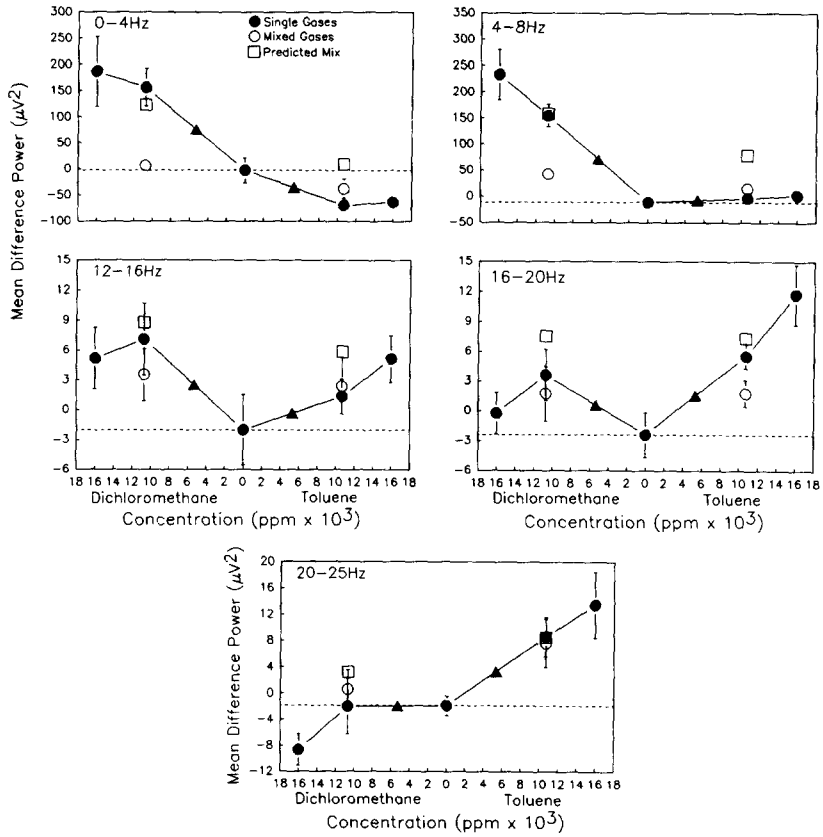

FIG. 5. Concentration-response functions for bipolar EEG power in frequency bands exhibiting significant overall Condition × Test interactions, measured after 55 min of exposure. Note the counterclockwise rotation of the functions as EEG frequency increases. Combined effects are shown by results of the mixed gases in relation to predicted values.

TOL eliminated N3 and later components; DCM had no effect. A synergistic effect like that observed for low frequency EEG was evident for late components.

SEP. DCM specifically eliminated the N1b component; TOL had a slight, indirect effect. TOL dramatically increased middle component amplitudes; DCM greatly reduced most amplitudes. TOL synergistically counteracted DCM's effect on N1P1 amplitude.

Combinations of these solvents produced interactions of all major types—independence, additivity and synergism. Independent and additive interactions each occurred about 36% of the time, whereas synergisms occurred about 24% of the time; ambiguities prevented clear categorization in the remaining cases. The EEG and SEP evidenced the clearest instances of opposite effects of the two solvents.

Electroencephalogram.

Frequency. When recorded with respect to the anterior reference electrode, the low frequency (0–4 Hz) EEG was dominated by olfactory bulb activity. Because of spectral scaling it was not possible to obtain reliable frequency data above 12 Hz from those records. Therefore, bipolar records were also obtained by taking the difference between the two referentially obtained EEGs.

In the bipolar records there were significant overall Condition × Test interactions for EEG bands 1 (0–4 Hz), 2 (4–8 Hz), and 7 (0–25 Hz), as shown in Fig. 3A–C. In band 1, although DCM tended to increase the frequency, only the combination of TOL/DCM in a ratio of 33/67 showed a significant ($p<0.0001$) T1/T3 interaction; frequency decreased in this condition. The patterns of change were similar to each other for the 4–8 Hz and whole band (0–25 Hz) ranges. The T1/T3 interactions for 16,000 ppm alone were significant ($ps<0.002$) for both TOL and DCM in both bands. The combined gas conditions were significant ($ps<0.05$) for the T1/T3 analysis only in the 4–8 Hz band. The two solvents had opposite effects—toluene increased frequency and DCM decreased it.

Concentration-response curves for bipolar 0–4 Hz EEG frequency scores, including the 10,700 ppm conditions alone, are shown in Fig. 3D. Although TOL alone had no effect on EEG frequency in band 1, and DCM tended to increase it, the 33/67 (TOL/DCM) combination had a depressant effect, i.e., frequency was decreased considerably below that for 10,700 ppm DCM alone, $t\text{DCM}(11)=4.9$, $p<0.0001$, and the predicted value ($t=6.1$, $p<0.0001$). This represented a strong synergistic antagonism. The result for the TOL 67/33 mix was ambiguous; it was not significantly different from the effect of 10,700 ppm TOL alone or the predicted value, indicating either independence or additivity in this direction. In the 4–8 Hz and 0–25 Hz bands, the effects of the mixed gases were what would be expected from additivity, i.e., the combined effects were less than the gases alone because of the opposite effects of the two solvents.

Power. Changes in EEG power induced by the solvents appeared to occur predominantly in visual cortex, although in some cases similar trends were evident in somatosensory cortex as well. However, we systematically analyzed power of the bipolar EEG to

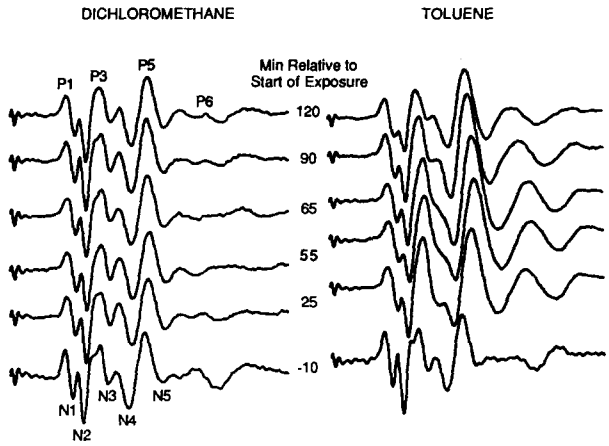

FIG. 6. Group-averaged brainstem auditory-evoked responses during exposure to 16,000 ppm dichloromethane (left column) and toluene.

eliminate influences of the olfactory bulbs; this also eliminated activity common to somatosensory and visual cortices.

In the 0–12 Hz bipolar EEG bands, the major deviation from normality was enhancement of power by 16,000 ppm DCM aone, e.g., 4–8 Hz (Fig. 4A). Examination of the referentially recorded plots for somatosensory and visual cortices indicated localization of this effect to visual cortex.

At the higher frequencies (16–25 Hz), TOL tended to enhance power, $F_{T1/T3}(20\text{–}25) = 2.4$, $p = 0.05$, whereas DCM decreased it ($F_{T1/T3} = 7.2$, $p = 0.02$) (Fig. 4B, 20–25 Hz).

As shown in the concentration-response curves (Fig. 5), DCM enhanced bipolar EEG power in the lowest band, whereas TOL slightly suppressed it. With increasing EEG frequency, the effect of the solvents gradually reversed; the curves rotated counterclockwise. At 0–8 Hz, where TOL alone had either mildly suppressant (0–4 Hz) or no effect (4–8 Hz), its presence eliminated or suppressed, respectively, the enhancing effect of DCM. The differences between the gas alone at 10,700 ppm and the mixed conditions were significant for both bands when the major gas was DCM, $t_{0\text{–}4}(11) = 3.5$, $p = 0.005$; $t_{4\text{–}8}(11) = 4.5$, $p = 0.001$, but not when TOL was the major gas. In both bands the value produced by the 67% mixture differed significantly from the predicted effect. An asymmetric antagonistic synergism is suggested by these data, i.e., whereas TOL synergistically reduced the DCM effect, 33% DCM had no effect on the response to TOL.

