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Evidence of Tolerance Following Repeated Exposure to Toluene in the Rat[1]

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REES, D. C., R. W. WOOD AND V. G. LATIES. *Evidence of tolerance following repeated exposure to toluene in the rat.* PHARMACOL BIOCHEM BEHAV 32(1) 283–291, 1989.—Toluene shares pharmacological properties with other abused central nervous system depressants such as ethanol and the barbiturates. Although tolerance has been clearly demonstrated for these classic CNS depressants, evidence of tolerance following repeated toluene exposure is equivocal. The present work examined if tolerance would develop to the effects of repeated toluene exposure on learned behavior and examined the possibility that external discriminative stimuli could influence these effects. Two variants of a fixed-consecutive-number schedule of reinforcement were used as components in a multiple schedule. The components differed in whether or not behavior within them was under the control of external discriminative stimuli. Rats were exposed daily for two hours to toluene (1780 to 4500 ppm). Different patterns of effects emerged from repeated exposure; some rats displayed tolerance while the performance of others deteriorated. Behavior controlled by external discriminative stimuli was more resistant to disruption and showed tolerance more readily than did behavior not under such control.

Stimulus control Toluene Behavioral tolerance Rats Depressants Abuse

ALTHOUGH the determinants of inhalant abuse potential remain largely unknown, inhalants may produce a type of intoxication similar to that produced by central nervous system (CNS) depressants such as the barbiturates, alcohols and benzodiazepines (1). Consistent with this hypothesis is evidence demonstrating that abused inhalants share pharmacological properties with these CNS depressants. For example, the aromatic hydrocarbon toluene is abused (28) and has reinforcing properties in an animal model (33,34) as well as antipunishment (5,36) and anticonvulsant actions (36). Toluene and other volatile inhalants share discriminative stimulus properties with barbiturates (22, 25, 27). In addition, toluene's effects on motor-performance resemble those of the general anesthetics, alcohols and barbiturates [e.g., (14–16)].

Another pharmacological property common among classic CNS depressants is the development of tolerance [cf. review (29)]. Tolerance has been defined as a "reduction in any given effect of a drug when the same dose is administered repeatedly, and to the need to give an increased dose to obtain the original effect" (2). Since toluene may produce a depressant-like intoxication, tolerance might be expected to develop to its behavioral effects. Tolerance development has been reported in human inhalant abuse (6,17). Experimental evidence of such tolerance development in animal models, however, is equivocal and contradictory. Moser and Balster (14) found no evidence of tolerance, and, instead found increased sensitivity (reverse tolerance). Some studies have reported both tolerance and reverse tolerance [e.g., (7)], while others reported only tolerance [e.g., (30)].

The reasons for these disparate findings are not clear. Previous work from this laboratory has shown that animals can differ in their sensitivity to acute toluene exposure (35) and in the peak blood levels achieved during exposure (24). Between-group experimental designs, therefore, might allow individual differences to obscure detection of tolerance de-

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TABLE 1
SUMMARY OF TREATMENT CONDITIONS FOR REPEATED TOLUENE EXPOSURES

Exposure (ppm)	Animal No.	Acute (N=11)	Days 1–5	6–11	12–23	24–28	29–38	39–41
1780	43,44	(4)	T-A	T-A	T-A	T-A	T-A	A-A
3000	10		T-A	Z	A-A			
400	18,22,23	(5)	T-A	T-A	T-A	T-A	T-A	A-A
	19,21,25	(5)	A-T	A-T	A-T	A-T	T-A[a]	A-A*
4500	65,71	(5)	T-A	T-A	T-A	T-A	T-A	A-A*
	70,80	(4)	A-T	A-T	A-T	T-A[a]	T-A	A-A[b]

Z: Increased exposure concentration to 4000 ppm.
*Four days of A-A.
[a]Crossover condition.
[b]Two days of A-A.
Toluene before, air after session: T-A; Air before, toluene after session: A-T; air before, air after session: A-A.

velopment. On the other hand, if tolerance does not develop or is of limited magnitude compared with other abused CNS depressants, then toluene may produce a different type of intoxication.

Use of operant procedures can generate stable baselines upon which to detect changes over time (10). The present study used a multiple schedule of reinforcement to examine the behavioral effects of daily toluene exposure, and paid particular attention to performance of individual animals. In one component, a lever press was reinforced only if preceded by a minimum number of consecutive responses on another lever; no cues indicated that the minimum number had been reached. In the other component, discriminative stimuli signalled completion of the response requirement.

Our previous work with the same schedule of reinforcement found that behavior under the control of external discriminative stimuli was more resistant than behavior not under such control to behavioral disruption caused by acute *d*-amphetamine administration (9,23) or by acute exposure to toluene (35). The present study examined whether behavior under such control would remain more resistant to the effects of repeated exposure to toluene as well, as was the case in our previous work with repeated *d*-amphetamine administration (26).

