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The effect of nitrous oxide on time estimation in rats

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Rats were trained on a temporal discrimination task in a two-way shuttlebox while inhaling air and tested while inhaling various concentrations of nitrous oxide. The animals successfully performed the tasks even while inhaling 50% nitrous oxide. At this concentration, response latencies increased. At 20% and 35% nitrous oxide, response latencies decreased. An interaction between conditioned fear in the shuttlebox and the sedative effect of nitrous oxide could explain these results. The ability of the rats to perform successfully a temporal task contrasts with results obtained from human subjects by other investigators. This could reflect the effect of nitrous oxide on alertness maintained by fear in rats as against different motivations in human subjects.

At least several different species can estimate the passage of time, but the mechanisms employed are still under investigation (Adam, Rosner, Hosisk, & Clark, 1971; Barrett, Leith, & Ray, 1971; Bell, 1966; Dimond, 1964; Nelson, Bartley, & Jordan, 1963; Robson, Burns, & Welt, 1960; Zelkind, 1973). Nitrous oxide (N_2O) has been used in attempts to identify the physiological basis of time estimation (Robson et al., 1960; Steinberg, 1955), since its interference with this function may place constraints on possible underlying mechanisms.

In humans, inhalation of N_2O prolongs the subjective passage of time (Robson et al., 1960). Use of human subjects, however, is expensive and limits the type of experimentation that can be done. It would be advantageous to continue this research using a species other than man, such as the rat, which can perform tasks requiring an estimation of time (Sidman, 1966). We therefore studied the effect on rats of inhalation of N_2O under pharmacological conditions which closely duplicated those used with humans (Henrie, Parkhouse, & Bickford, 1961; Parkhouse, Henrie, Duncan, & Rome, 1960; Robson et al., 1960).

METHODS AND APPARATUS

The avoidance apparatus and procedure have been described (Wallenstein & Rosner, 1976). Briefly, a box with a grid floor was mounted on a fulcrum. Movement of a rat from one side of the box to the other tilted the box and tripped a microswitch. Differential reinforcement of a low-rate schedule with a limited-hold specification was used. The US was shock. The CS was a light which came on at the beginning of each trial and stayed on until the rat had crossed the barrier during or after the temporal "window." The grid floor was connected to the shock scrambler so that the grids on the half of the shuttlebox away from the rat were activated du ng the first 7 sec of a trial. Then no shock was applied to either ide for the next 5 sec. If the rat had not crossed the barrier by the 12th sec, it received shock.

Each session consisted of two groups of 50 trials; a 15-min rest period separated the groups. Each rat required about 4 days to reach the 70% criterion. Once the rat reached criterion, the next 15-min break was used to change the atmosphere. Twenty extinction trials were then performed.

Premixed nitrous oxide:oxygen ($N_2O:O_2$) combinations (Air Products Inc.), e. g., 35% N_2O:65% O_2, were used at an inflow rate of 8 liters/min for the first 15 min, and 6 liters/min for the rest of the experiment. Measurements within the chamber through a Beckman oxygen meter revealed that these flow rates gave a plateau for percentage of O_2 within 10 min. This level of O_2 was constant within the chamber, but less than the O_2 percentage flowing into the chamber. Therefore, the concentration of N_2O in the chamber was computed from the percentage of O_2 in the air and in the $N_2O:O_2$ mixture entering the chamber. For example, when the 50% $N_2O:O_2$ mixture flowed into the chamber, the oxygen meter read 41%. With this mixture, for every 1% atmospheric O_2 lost, 2.5% O_2 entered. Thus, 35% O_2 from the N_2O mixture and 6% O_2 from the remaining air made up the 41% O_2 registered by the oxygen meter. The estimate that .70 parts of the atmosphere in the chamber came from the $N_2O:O_2$ mixture held for all $N_2O:O_2$ combinations tested (i. e., .70 of 50% N_2O = 35%). Therefore, all percentages of N_2O referred to in the results are estimated from these computations.

RESULTS

Temporal discrimination limited to a "window" (DRL) is a difficult task. The animal must wait 7 sec after CS onset and then has only 5 sec in which to respond. Nevertheless, rats could master this task while inhaling 50% N_2O (see Figure 1).

When rats learned the DRL task while inhaling air (100 trials per day) and then were tested under various concentrations of N_2O, some differences appeared in response latencies (see Table 1). The rats represented in Table 1 served as their own controls. During each successive day the animal was brought back up to criterion, and again the last group of trials was extinction. The order of concentrations of N_2O was selected before learning began and was different for each rat. As Table 1 shows, extinction trials performed while inhaling air produced almost no shift in response latency. Extinction trials performed while inhaling 20% N_2O produced inconsistent shifts in latency. These shifts were significant at $p < .01$ for four out of the five rats tested with 20% N_2O. The three rats tested while inhaling 35% N_2O all showed shorter latencies. Four out