Above 12 Hz effects of the combined gases were generally not significantly different from effects of the single gases at 10,700 ppm or they were close to the expected value, either independent or additive properties were expressed. An exception was the 67/33 (TOL/DCM) mix in the 16–20 Hz band where power was significantly less than the predicted value, $t(11) = 4.2$, $p = 0.004$, indicating a synergistic antagonism.

Brainstem Auditory-Evoked Response

Latency. For clarity we report data for just P1 and P5 latencies and the P1–P5 interwave time. Group-averaged waveforms contrasting the effects of 10,000 ppm TOL and DCM are shown in Fig. 6. Significant (maximum $p = 0.001$) overall Condition × Test interactions were obtained for both latencies and the interwave time (Fig. 7). Significant T1/T3 Condition × Test interactions, involving just the baseline and 55-min exposure tests and comparing the pseudoexposure with each other condition separately, were obtained for both P1 and P5 in all exposure conditions. P1–P5 time T1/T3 interactions were significant only for TOL alone and the 67/33 (TOL/DCM) condition. As shown in Fig. 7, P1 latency was prolonged in all exposure conditions, but the effect was most pronounced for TOL and the effect increased as the concentration of TOL increased. TOL alone and the 67/33 (TOL/DCM) mix increased P5 latency and the P1–P5 interwave time as well (Fig. 7). DCM also increased P5 latency, but not P1–P5 time.

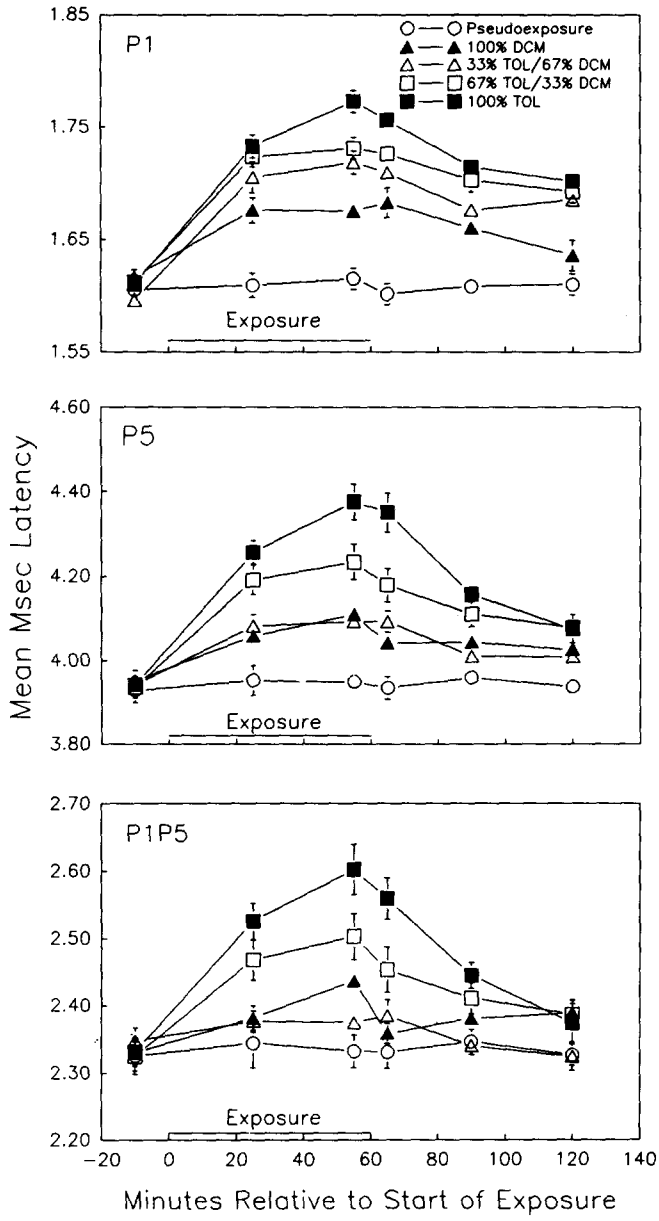

FIG. 7. Latencies and interwave time of brainstem auditory-evoked response components P1, P5, and P1–P5 as a function of time relative to the start of exposure and the several exposure conditions.

As shown in the concentration-response function for P1 latency (Fig. 8), there was a more linear function for TOL than DCM. The effect of the 67/33 (TOL/DCM) mixture did not differ from that of

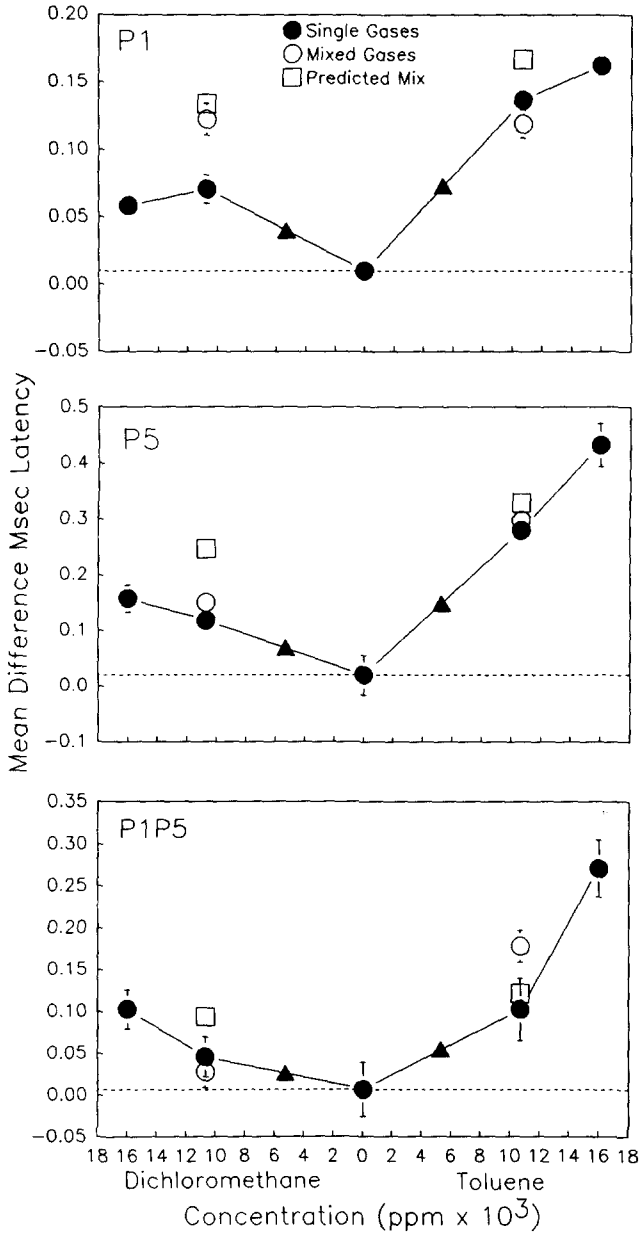

FIG. 8. Concentration-response functions of latency and interwave time for brainstem auditory-evoked response components P1, P5, and P1–P5 measured after 55 min of exposure. Combined effects are shown by the results of the mixed gases in relation to predicted values.