To ascertain if a history of reinforcement under the influence of toluene would alter the behavioral effects of repeated toluene exposure, some rats were exposed to toluene preceding the behavioral testing and others were exposed following it. A similar strategy has been used to test a variety of psychoactive agents including other CNS depressants (2).

METHOD

Subjects

Thirteen male adult Long-Evans rats were kept at 300±20 g for the duration of the study. Animals had different drug-treatment histories: prior exposure to toluene (rat Nos. 10, 18, 19, 22, 23, 24, 25, 43 and 44), prior injections of either *d*-amphetamine (rat Nos. 65, 70 and 80) or chlorpromazine (No. 71). Prior exposures to toluene were always separated by at least 72 hours and occurred at least four months before initiation of these studies. Sensitivity to toluene's behavioral effects was not associated with prior drug treatment.

Apparatus

Lehigh Valley Electronics rat chambers with two Gerbrands levers mounted on the front wall, a white jewel light above each, were used for these experiments. A 76 dB white noise was always present. Sweetened-condensed milk diluted with two parts water was used as the reinforcer; 0.1 ml was presented for three sec. Approximately 0.26 N was required to move the right lever and record a response; only 0.18 N was required for the left lever. Differences in lever force served to accentuate the differences between the two levers.

Procedure

The training procedures and contingencies of the multiple schedule baseline have been described in detail elsewhere (23). Briefly, animals were trained to perform on a fixed-consecutive-number schedule of reinforcement (12). At least eight consecutive left-lever responses were always required before a right-lever response would produce milk delivery. In one component, the FCN-S^D or signalled variant, a light-tone complex served as a discriminative stimulus. The left-lever light set the occasion for left-lever responding. With the completion of the minimum response requirement, termination of the left light, simultaneous illumination of the right-lever light and presentation of a tone signalled that a right-lever response would be reinforced. In the other component, the FCN or unsignalled variant, no external cues were presented to signal food availability. In both components, if fewer than eight responses on the left lever preceded a right-lever response, the requirement was reset.

Each session began with the discriminated component, which remained in effect for 11 runs, a run being defined as at least one left-lever response followed by a right-lever response. The FCN-S^D and FCN components alternated every ten runs until 100 runs (50 per component) had been completed. (Data from the first run were excluded from the analysis.) The criterion for stability of baseline performance was, generally, at least 12 successive sessions without systematic changes in performance measures [(23), p. 245].

Since previous work [e.g., (9, 11, 23)] indicated that rats usually receive reinforcement for approximately half the response runs during FCN, but would almost always satisfy

TABLE 2

EFFECTS OF TOLUENE ON PERCENT RUNS ≥8% (±2 S.E.) FOR FCN-S^D AND FCN COMPONENTS

Toluene Exposure (ppm)	Animal No.	Control		Acute Exposure				Repeated Exposure[c] (Presession and last 10 days)		Crossover Day		% Change[a]	
		FCN-S^D	FCN	FCN-S^D Mean	FCN-S^D First	FCN-S^D Last	FCN Mean	FCN-S^D	FCN	FCN-S^D	FCN	FCN-S^D	FCN
1780	44	100 ± 0	51 ± 25	100 ± 0	100	100	0	96 ± 2	5 ± 2	*	*	*	*
	43	98 ± 2	83 ± 3	84 ± 3	88	80	13 ± 15	100 ± 0	0 ± 0	*	*	*	*
4000	23	95 ± 5	44 ± 21	41 ± 11	24	48	0 ± 0	54 ± 11	4 ± 3	56	4	+400	−33
	22	99 ± 3	51 ± 22	77 ± 15	64	64	0 ± 0	99 ± 1	7 ± 4	100	18	−26	0
	18	99 ± 1	38 ± 20	95 ± 8	98	82	10 ± 6	96 ± 3	12 ± 6	100	30	+20	+10
	21[b]	95 ± 5	48 ± 19	77 ± 18	86	80	10 ± 9	77 ± 4	5 ± 2	76	0	−100	−21
	19[b]	100 ± 0	49 ± 19	69 ± 34	44	100	0 ± 0	63 ± 24	5 ± 3	100	8	−87	0
	25[b]	97 ± 14	23 ± 13	81 ± 22	10	88	8 ± 7	80 ± 8	16 ± 8	54	6	−87	+17
4500	65	98 ± 1	61 ± 4	99 ± 2	100	96	2 ± 2	68 ± 15	3 ± 1	98	16	0	+122
	71	97 ± 3	53 ± 10	62 ± 24	64	80	4 ± 6	85 ± 9	4 ± 2	84	0	+17	−100
	70[b]	100 ± 0	80 ± 3	53 ± 35	41	14	5 ± 5	76 ± 9	10 ± 4	94	16	+11	−79
	80[b]	99 ± 1	32 ± 6	97 ± 3	98	92	3 ± 3	76 ± 13	2 ± 2	90	2	−10	−96

*Not applicable.