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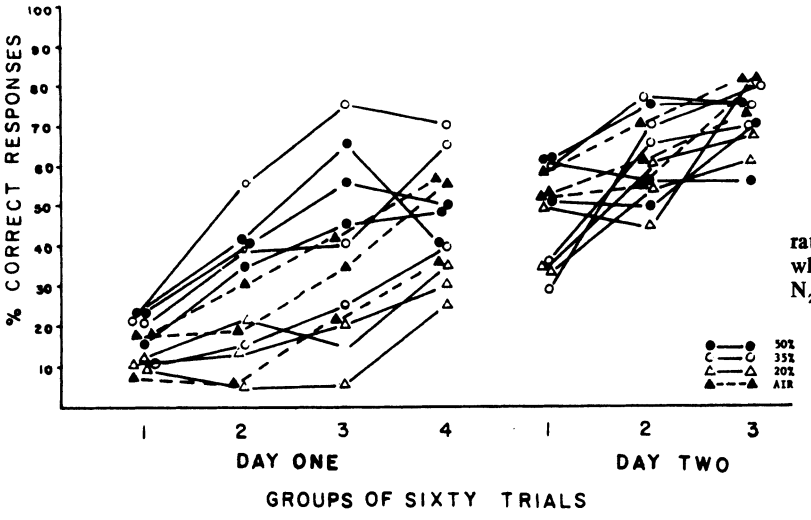

Figure 1. Learning rates by individual rats for the DRL with limited hold task while inhaling various concentrations of N_2O and air.

of the six rats tested while inhaling 50% N_2O showed increased response latencies. In all four cases, the shift was significant at $p < .01$.

DISCUSSION

Although the increase in response latency with 50% N_2O was significant, most rats crossed the barrier in time. This suggests that retention of the task and maintenance of motivation persisted despite the high concentration of N_2O. With human subjects, concentration of N_2O above 30% caused significant impairment of memory and motor performance (Henrie et al., 1961; Parkhouse et al., 1960; Robson et al., 1960). With 50% N_2O, most human subjects did not respond to external stimuli. The shift in response latency by the rats during the DRL task (Table 1) was not caused by impairment of the motor system, since rats inhaling 35% N_2O showed shorter response latencies than rats inhaling air, and rats inhaling 50% N_2O performed this task perfectly well (Figure 1).

Rats inhaling 20% and 35% N_2O showed shorter response latencies than during control testing. This was particularly prominent at 35% N_2O. In contrast, human subjects inhaling 20% N_2O produced an estimation of time which was double the control estimation, while inhalation of 30% N_2O caused an estimation which was three times longer in duration (Robson et al., 1960).

Learning in a two-way shuttlebox requires the rat to return to a side of the box in which he had just been shocked. Thus, the rat must overcome fear of that side (Seligman, Note 1). A sedative effect of N_2O might subjectively decrease the intensity of the shock for the rat and, in turn, decrease the level of fear, thus facilitating two-way avoidance behavior. N_2O is known to have a sedative effect on human subjects (Parbrook, 1967). Figure 1 suggests that such a sedative effect did occur in the rats. Rats performing the task while inhaling 35% and 50% N_2O actually learned more rapidly than did controls inhaling air.

While inhalation of N_2O did change temporal behavior in rats, they generally performed well under concentrations of N_2O which seriously impair performance of human subjects. We suggest the reason is methodological rather than physiological. In our avoidance task, fear has physiological correlates (e.g., elevated levels of adrenalin, mineralocorticoids, glucocorticoids, and O_2 intake). The hormonal factors are relatively

Table 1

Rat	Control	Air	Control	20% N_2O	Control	35% N_2O	Control	50% N_2O
51	6.7 ± .7	6.7 ± .7	6.4 ± 1.4	8.1 ± 2.8 ($p < .01$)			6.5 ± 1.4	10.8 ± 3.6 ($p < .05$)
55	6.5 ± 1.6	7.7 ± 1.5					6.6 ± 1.0	11.2 ± 5.8 ($p < .05$)
57	7.8 ± 1.1	9.0 ± 1.2	7.2 ± 1.6	4.1 ± .7 ($p < .001$)	7.0 ± 1.8	5.3 ± 1.0	6.5 ± .8	3.4 ± .9 ($p < .001$)
58	6.8 ± 1.9	6.3 ± 1.3	6.1 ± 2.2	3.4 ± 1.2 ($p < .01$)			6.3 ± 1.6	11.8 ± 5.8 ($p < .001$)
69	7.3 ± 1.1	6.5 ± .5	7.4 ± 1.0	5.7 ± 1.2	7.0 ± 1.4	5.4 ± 1.5	7.0 ± .9	13.4 ± 4.8 ($p < .001$)
72	6.7 ± 1.4	6.4 ± .8	6.9 ± .8	9.1 ± 2.9 ($p < .01$)	7.2 ± 1.2	6.2 ± 1.1	7.4 ± 1.1	7.1 ± 2.9

long lasting and could carry over into a period of extinction trials where they would be maintained by reappearance of the CS. Robson et al. (1960) noted that, if a human subject under N_2O felt discomfort from an external stimulus during an experiment, the prolongation of the subjective passage of time abruptly disappeared. With human subjects, maintenance of alertness could be decreased by the "euphoric" side effects of N_2O (Henrie et al., 1961).

These observations suggest that a subject's state of alertness or vigilance may be a significant factor in changes in the estimation of time. We propose that N_2O may not cause temporal prolongation by physiologically affecting an "internal clock" (Bell, 1966; Zelkind, 1963) but by reducing the subject's state of alertness. Of course, this in turn may cause the subject to reduce his monitoring of an internal clock. There is no way of determining this from our experiments. Appropriate controls for effects on alertness seem necessary in future studies of interference with time estimation in man.

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