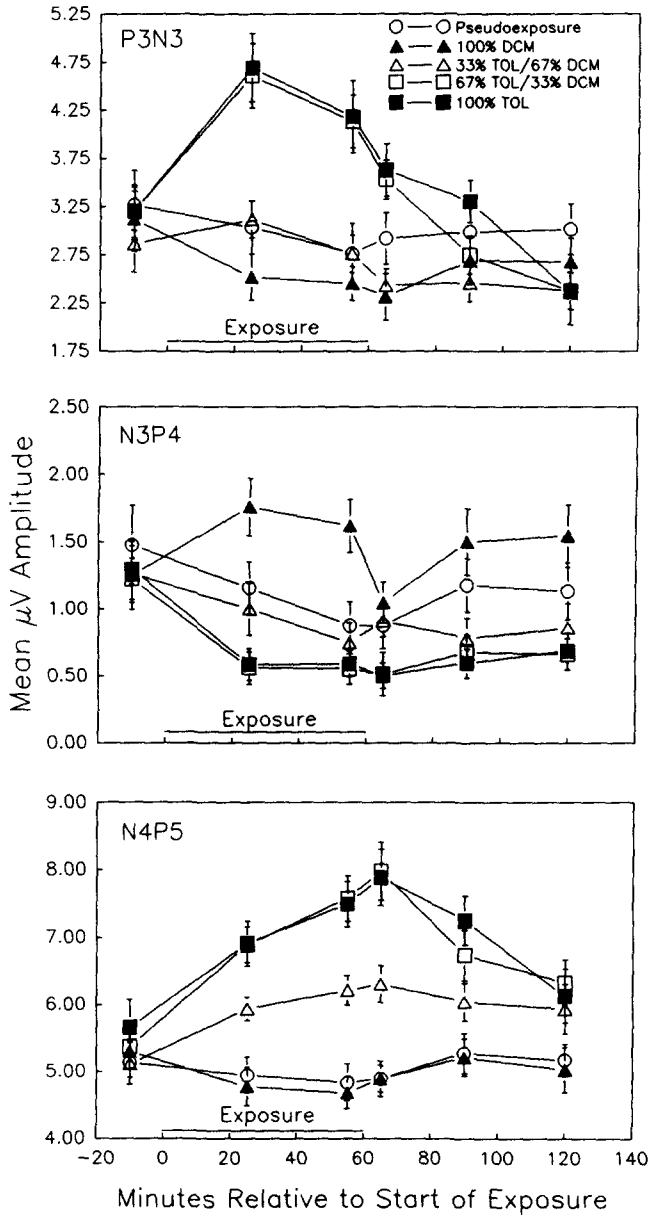

FIG. 9. Peak-to-peak amplitudes of selected brainstem auditory-evoked response components as a function of time relative to the start of exposure and the several exposure conditions.

10,700 ppm TOL alone, i.e., the effect of TOL was not influenced by DCM.

When DCM was the major gas, the mixed exposure resulted in P1 latency prolongation significantly greater than that caused by 10,700 ppm DCM alone, $t10K(11) = 3.2$, $p = 0.008$. The magnitude of this combined effect was equivalent to the predicted effect, indicating additivity in this relationship.

When TOL was the major gas, the effect of the mixture on P5 latency (Fig. 8) was equal to the effect of 10,700 ppm TOL alone, indicative of independence of the solvents. The same conclusion was indicated when DCM was the major gas; the effect of the mixture was significantly less than the predicted value, but equivalent to 10,700 ppm DCM alone.

P1–P5 time (Fig. 8) in the mixed conditions did not differ significantly from those of 10,700 ppm of the gases alone. These results indicate independent actions. However, there was a tendency for the 67/33 (TOL/DCM) mix to be greater than 10,700 ppm TOL alone, suggestive of a slight synergism. This was supported by the fact that the mixed effect was significantly greater than the predicted value, $t(11) = 3.0$, $p = 0.01$.

Amplitude. Significant (maximum $p = 0.001$) overall Condition × Test interactions for PBAER amplitudes were obtained for all components except P1 and P1N1. TOL alone increased N2P3 (not shown) and P3N3 amplitudes (Fig. 9), and although DCM had no

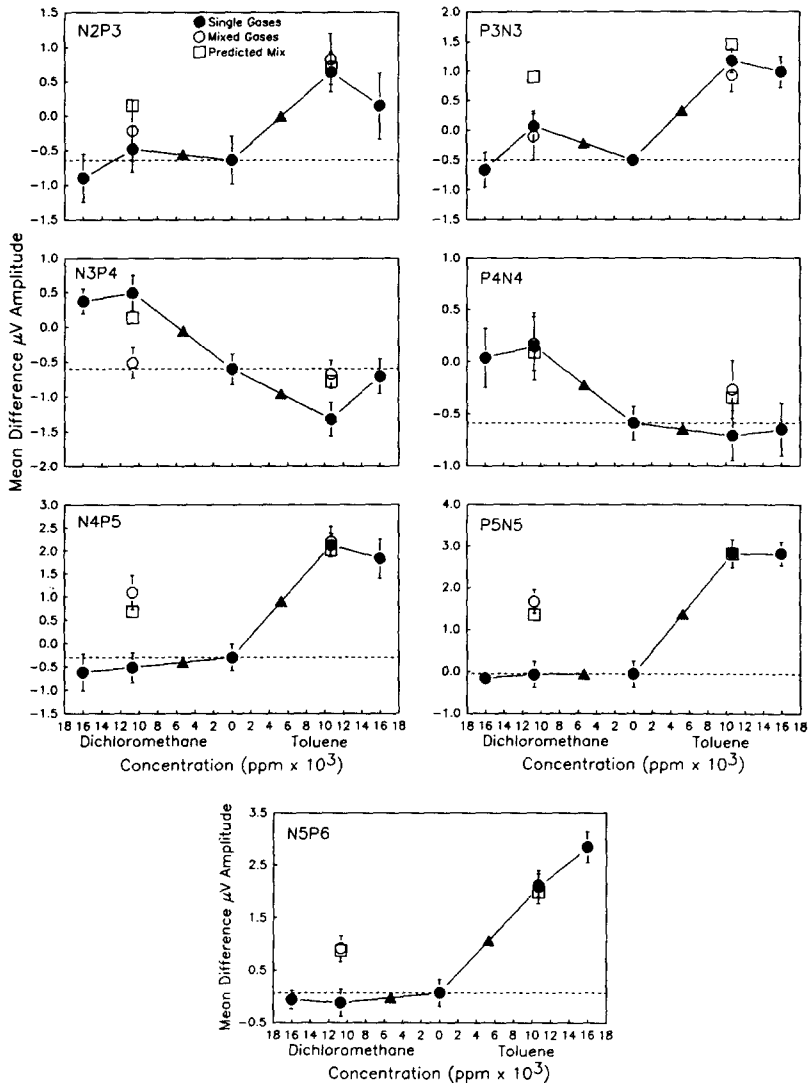

FIG. 10. Concentration-response functions for amplitudes of several brainstem auditory-evoked response components measured after 55 min of exposure. Combined effects are shown by the results of the mixed gases in relation to predicted values.

significant effect on either N2P3 or P3N3 components, it appeared to synergize the TOL effect, e.g., the effect of the 67/33 (TOL/DCM) combination was as great as 16,000 ppm TOL alone on the P3N3 component (but it was also like the 10,700 ppm TOL alone, which was as potent as the 16,000 ppm exposure, see Fig. 10).

TOL appeared to depress components N3P4 and P4N4 (not shown), especially at the 25-min test, but the T1/T3 interactions were not significant due to a similar trend in the pseudoexposure condition. DCM alone significantly enhanced N3P4 amplitude, but all combinations were without effect (Fig. 9).