[a]Expressed as percent Crossover Day of Mean of 5 preceding days. Crossover day represents the day on which animals who had previously been exposed to toluene after the behavioral session were exposed to toluene before testing. + Indicates an increase in runs ≥8(%); − indicates a decrease.

[b]Repeatedly exposed after behavioral session.

[c]I.e., days 29 through 38 with toluene before, air after sessions, which had followed 28 days with air before, toluene after sessions (cf., Fig. 5).

the minimum requirement during FCN-S^D, differences in reinforcement frequency between the components were minimized. The probability of food reinforcement during the FCN-S^D was set to 0.5. Thus, on the average, runs were reinforced with milk one out of every two times that the minimum response requirement was satisfied in the external discriminative stimulus condition.

Exposure Protocol

Rats were exposed in 28-liter chromatography jars into which was introduced approximately 30 liters/min of clean compressed air that had first been passed through an air purifier (Del-Monox, Deltec Engineering, Inc.). Different toluene vapor concentrations were generated by varying the proportion of air shunted through a fritted-glass disk in a 500-ml gas-washing bottle containing spectrophotometrically pure toluene (Fisher T330). The bottle sat in an ice bath. Glass wool at the top of the bubbler prevented the delivery of aerosolized toluene. Toluene concentrations were monitored by an infrared spectrophotometer (Foxboro, Miran 1A) and were within five percent of the nominal values.

Behavioral effects of toluene were assessed after removing the animal from the exposure chamber after two hours and carrying it immediately to the test chamber 15 meters away, a procedure that caused approximately 90-sec delay before behavioral evaluation commenced. Sessions continued until subjects completed 100 runs (excluding the first run in the FCN-S^D component). In general, most sessions were completed within 60 min. The longest session was approximately 90 min. Acute exposures usually occurred on Tuesdays and Fridays; control sessions or air exposures occurred on the remaining weekdays.

In a preliminary experiment, rat 10 was exposed daily for 5 times to two hours of 3000 ppm toluene. Because the behavioral effects diminished, the exposure level was raised to 4000 ppm on day 6 for an additional 6 days to see if the original effect could be reinstated. Additional animals were assigned to the different treatment conditions summarized in Table 1. A range of concentrations were selected to examine the concentration dependency of tolerance development; the concentrations ranged from nearly anesthetic down to levels demonstrated to produce selective performance impairment without large effects on response rate (35). Four or five acute-effect determinations were performed twice-weekly; then the rats were exposed daily, 7 days/week. Those rats given 4000 or 4500 ppm were divided into subgroups, with half under each condition given toluene before each behavioral session and air afterwards. The other group was exposed to air before each behavioral session and to toluene within 90 sec afterwards for two hours. After signs of tolerance were observed in the before-session exposure subgroup at each exposure concentration, animals in the after-session subgroup were then exposed to toluene before the session and air afterwards. The reversal of the order of exposure occurred on day 29 (i.e., "crossover" day) for the 4000 ppm group, and on day 24 for the 4500 ppm group. Following completion of the different exposure regimens, animals were exposed to air both before and after behavioral sessions.

Data Analysis

The principal dependent variable was runs ≥8(%), the

percentage of runs that had met the eight consecutive response requirements for reinforcement. It is a measure of run length that has proven to be adequately sensitive for the identification of effects modulated by stimulus control (11,19). For the FCN schedule, this measure is directly proportional to food reinforcement frequency, which can be calculated by dividing runs ≥8(%) by 2 since there were 50 runs per component per session. For the FCN-S^D schedule, this measure is approximately four times the reinforcement frequency since the probability that food reinforcement would be delivered upon completion of a run meeting the minimum requirement was set to 0.5. Our criteria for indicating tolerance was that performance must show a consistent and progressive return towards baseline levels and that the session lengths must be equal to or less than that observed following acute exposures.

Overall rate was defined as the total number of left lever responses during a whole session excluding the time occupied by the food presentation and the first run of each session. Total session time was also recorded.

RESULTS

Although the behavioral effects of toluene varied among animals, consistent patterns of effects emerged and were repeatable in different animals. For some animals, diminution of the acute behavioral effects occurred following repeated exposure, whereas other animals showed progressive deterioration of performance.

Control Performance and Acute Exposure Effects

Following air exposure, animals on average met the consecutive response requirement 98% of the time (±1.0%; ±2 S.E.) in the FCN-S^D component and 51% (±10.0) during the FCN component (Table 2). Overall response rates (responses/sec) were comparable between components under control conditions; FCN-S^D, 1.38 (±0.20) and FCN, 1.36 (±0.38). Mean total session times varied among animals ranging from 9 to 25 min (Table 3).