Solvent effects on components N4P5 through N5P6 were similar to those on some earlier components; DCM alone was without effect, TOL enhanced the components, and the 67/33 combination was as, or almost as, effective as TOL alone (see, e.g., N4P5, Fig. 9). The enhancement of N5P6 by TOL was particularly dramatic as this component was barely discernible during exposure to air only or DCM (see Figs. 1 and 6), and may be related to TOL ototoxicity (15,20).

Figure 10 shows concentration-response functions for PBAER component amplitudes. Only one (N3P4) of the component *t*-tests comparing the 67/33 (TOL/DCM) condition with 10,700 ppm TOL alone was significant, so, for the most part, 33% DCM exerted no effect (independence); the mixed value for component N3P4 when TOL was the major gas was equivalent to the predicted score, indicating additivity. In contrast, for components N3P4, N4P5, P5N5, and N5P6, toluene subtracted from or added to the effects of DCM when it was the major gas (DCM had negligible effects on the last three components). The deviations of the mixture from 10,700 ppm DCM alone were significant for each of these four components (*p*s ranged from <0.004 to 0.02). Except for component N3P4 the deviations were all close to the predicted values, indicating additivity of the solvents. However, the mix produced a lower amplitude of component N3P4 than expected when DCM was the major gas, indicating synergistic antagonism, $t(11)=2.9$, $p=0.01$.

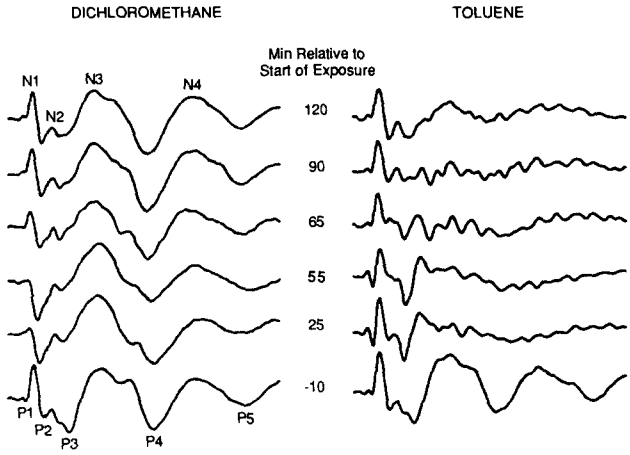

FIG. 11. Group-averaged flash-evoked potentials during exposure to 16,000 ppm dichloromethane (left column) and toluene.

Flash-evoked potential.

Amplitude. Although the P1 component was small and difficult to score, we quantified it as well as possible. As shown in the group-averaged waveforms (Fig. 11), this component was not evident during exposures to 16,000 ppm DCM alone, but reappeared in the recovery phase. TOL seemed to enhance P1 amplitude. The overall Condition × Test interaction was significant, but only the decrease caused by 16,000 ppm DCM alone (T1/T3 interaction) approached significance, $F_{DCM}16K(1,11) = 5.0$, $p = 0.05$ (Fig. 12, P1).

TOL had no effect on N1 amplitude, but DCM virtually eliminated the component (Figs. 11 and 12), revealing a normally hidden positive wave. The decline in amplitude was significant in all conditions involving DCM [e.g., TOL/DCM F67/33(1,11) = 6.7, $p = 0.02$]. Because of distortion of the waveform, middle components could not be reliably scored, and there was not good agreement between peak-to-peak scores and an integrated amplitude score, so the graphic representation of differential effects of TOL and DCM (Fig. 11) must suffice. Late components (N3–N4) were not significantly affected by 16,000 ppm DCM alone, but were depressed by TOL alone (e.g., N3P4, Fig. 12) and both combinations [e.g., for 33/67 TOL/DCM, $F_{N3P4}(1,11) = 23.0$, $p = 0.001$]. Because TOL almost completely eliminated these components, their individual scoring was somewhat arbitrary. However, integration from P3 through P5 showed the same pattern of graphic and statistical effects (Fig. 12).

Concentration-response functions for selected FEP component amplitudes are shown in Fig. 13. For P1 amplitude, despite the lower or absent statistical significance in the initial analyses, the 10,700 ppm-alone exposures were compatible with concentration-related effects of both gases, and the effects were opposite one another. Neither mixture caused a significant deviation from the 10,700 ppm exposures, indicating independent actions. For P1N1 amplitude, the 67/33 mixture's effect was equivalent to the predicted effect, indicating the additively counteractive effect of DCM. Because of differential potencies, the effect of the 33/67 TOL/DCM mixture was negligible, but, as predicted, it was slightly less than 10,700 ppm DCM alone. Results for the N3P4 and P4N4 components were alike but somewhat variable. Therefore, the scores for these were averaged for each rat and analyses were carried out on the combined scores. As DCM alone had essentially no effect, the score from the 67/33 (TOL/DCM) mixed condition was equivalent to both the 10,700 ppm TOL alone condition and the predicted value. In contrast, when DCM was the major gas there was a substantial synergism; the small amount of toluene produced a significant departure of the mixture's effect from both 10,700 ppm and the predicted value ($t_{PRED} = 5.4$, $p = 0.002$).

Latency. DCM appeared to decrease P1 latency (Fig. 14A), but the T1/T3 interaction was not significant; however, P1 was significantly prolonged (T1/T3 interaction) by 16,000 ppm TOL and the 67/33 (TOL/DCM) mixture, e.g., F16K(1,11) = 14.7, $p = 0.003$. There was a slight increase in N1 latency during exposure to toluene alone (Fig. 14B); this trend, coupled to a decrease during the pseudoexposure condition, resulted in significant T1/T3 interactions for the major TOL conditions, e.g., F67/33(1,11) = 13.2,

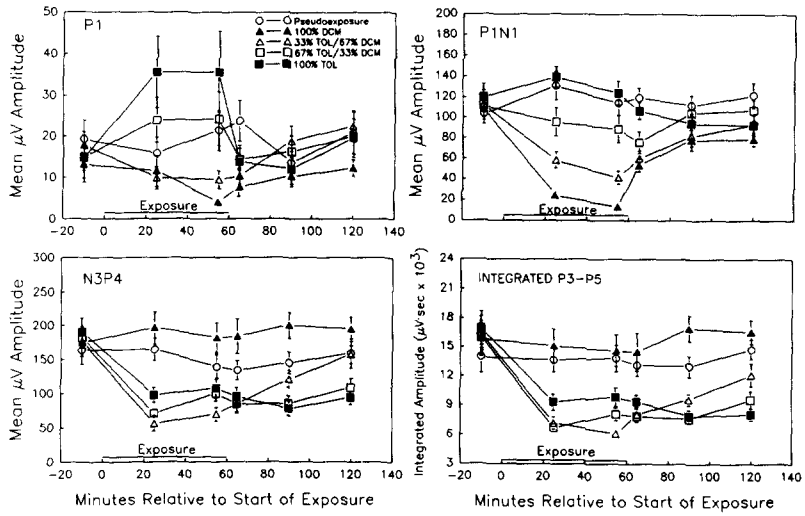

FIG. 12. Peak-to-peak amplitudes of several flash-evoked potential components and integrated amplitude of late components as a function of time relative to the start of exposure and the several exposure conditions. Integrated amplitude showed the same effects as scoring individual late components, which could not be done with complete confidence.

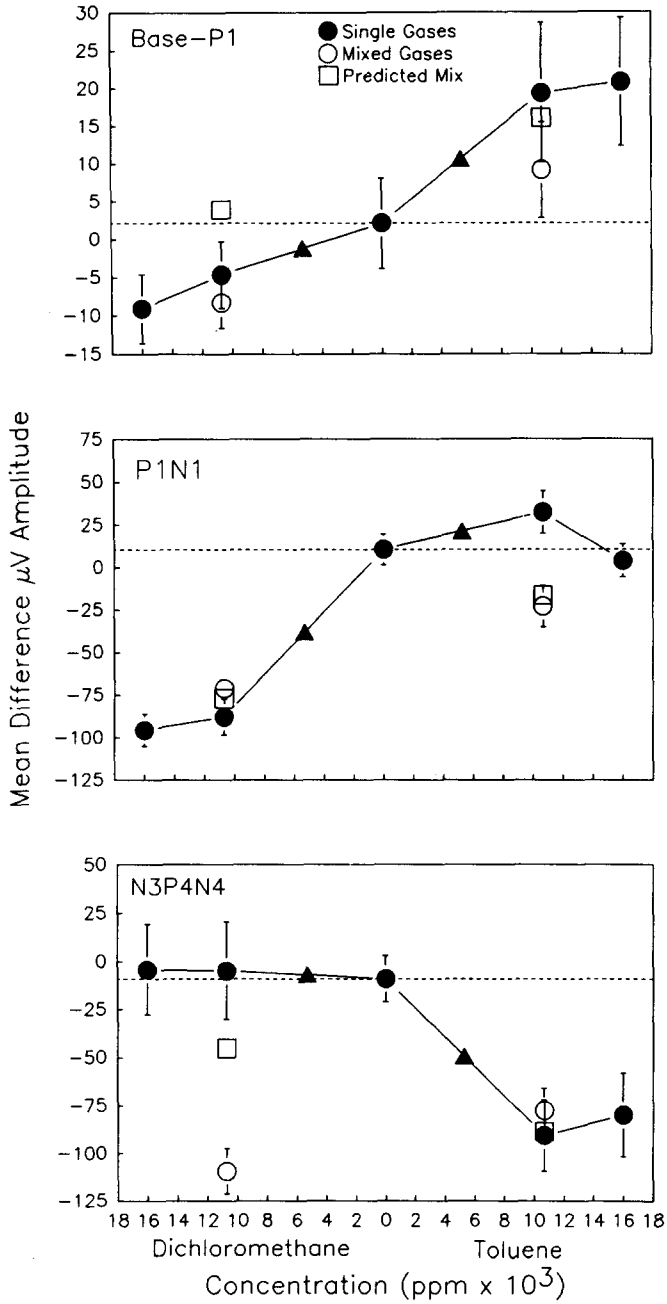

FIG. 13. Concentration-response functions for amplitudes of selected flash-evoked potential components measured after 55 min of exposure. Combined effects are shown by the results of mixed gases in relation to predicted values.

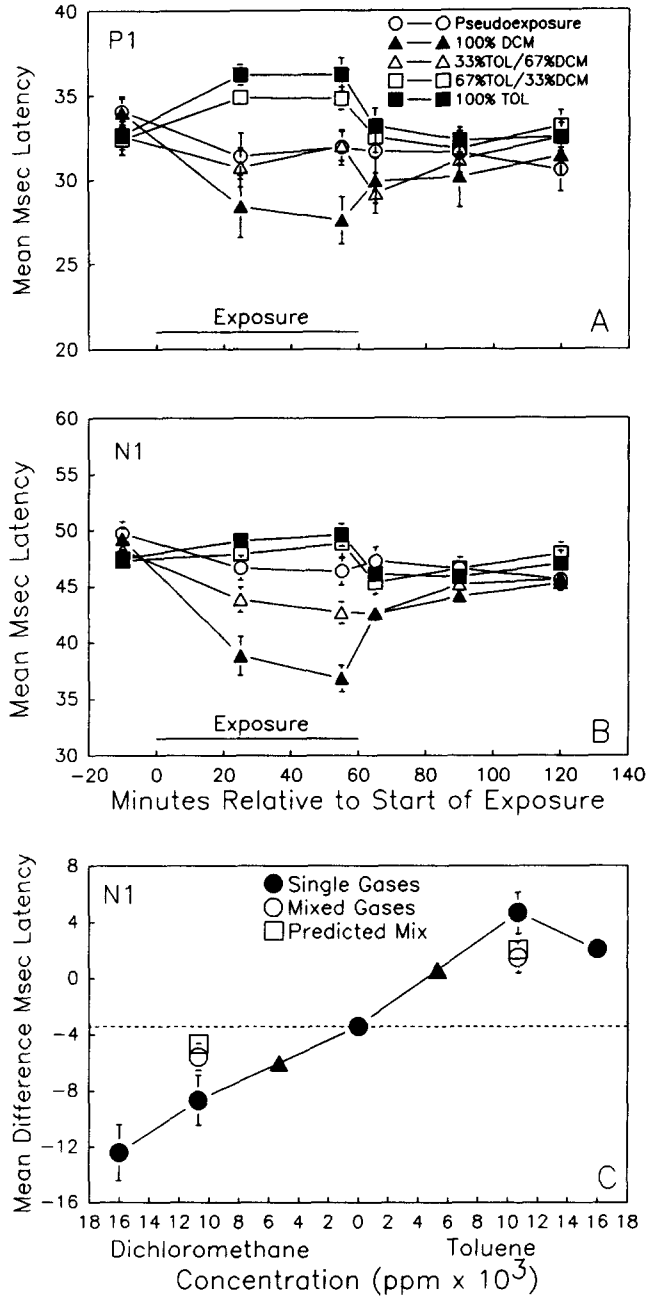

FIG. 14. (A, B) Mean latencies of components P1 and N1 of the flash-evoked potential as a function of time relative to the start of exposure and the several exposure conditions. (C) Concentration-response function for latency of the N1 component. Combined effects are shown by the results of mixed gases in relation to predicted values.

$p = 0.004$. DCM at 16,000 ppm alone, and as the major gas in the mix, appeared to decrease N1 latency, e.g., $F16K(1,11) = 13.1$, $p = 0.004$, but this may have been due simply to scoring the beginning of the positive wave revealed by the disappearance of N1.

As indicated before, middle components could not be quantified with confidence for either solvent and there were no systematic trends in the Condition × Test plots (now shown). Late component latencies could not be scored reliably either during exposure to TOL because they were virtually eliminated (Fig. 11). Also, during the recovery phase when the components began to reappear no systematic effects could be discerned. There was only one significant effect of DCM on late components; at 16,000 ppm alone it caused N4 latency to increase relative to the pseudoexposure condition, $F_{T1/T3}(1,11) = 6.5$, $p = 0.03$.

The concentration-response function is shown only for FEP N1 latency, as its latency could be most reliably scored (Fig. 14C).

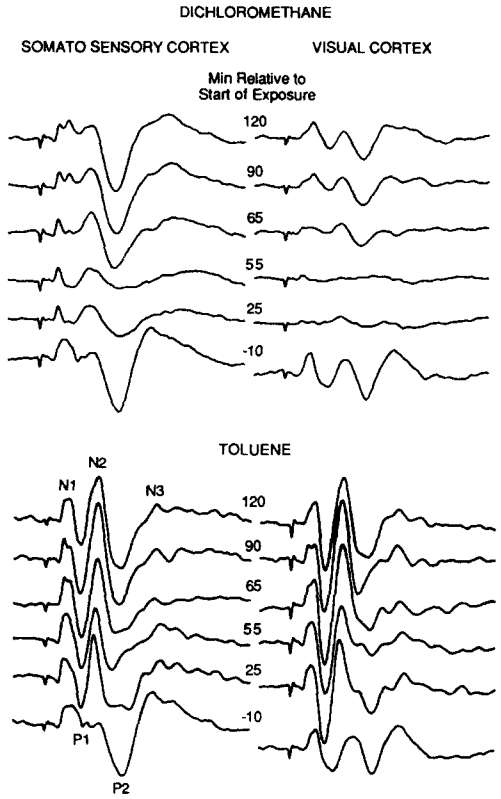

FIG. 15. Group-averaged somatosensory-evoked potentials in somatosensory (left column) and visual cortices during exposure to 16,000 ppm dichloromethane (upper) and toluene.