Acute toluene exposures were given twice per week and produced large decreases in the obtained number of reinforcements at all three concentrations (Table 2). Signalled performance generally remained more resistant to toluene's acute effects than did unsignalled performance, as can be seen by comparing columns 3 and 5 and columns 4 and 8. Similar results were observed for rat 10 (Fig. 1) who was studied before the others and for whom the sequence of testing differed. The cumulative record shows that on the first day of toluene exposure (equivalent to an acute exposure), total session time increased substantially and performance in both components was initially impaired.

Overall response rates were, in general, comparably affected between components for all treatment conditions. For example, in the 4000 ppm group the response rates (mean responses/sec; ±2 S.E.) following acute exposure were in FCN-S^D (0.32±0.17) and in FCN (0.32±0.09). The total time to complete a behavioral session increased for all animals following acute exposures at the two highest concentrations (column 4, Table 3). At the lowest concentration total session times remained the same or decreased.

Signs of recovery between the first and last acute exposures were evident for some animals and were characterized as a decrease in total session time (columns 5 and 6, Table 3). Total session times decreased by over eight min in five of the

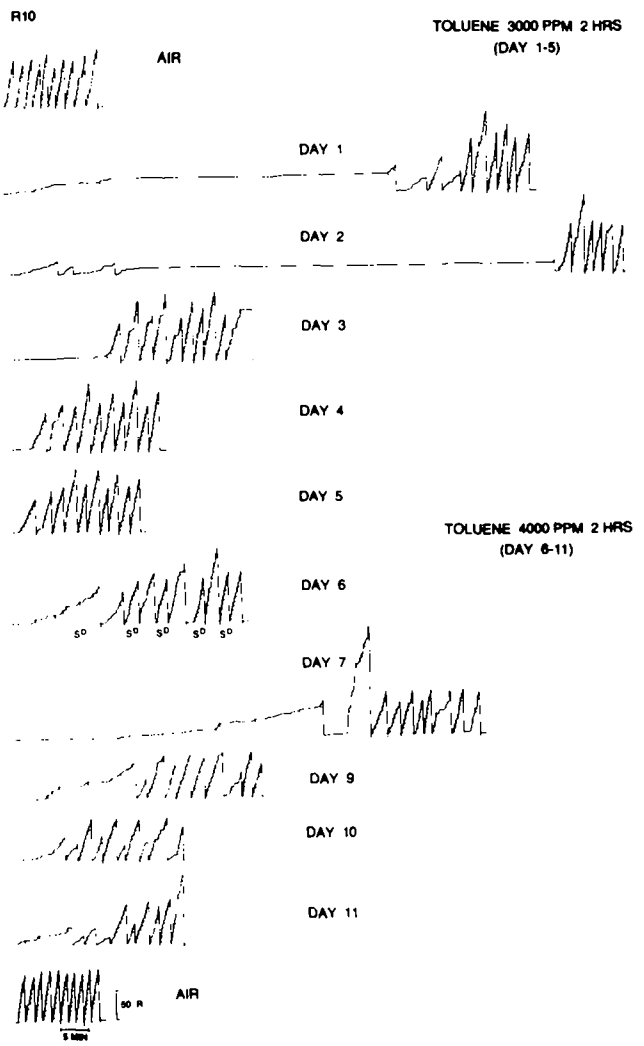

FIG. 1. Cumulative records of performance for rat 10 following two-hour exposures to 3000 ppm (days 1–5) and later (days 6–11) to 4000 ppm of toluene. Posttoluene air exposure is for day 12. The FCN-S^D component is indicated by "S^D." All toluene exposures occurred before behavioral assessment and were followed by two hours of air exposure. Responses on the left lever moved the pen upward as it moved to the right with time. Runs greater than or equal to 8 are indicated by short diagonal lines. The pen reset to the baseline with completion of the tenth run in each component.

animals (Nos. 23, 22, 18, 21 and 25) tested at 4000 ppm. Signs of recovery following acute exposure were also apparent for some animals in FCN-S^D runs ≥8(%) (Table 2), despite animals performing earlier in the session. For example, FCN-S^D runs ≥8(%) for rat 23 increased from 24 percent to 48 percent (Table 2) while total session time decreased from 64 to 38 minutes (Table 3). This is also evident in Fig. 2 by comparing the cumulative records for acute and for day 1, which was the last acute exposure day for this rat. For other animals, a different pattern of recovery was observed. It was characterized by a large decrease in total session time and no improvement in FCN-S^D performance. For example,