Values for both mixtures were equivalent to the predicted values, indicating additivity of the effects.

Spectral composition. As evident in Fig. 11, and as reported before (21), TOL induces oscillations in the FEP. These are not time-locked oscillations (22) and probably reflect the same phenomenon described above for visual cortex EEG. Results were very comparable to those shown in Fig. 4B.

Somatosensory-evoked potential.

Amplitude. Group-averaged waveforms are shown in Fig. 15. Whereas the waveforms were somewhat different in somatosensory and visual cortices, the effects of the solvents were almost the same (visual cortex actually appeared to be more sensitive to the depressant effects of DCM), so only data from somatosensory cortex are considered here. Because SEP components had a greater tendency than those from other modalities to exhibit reversals of trend during the later part of the exposure period (Fig. 16), we analyzed these data from the 25-min rather than 55-min test. All overall Condition × Test interactions were significant at $p<0.0001$. Component N1 was reduced in size in a concentration-related way by all conditions involving DCM [e.g., T1/T2 interaction for the 67/33 (TOL/DCM) ratio $F(1,11)=5.2$, $p=0.04$]. TOL alone had no effect. DCM alone at 16,000 ppm reduced all later components, e.g., $FP2N3(1,11)=30.4$, $p<0.0001$, except P1N2. In fact, in the group-averaged waveforms (Fig. 15), P1N2 appeared to be slightly enlarged by DCM. All TOL conditions increased the sizes of components N1P1 and P1N2. No condition in these analyses involving TOL affected N2P2 (however, as shown below, N2P2 amplitude was increased by 10,700 ppm TOL), and all TOL conditions significantly decreased P2N3 amplitude.

In the concentration-response functions (Fig. 17), the mixture with 67% DCM was equivalent to the predicted value except in one case; there was a synergistic response for N1P1, $t(11)=4.5$, $p=0.009$. Slight synergism was evident for components P1N2 and N2P2 when TOL was the major gas in the mix (MIX vs. PRE: $ps=0.03$ and 0.04, respectively), otherwise the effects of 33% DCM were additive or ambiguous (P2N3).

Close inspection of the group-averaged waveforms (Fig. 15) indicated that the N1 component actually comprised two components, N1a and N1b. Both solvents preferentially affected N1b, but, apparently, by different mechanisms. DCM rather specifically eliminated N1b, whereas TOL seemed to affect it indirectly because of its enhancement of the positive-going P1 component.

It is not entirely clear that the increase in the size of component P1N2 during exposure to TOL was actually a change in that component. As is most evident in visual cortex after 25 min of exposure (Fig. 15), the hump on the trailing edge of the putative P1N2 wave might be the component; TOL may have induced the emergence of a normally hidden component. We believe a splitting of component N3 also occurred with exposure to TOL. This is suggested by Fig. 15, but was especially clear during exposure to 10,700 ppm TOL alone (not shown).

Latency. Although the overall Condition × Test interactions were significant for all components except N2 and there were several significant T1/T2 Condition × Test interactions, we do not believe most of the changes reflect a true change in conduction time or synaptic delay, but were a consequence of the disappearance or appearance of components. One probable exception is the change in N3 latency during exposure to DCM. That wave changed in a way compatible with later arrival of neural impulses to the generator site, elevating the later part of the wave and therefore delaying the peak.

The concentration-response function for N3 latency is shown in Fig. 18. The change in latency produced by TOL is due to the split of the component, the two subcomponents occurring earlier and later than the original peak, and the later one being generally larger and therefore most likely to be identified as the peak during scoring. The concentration-response curve was nonlinear for both solvents; at 10,700 ppm, TOL alone tended to decrease N3 latency, but prolonged it at 16,000 ppm. Both mixes produced apparently synergistic effects, i.e., the value in the mixed exposures were significantly different than the predicted values ($ps=0.004$ and 0.03 for DCM and TOL as major gases, respectively). However, because of the reversals in the concentration-response functions the interpretation is somewhat ambiguous.

DISCUSSION

Changes in the EEG and EPs due to TOL and DCM alone were essentially like those observed before (21–23). In this study, however, we more thoroughly analyzed the EEG, evaluating frequency changes within bands as well as power, providing additional information about the electrophysiologic effects of these solvents. As would be expected from general considerations about the reciprocal relationship between EEG frequency and power, these solvents sometimes affected these parameters reciprocally, e.g., DCM decreased frequency in the 4–8 Hz band and increased power. Effects of the solvents varied among the several frequency bands, indicating both the diversity of mechanisms controlling different aspects of the EEG, and the specificity of different solvents in their profiles of effects. With respect to the EEG, TOL and DCM acted oppositely in many respects. This was clear for both frequency and power. Particularly striking was the reciprocity as a function of frequency band, the effects of the solvents on EEG

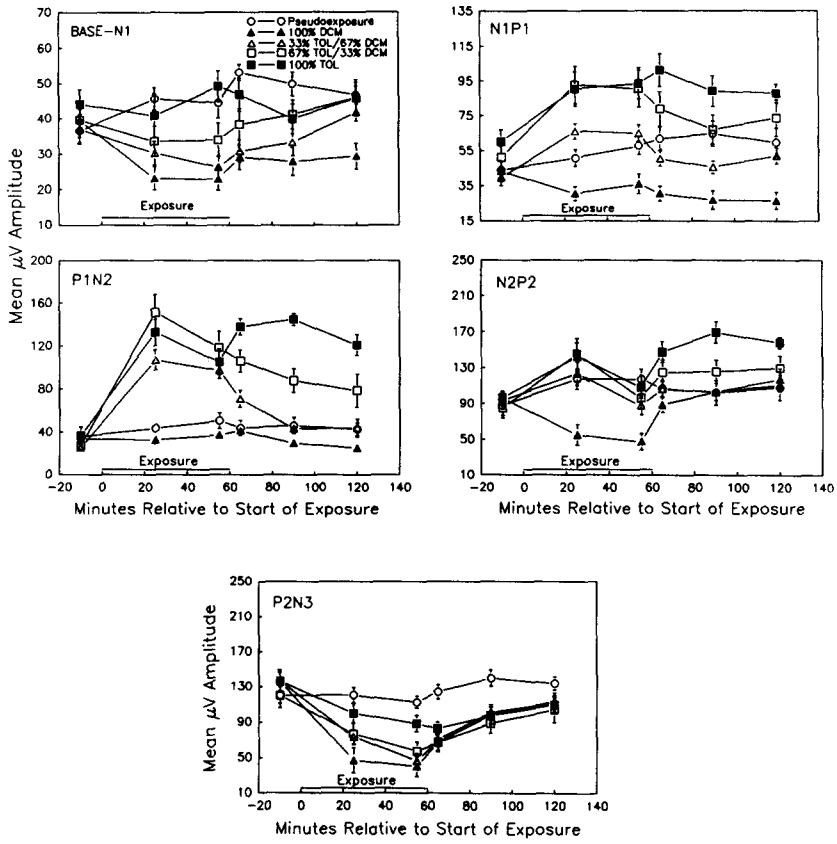

FIG. 16. Mean peak-to-peak amplitudes of somatosensory-evoked potential components as a function of time relative to the start of exposure and the several exposure conditions.

power reversing as frequency increased (Fig. 5).