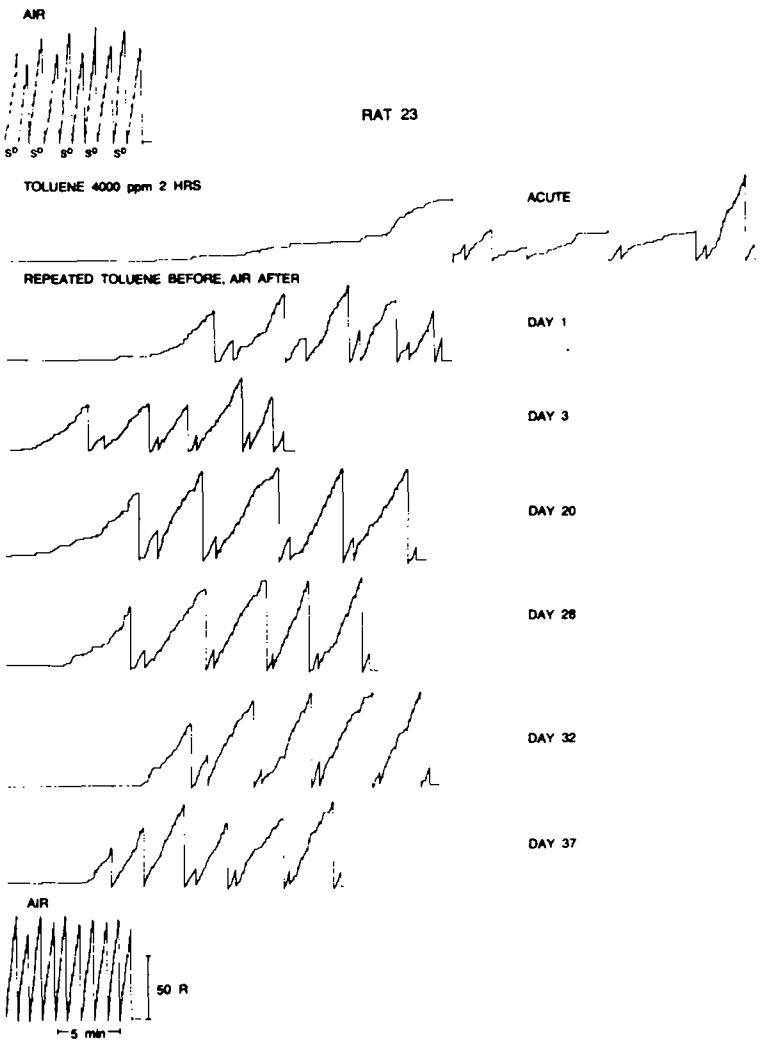

FIG. 2. Representative cumulative records of performance for rat 23 following repeated two-hour exposures to 4000 ppm toluene. Posttoluene air exposure is for day 39. See Fig. 1 legend for greater detail.

FCN-S^D performance for rat 18 did not improve (Table 2), while decreases in total session time were observed concurrently (Table 3).

Effects of Repeated Daily Exposure

Performance improved with repeated exposure in some animals. Representative cumulative records for selected animals illustrate this finding. Acute exposure of rat 43 (Fig. 3) to the lowest concentration studied, 1780 ppm, resulted in a decreased number of runs meeting the criterion for reinforcement in both components, as can be seen in the records labelled "Acute" and "Day 1." By the sixteenth day, signalled performance had recovered, whereas unsignalled performance remained impaired through day 38, with many fewer responses and no runs meeting the minimum requirement on that day. For rat 10 (Fig. 1), the first few days of exposure to 3000 ppm produced rate decreases in both components and an increase in total session time. Early in these sessions, few runs in either component were long enough to insure reinforcement for a right-lever response. Progressive improvement in performance occurred with repeated daily exposure through day 5. When the exposure concentration was increased on day 6 to 4000 ppm, performance disruption was reinstated. As exposure at this concentration continued, more runs met the minimum response requirement and session length shortened. Signalled performance recovered more quickly from toluene's behaviorally-disruptive effects.

For rat 23 (Fig. 2), acute exposure to the high concentration, 4000 ppm, resulted in decreased response rate and impaired performance in both components. Performance improvement was most prominent in the signalled component. This was characterized as an increase in runs $\geq$8(%) occurring earlier in the session. It started to disappear by the end of the daily series of exposures, as will be seen more clearly in Fig. 5 (which will be described below).