The most striking synergistic interaction on the EEG was the depression by 33% TOL of the power-enhancing effect of DCM in the 0–8 Hz bands (Fig. 5). Effects in the 4–8 Hz and 20–25 Hz bands made an interesting contrast. Whereas there were essentially no effects of TOL in the low band and none for DCM up to 10,700 ppm in the high band, 33% TOL synergistically affected the response to DCM, but 33% DCM had no effect on the response to TOL. Thus, in the 4–8 Hz band TOL had a latent or hidden influence, evident in conjunction with the other solvent. For DCM, however, there was a real noneffect. A similar synergism was the depression by 33% TOL of DCM's slight increase of 0–4 Hz bipolar EEG frequency.

These solvents also acted reciprocally with respect to their major effect on the SEP (TOL enhancing, DCM depressing amplitudes). For this response, too, the direction of the major synergism (component N1P1) was for 33% TOL to affect the response to DCM (Fig. 17). Of the earliest components, DCM had a very specific affinity for the N1b component. It is tempting to speculate that DCM's effect on the SEP N1b and the FEP N1 components were mediated through a similar mechanism.

Whereas in this and previous experiments TOL at 10,000 ppm dramatically decreased FEP N3 amplitude, DCM at that level had no effect. The synergistic interaction on late FEP components was asymmetric such that 33% TOL depressed the components in the presence of DCM, but 33% DCM exerted no effect in combination with TOL (another true noneffect of DCM alone). It is likely that the generators of FEP late components, especially the "after-discharge" following component N3, are controlled by brainstem and thalamic nuclei that also control oscillatory parameters of the spontaneous EEG (3). The considerable similarities in the results for low frequency EEG changes and FEP late components [Figs. 5 (0–8 Hz) and 13 (N3P4N4)] in the direction and extent of the synergism make it likely that similar mechanisms are involved in the EEG and FEP effects.

Synergisms evident on PBAER component amplitudes were, as on other EEG and EP parameters, asymmetric, 33% TOL synergistically affected responses to the mixture when DCM was the major gas, but no reciprocal synergisms were evident.

For the most part, these solvents acted independently or additively in their acute neurophysiologic effects, but 24% of the interactions were synergistic, and most of the time the synergisms were asymmetric in favor of TOL. We considered whether this was simply due to different potencies, DCM perhaps being less potent so that its 33% level in the mix would never exceed a threshold for effect. However, this was not generally true. DCM was more potent than TOL in its effects on 0–4 Hz EEG frequency; it was approximately equipotent as TOL in its effects on 4–8 Hz referential EEG frequency; DCM was much more potent in enhancing 4–8 Hz power in bipolar derivations; it enhanced PBAEP N3P4 amplitude as much as TOL depressed that component; DCM was equipotent with respect to changing FEP N1 latency; it was more potent than TOL in depressing SEP P2N3 amplitude; and DCM was more potent than TOL at 10,700 ppm in changing SEP N3 latency.

There are several ways that these solvents could alter the EEG

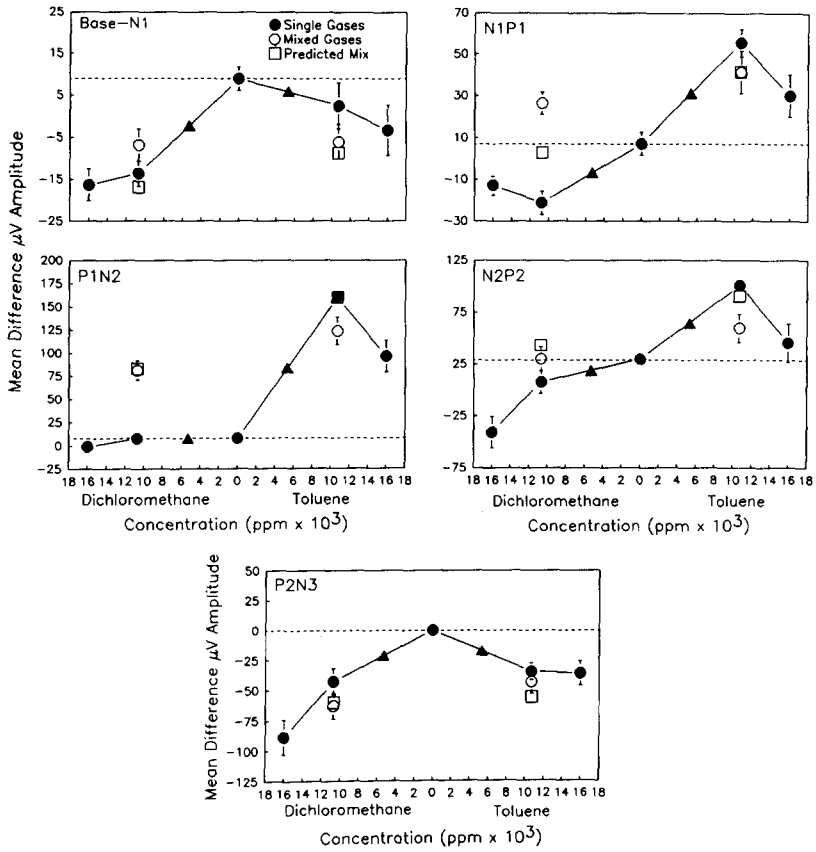

FIG. 17. Concentration-response functions for peak-to-peak amplitude of somatosensory-evoked potential components measured after 25 min of exposure. Combined effects are shown by the results of mixed gases in relation to predicted values.

and evoked potentials. It has been suggested that volatile solvents can alter membrane fluidity (28). This, in turn, can modify cellular functions in a variety of ways; nonspecific effects can be mediated by a general de- or hyperpolarization of the membrane if the membrane is made more permeable (14). On the other hand, by changing the relative exposure of various receptor complexes in the membrane, altered fluidity can induce specific changes in the efficacy of different neurotransmitters (1). Several aliphatic benzene derivatives have also been shown to uncouple oxidative phosphorylation in mitochondria, associated with their hydrophobicity (13). Finally, it is possible that the solvents, or their metabolites, could have direct effects on neurotransmission as suggested in the introduction, e.g., by affecting postsynaptic receptor sites.

The specificities and reciprocities in the effects of TOL and DCM suggest to us that these solvents might act as agonists and antagonists of a particular neurotransmitter, or, more likely, affect different but reciprocally related neurotransmitters. Determining this is complicated by the fact that little is known about the neurotransmitters that mediate specific parameters of the EEG or evoked potentials, so inferences cannot be made directly on the basis of changes in those parameters. The specific effects noted in this experiment that seem to imply changes in neurotransmission include the opposite effects on EEG power and changes with EEG frequency bands (Fig. 5), the specific affinity of DCM for the SEP N1b and FEP N1 components, TOL's affinity for late components of the FEP, and the fact that the combined effects on BAER P1–P5 time were the same as the individual effects of the gases—implying either effects on different fibers or neurotransmitters. The fact that only DCM affected FEP N1 and only TOL affected FEP N3 has the same implication. Although specific pharmaco-

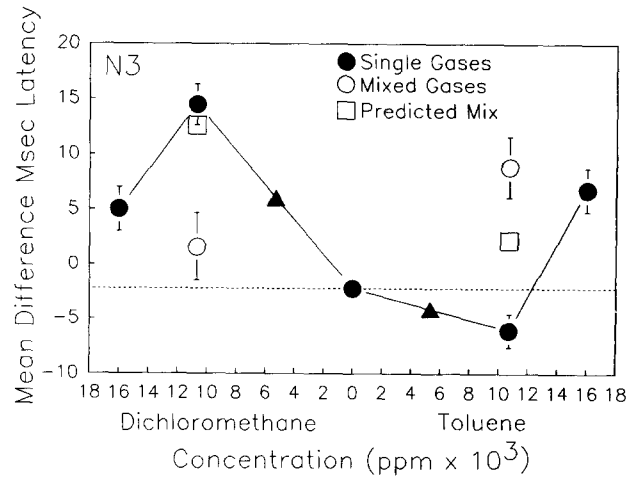

FIG. 18. Concentration-response function for latency of the N1 component of the somatosensory-evoked potential measured after 25 min of exposure. Combined effects are shown by the results of mixed gases in relation to predicted values.

logic agents have been examined with respect to their effects on EPs (5,25), we are not aware of any results that exhibit the degree of specificity for FEP N1 shown by dichloromethane (at certain concentrations); thus, it is also difficult to conclude on the basis of similar effects what neurotransmitters or receptors might be affected by these solvents.