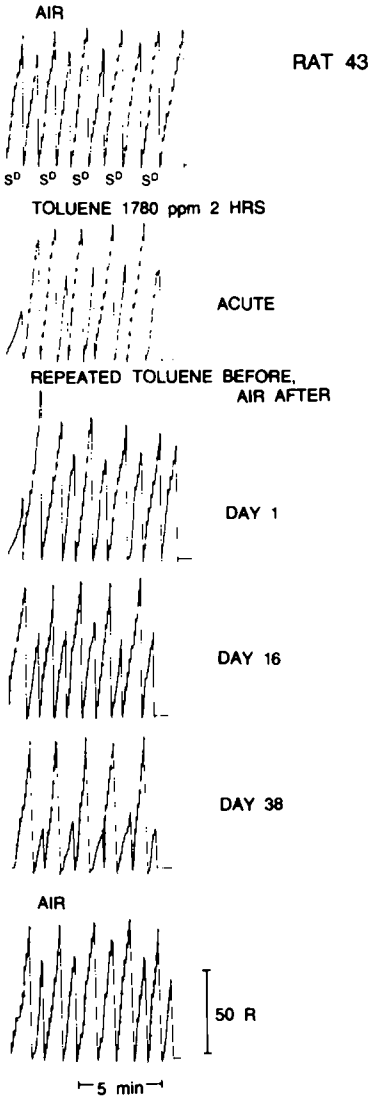

FIG. 3. Representative cumulative records of performance for rat 43 following repeated two-hour exposures to 1780 ppm of toluene. Posttoluene air exposure is for day 40. See Fig. 1 legend for greater detail.

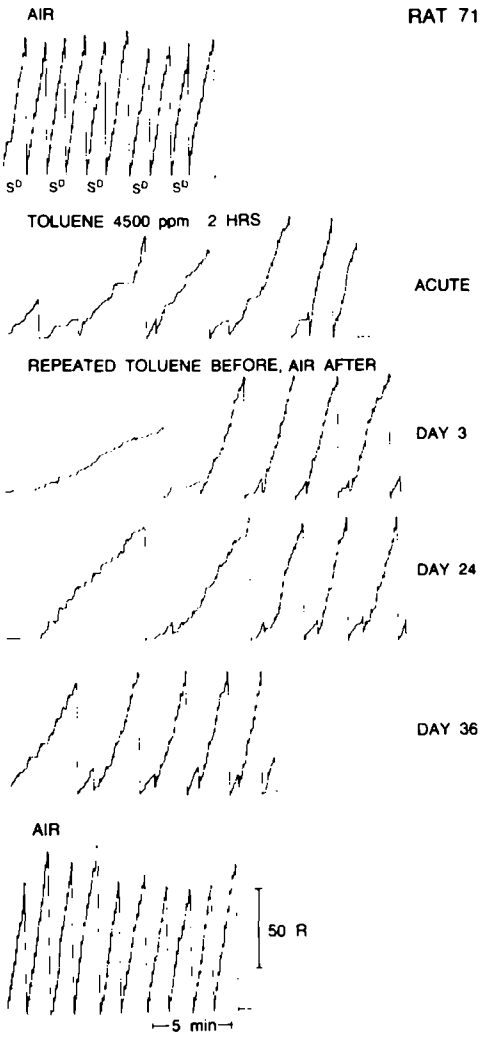

FIG. 4. Representative cumulative records of performance for rat 71. Posttoluene air exposure is for day 42. See Fig. 1 legend for details.

Acute exposure for Rat 71 to 4500 ppm resulted in disruption of performance in both components (Fig. 4) and was characterized by periods of pausing and decreases in runs ≥8(%). By day 3 of repeated daily exposure, FCN-S^D performance was showing improvement that continued across successive exposures. FCN performance remained impaired through day 36.

When tolerance was observed, it depended in part on the opportunity to behave during toluene exposure (Fig. 5). Rats 23, 22 and 18, which were exposed daily to 4000 ppm toluene before each session, all displayed tolerance although the pattern differed among them. For the last ten days of repeated exposure (days 29–38), percentages of runs for rats 18 and 23 met the FCN-S^D response requirement at least as well if not better than during the acute sessions (Table 2). This level of performance for the signalled component occurred despite the fact that the animals were responding earlier in the session and thus sooner after exposure had ended (Table 3). For rat 22, FCN-S^D performance improved although no consistent reduction in total session time was observed. No consistent signs of recovery were observed for any animal in the unsignalled component.

When 4000 ppm toluene exposures were changed from after to before behavioral sessions for rats 21, 19 and 25, the "crossover" day (day 29) behavior was disrupted comparably to that during acute-effect determinations. Time to complete the behavioral session was increased and was at least as great as following acute exposure (Table 3). These effects occurred despite the history of daily toluene exposure these rats had experienced.

Performance in the signalled component remained more resistant to toluene's disruptive effects than performance in the unsignalled component. This finding can be seen by comparing performance in the two components during the last ten days of repeated exposure (Table 2). For all animals, regardless of the concentration, behavior under the control

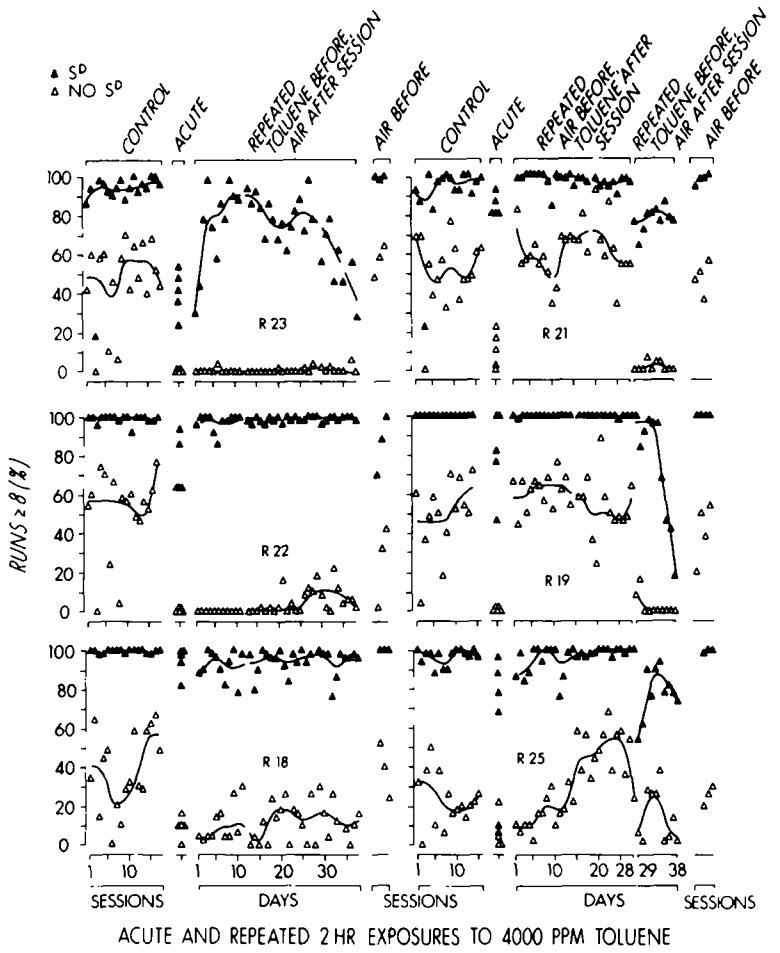

FIG. 5. Percent of total daily runs meeting the multiple-schedule response requirements. Rats 18, 22 and 23 were exposed to toluene (4000 ppm) before behavioral assessment and air afterwards. Rats 19, 21 and 25 were exposed to air before and toluene (4000 ppm) afterwards (days 1–28); they were exposed to toluene before and air afterwards (days 29–38). FCN-S^D, ▲; FCN, △. Daily data were smoothed using a 4254H twice digital filter (32, 32) that emphasizes systematic trends in the data. The results of this smoothing algorithm are plotted as a solid line superimposed on the raw data points. Briefly, data sequences are smoothed by calculating running medians based on different sample sizes, the residuals remaining from this procedure also being smoothed. Recombining both smoothed data sets ("reroughing") results in a more adequate description of the original data set. This procedure is not inferential in nature, and is only intended to make trends in the data easily apparent.

of the external discriminative stimuli was more resistant to disruption than behavior not under such control.

Although tolerance was observed in some animals, others displayed deterioration in performance. Rat 44 showed selective and irreversible loss in baseline in the FCN component (Table 2). A similar but abbreviated trend was observed for rat 43 (Fig. 3). Rat 19 displayed a progressive and profound impairment of FCN-S^D performance from days 35 to 38 of repeated exposure; rat 23 displayed a more gradual deterioration of performance from day 25 on (Fig. 5).

DISCUSSION

Substance abuse in humans is frequently associated with the development of tolerance and inhalant abuse is no exception (6, 17, 28). Evidence of tolerance in animal models, however, has been contradictory. Since the effects of toluene can differ widely across animals (35), differences in animal sensitivity may have obscured previous attempts to observe tolerance development. In this study particular attention was placed on performance changes of individual animals following repeated exposure to toluene using a multiple fixed-consecutive-number schedule of reinforcement. A complex behavioral profile was observed, some animals developing tolerance, whereas others displayed progressive performance deterioration (e.g., Fig. 3). Tolerance was manifested in the percent of runs meeting the schedule requirements. Tolerance was observed in this measure despite the tolerance evident in the shortened session duration, and

TABLE 3

TOTAL SESSION TIME (MIN)