One finding of potential relevance is that etomidate, an hypnotic anesthetic, produces "giant" SEPs in humans (7) that appear to be much like the enhanced SEPs in rats exposed to toluene. Etomidate is a GABA-mimetic (8,12). How increased GABA-ergic activity would translate into enlarged EPs is conjectural, but it suggests a meaningful beginning to pharmacologic investigations of these solvents. It would be interesting, for example, to determine the extent to which the effects of etomidate on EP parameters paralleled that of toluene.

DCM is metabolized, in part, to carbon monoxide (CO), but it is unlikely that CO mediates the effects of DCM on EPs, as we have indicated before (23). Arterial carboxyhemoglobin levels above 20% are necessary to alter EPs and the metabolism of DCM is a saturable pathway so that high COHb levels are not produced even with exposure to high levels of DCM; for example, we obtained only 7% COHb in rats exposed for 1 hr to 15,000 ppm DCM.

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REFERENCES

1. Antonian, L.; Shinitzky, M.; Samuel, D.; Lippa, A. S. AL721, a novel membrane fluidizer. Neurosci. Biobehav. Rev. 11:399–413; 1987.
2. Arito, H.; Tsuruta, H.; Nakagaki, K. Acute effects of toluene on circadian rhythms of sleep-wakefulness and brain monoamine metabolism in rats. Toxicology 33:291–301; 1984.
3. Bigler, E. D. Neurophysiology, neuropharmacology, and behavioral relationships of visual system evoked after-discharges: A review. Biobehav. Rev. 1:95–112; 1977.
4. Briving, C.; Hamberger, A.; Kjellstrand, P.; Rosengren, L.; Karlsson, J. E.; Haglid, K. G. Chronic effects of dichloromethane on amino acids, glutathione and phosphoethanolamine in gerbil brain. Scand. J. Work Environ. Health 12:216–220; 1986.
5. Dyer, R. S.; Bercegeay, M. S.; Mayo, L. M. Acute exposures to p-xylene and toluene alter visual information processing. Neurotoxicol. Teratol. 10:147–152; 1988.
6. Dyer, R. S.; Jensen, K. F.; Boyes, W. K. Focal lesions of visual cortex—effects on visual evoked potentials in rats. Exp. Neurol. 95:100–115; 1987.
7. Ebner, A.; Deuschl, G. Frontal and parietal components of enhanced somatosensory evoked potentials: a comparison between pathological and pharmacologically induced conditions. Electroencephalogr. Clin. Neurophysiology 71:170–179; 1988.
8. Evans, R. H.; Hill, R. G. GABA-mimetic action of etomidate. Experientia 34:1325–1327; 1978.
9. Finney, D. J. Probit analysis. London: Cambridge University Press; 1971.
10. Fuxe, G.; Hansson, T.; Ogren, S.-O.; Agnati, L. F.; Eneroth, P.; Härfstrand, A.; Gustafsson, J.-A. Effects of chronic toluene exposure on central monoamine and peptide receptors and their interactions in the adult male rat. Toxicology 52:103–126; 1988.
11. Grabski, D. A. Toluene sniffing producing cerebellar degeneration. Am. J. Psychiatry 118:461–462; 1961.
12. Hill, R. G.; Tabener, B. Z. Some neuropharmacological properties of the new non-barbiturate hypnotic etomidate (R(+)-ethyl-1-α-methylbenzyl) imidazole-5-carboxylate. Br. J. Pharmacol. 54:241; 1975.
13. Mickiewicz, W.; Rzecyzcki, W. Effect of styrene and other alkyl benzene derivatives on oxidation of FAD- and NAD-linked substrates in rat liver mitochrondria. Biochem. Pharmacol. 37:4439–4444; 1988.
14. Midgley, P. J.; Rutter, G. A.; Thomas, A. P.; Denton, R. M. Effects of Ca^{2+} and Mg^{2+} on the activity of pyruvate dehydrogenase phosphate phosphatase within toluene-permealized mitochondria. Biochem. J. 241:371–377; 1987.
15. Pryor, G. T.; Dickinson, J.; Howd, R. A.; Rebert, C. S. Transient cognitive deficits and high-frequency hearing loss in rats exposed to toluene. Neurobehav. Toxicol. Teratol. 5:53–57; 1983.
16. Rank, J. Xylene induced feeding and drinking behavior and central adrenergic receptor binding. Neurobehav. Toxicol. Teratol. 7:421–426; 1985.
17. Rea, T. M.; Nash, J. F.; Zabik, J. E.; Born, G. S.; Kessler, W. V. Effects of toluene inhalation on brain biogenic amines in the rat. Toxicology 31:143–150; 1984.
18. Rebert, C. S. Multisensory evoked potentials in experimental and applied neurotoxicology. Neurobehav. Toxicol. Teratol. 5:659–671; 1983.
19. Rebert, C. S.; Davis, E. E.; Juhos, L. T.; Jensen, R. A.; Pryor, G. T.; Robin, D. E. Effect of acute respiratory acidosis on multimodality sensory evoked potentials of Long Evans rats. Int. J. Psychophysiol. 8:155–168; 1989.
20. Rebert, C. S.; Day, V. L.; Matteucci, M. J.; Pryor, G. T. Sensory evoked potentials in rats chronically exposed to trichloroethylene: Predominant auditory dysfunction. Neurotoxicol. Teratol.; submitted.
21. Rebert, C. S.; Matteucci, M. J.; Pryor, G. T. Multimodal effects of acute exposure to toluene evidenced by sensory-evoked potentials from Fischer-344 rats. Pharmacol. Biochem. Behav. 32:757–768; 1989.
22. Rebert, C. S.; Matteucci, M. J.; Pryor, G. T. Acute electrophysiologic effects of inhaled toluene on adult male Long-Evans rats. Pharmacol. Biochem. Behav. 33:157–165; 1989.
23. Rebert, C. S.; Matteucci, M. J.; Pryor, G. T. Acute effects of inhaled dichloromethane on the EEG and sensory-evoked potentials of Fischer-344 rats. Pharmacol. Biochem. Behav. 34:619–629; 1989.
24. Rebert, C. S.; Sorenson, S. S.; Howd, R. A.; Pryor, G. T. Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. Neurobehav. Toxicol. Teratol. 5:59–62; 1983.
25. Rebert, C. S.; Tecce, J. J.; Marczynski, T. J.; Pirch, J. H.; Thompson, J. W. Neural anatomy, chemistry, and event-related brain potentials: an approach to understanding the substrates of mind. In: McCallum, W. C.; Zappoli, R.; Denoth, F., eds. Cerebral psychophysiology: Studies in event-related potentials. Amsterdam: Elsevier; 1986:343–392.
26. Sharp, C. W.; Brehm, M. L., eds. Review of inhalants: Euphoria to dysfunction. Washington, DC: U.S. Govt. Printing Office, NIDA Monograph 15; 1977.
27. WHO. Health Effects of Combined Exposure in the Work Environment. Technical Report Series 662. Geneva: WHO; 1981.
28. Wikberg, J. E. S.; Hede, A. R.; Post, C. Effects of halothane and other chlorinated hydrocarbons or α_2-adrenoceptors in the mouse cortex. Pharmacol. Toxicol. 61:271–227; 1987.