Toluene Exposure (ppm)	Animal No.	Air Control[a]	Acute Exposure Mean	First	Last	Repeated Exposure (Presession and last 10 days)	Crossover Day	% Change[b,c]
1780	44	15 ± 1	9 ± 1	9	8	9 ± 0	*	*
	43	11 ± 1	10 ± 1	10	10	10 ± 0	*	*
4000	23	11 ± 1	43 ± 7	64	38	30 ± 3	32	-4
	22	9 ± 1	22 ± 6	29	19	25 ± 3	24	-4
	18	10 ± 1	44 ± 18	101	25	22 ± 5	33	-27
	21	25 ± 1	43 ± 26	80	27	39 ± 4	46	+60
	19	14 ± 1	31 ± 5	24	33	41 ± 6	51	+318
	25	11 ± 0	34 ± 6	43	35	35 ± 2	41	+350
4500	65	11 ± 0	28 ± 11	9	11	23 ± 4	13	-27
	71	14 ± 0	31 ± 4	34	40	25 ± 2	26	-7
	70	13 ± 0	29 ± 2	31	29	28 ± 2	26	-26
	80	10 ± 0	45 ± 2	43	46	32 ± 4	34	+124

*Not applicable.

[a]Number of control sessions ranged from 16 to 22.

[b]Expressed as percent Crossover Day of Mean of 5 preceding days.

[c]– Indicates a decrease in total session time; + indicates an increase.

the probable accompanying higher blood toluene concentrations (24). Tolerance was also observed in some animals following multiple (twice weekly) acute exposures. This may have obscured subsequent attempts to detect tolerance associated with daily exposures; not all of the animals developed tolerance and, when it did occur, its incidence was not concentration-related.

Tolerance development was mediated by behavioral and environmental variables. Only animals exposed before the behavioral session developed tolerance despite the fact that animals in the after-session exposure group were equivalently exposed. Thus, prior experience with the behaviorally-disruptive effects of toluene can affect the ability of subsequent exposures to impair performance. Environmental variables that influence behavior were also found to modulate toluene's effects. Behavior under the control of external discriminative stimuli was most likely to exhibit tolerance. Together these results suggest that metabolic variables do not account for the recovery observed. Behavioral and environmental variables have also been shown to influence the development of tolerance to other CNS depressants (8). Tolerance development is another example of a shared pharmacological property between inhalants and CNS depressants. These results are consistent with the hypothesis that solvent abuse is related to the ability to produce a depressant-like intoxication (1,22). Further work is needed to characterize the concentration range over which tolerance can be produced.

Tolerance may develop in only some animals, because of differences in animal sensitivity. Rats differed in their sensitivity to the behavioral effects of toluene, using the same behavioral baseline as that reported here (35). This may be partially attributable to differences in their respective blood or tissue levels. Our previous work demonstrated large differences in blood levels of rats acutely exposed to toluene (24). Individual variations in the severity of physical dependence and drug elimination half-lives have been reported for other abused CNS depressants (18,19). Thus, it is possible that pharmacokinetic processes may generate differences in behavioral sensitivity and that both may determine whether tolerance develops or performance progressively deteriorates. Since blood levels for these animals were not determined, the role of pharmacokinetic variables to account for individual animal differences cannot be resolved.

These results as well as others (3, 4, 21) suggest health effects following chronic solvent exposure may not be predicted adequately from acute testing procedures. Since the magnitude of behavioral effects as well as peak blood levels may vary across animals, attention should be given whenever possible to the sensitivity of individual animals when selecting the concentrations for chronic exposure protocols. Studies designed to measure changes expressed as a proportion of control values benefit from establishing comparable magnitudes of effect between subjects. Although using different exposure levels designed to produce equivalent effects would be more difficult and costly to conduct, it might enable the detection of more subtle drug effects and a greater understanding of the determinants of tolerance development. Since adjusting exposure concentrations for individual animals is not always practical, awareness that animals may differ in sensitivity may aid in data analysis and interpretation. Future studies of the development of tolerance to the behavioral effects of inhalants would profit from the evaluation of effects when blood and brain levels are not in a state of transition, but at steady-state levels during exposure. Evidence of tolerance was observed between 1780 and 4500 ppm. Further work is needed to more systematically characterize tolerance development following volatile inhalant exposure including determining the lowest concentration at which such effects could be determined.

We previously showed that behavior under the control of external discriminative stimuli is generally less disrupted by

acute exposure to toluene than behavior not under such control (35). The present study extends those findings to include repeated toluene exposures. In addition, external discriminative stimuli were found to influence the development of tolerance: when tolerance was observed, it generally occurred in the FCN-S^D component. Development of tolerance is another example of shared pharmacological properties between toluene and abused CNS depressants. However, it remains to be determined whether tolerance of comparable magnitude to that produced by other CNS depressants can be produced by inhalants, and whether the ability to generate tolerance is characteristic of other types of inhalants.

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