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Nitrous Oxide-Induced Conditioned Taste Aversions in Rats: The Role of Duration of Drug Exposure and Its Relation to the Taste Aversion–Self-Administration "Paradox"

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GOUDIE, A. J. AND D. W. DICKINS. *Nitrous oxide-induced conditioned taste aversions in rats: The role of duration of drug exposure and its relation to the taste aversion–self-administration "paradox"*. PHARMAC. BIOCHEM. BEHAV. 9(5) 587–592, 1978.—Inhalation by rats of nitrous oxide immediately after ingestion of a solution of 0.1% saccharin resulted in a conditioned avoidance of the solution following recovery from the drug (Conditioned Taste Aversion). With a constant duration of inhalation (30 min), aversive properties of nitrous oxide in this behavioral paradigm were related to concentration over the range 0–80%. At constant concentrations (70% and 80%), the aversive potency of nitrous oxide was directly related to duration of inhalation of the gas over the range 0–4 hr. These data provide the first direct support for the hypothesis [5,6] that a major determinant of aversive potency of a drug in the taste aversion paradigm is its duration of action. Different temporal components of drug action may mediate self-administration and conditioned aversion.

Nitrous oxide Conditioned taste aversion Duration of action Self-administration Drug abuse

CONDITIONED taste aversions (C.T.A.s) induced by drugs of abuse have been studied in recent years in the hope that they will shed some light on the behavioural and neurochemical mechanisms involved in drug abuse and self-administration [3, 5–7, 10, 12, 15–19, 27, 30, 31, 32, 36–38, 41]. Much of the work in this area has been motivated by the "paradoxical" observation that the same doses of drugs of abuse which are self-administered can be shown to condition taste aversions (see [5, 6, 12] for reviews). In some recent elegant studies [24, 38, 41] it has been reported that drug injections in one group of animals can have reinforcing effects on operant behaviours (such as maze running and lever pressing), *and* aversive effects, as shown by the development of C.T.A. to fluids presented just before experimental sessions. These findings, in conjunction with studies demonstrating that the neurochemical systems mediating C.T.A. and self-administration are either the same or remarkably similar [16, 26, 27, 29, 30, 36], highlight the paradoxical nature of C.T.A.s induced by self-administered drugs. Some authors have pointed out that it is in no way surprising that drugs can have different types of effects in different situations [6,12]. However, the paradox clearly remains unresolved since the variables which determine which type of effect a drug will have in any one specific behavioural paradigm remain undefined [10].

Research aimed at resolving the paradoxical nature of drug-induced C.T.A. *may* have remained inconclusive largely because relatively little work has been directed at specifying which parameters of drug action are important determinants of a drug's aversive potency. Amongst the factors which *have* been shown to be important are the dose [3, 5, 6, 10, 19] and route [7] of drug administration. However, manipulations of dosage and route of administration are confounded by the fact that both these manipulations inevitably influence duration of drug action, a factor which has been noted to be "consistently correlated" with the aversive potency of drugs [5]. The work reported in this paper provides an empirical test of the hypothesis that duration of drug action is a critical determinant of the aversive potency of a drug [5,6] by studying the role of duration of action of one specific agent, nitrous oxide, in the C.T.A. paradigm.

A gaseous agent such as nitrous oxide possesses a distinct advantage over more conventional agents administered by injection for studying the role of duration of drug action because the drug's action can be limited simply by terminating administration. Of all conventional inhalation agents, nitrous oxide would appear to be the drug of choice for studying the role of duration of action since it is relatively insoluble in plasma and therefore saturates the blood rapidly, and as it is also non-metabolized it consequently equilibrates very rapidly (within 10–15 min) with brain tissue [14, 21, 22]. Due to these physicochemical properties of the gas, once equilibration has occurred measurements of the drug's effect on behaviour can be made in what is effectively a steady state [22,39]. Since the drug is also eliminated from the brain within minutes [22], for all practical purposes, apart from a brief period of equilibration, duration of drug action can be effectively equated with duration of inhalation of the drug. The work

reported in this paper was consequently designed to assess the role of duration of drug action in C.T.A. studies by utilising these relatively unique pharmacokinetic properties of nitrous oxide.

EXPERIMENT 1

This preliminary study was undertaken to obtain information on the concentration/response relationship of any conditioned aversion induced by nitrous oxide. Accordingly, the aversive properties of 80%, 60% and 0% nitrous oxide in oxygen were assessed over repeated conditioning trials. Only relatively high concentrations of nitrous oxide were chosen in this preliminary study since the analgesic and other behavioural effects of this agent in rodents are relatively weak or absent at low concentrations [1,9].

METHOD

Animals

Twenty-seven female albino rats, weighing between 240 and 330 g were used. They were derived from the breeding stock of Liverpool University Psychology Department, and were maintained in individual cages, in a temperature (70 ± 2°F) and illumination (12 hr cycle) controlled room. Animals were allocated to 1 of 3 groups (n=9), the groups being matched approximately for mean and variance of body weight.

Procedure

The experiment was run in two phases. Phase 1 consisted of a period of four days of adaptation to a 30 min per day regime of water access, followed by a series of 4 one-bottle conditioning trials each involving exposure for 30 min to 0.1% saccharin followed immediately by the relevant treatment (O_2, 60% or 80% N_2O) for a further 30 min. Conditioning trials were separated by two days of the baseline regime of restricted water access, as previously described in studies in this [17, 18, 19] and other [5,6] laboratories. Phase 2 consisted of a two bottle preference test between saccharin and water which lasted 45 min for each animal and was administered 3 days after the fourth conditioning trial, when all animals were at least 24 hr fluid deprived. This preference test was included because two-bottle tests have been found to be more sensitive in detecting aversions than one-bottle tests [20]. Bottle positions in the test were allotted randomly to each animal.

Administration of gases on conditioning trials involved transport of animals to a neighbouring experimental room where they were placed into 9×6×10 in. air tight modified operant chambers designed for administration of gaseous anaesthetic agents. Animals remained in these chambers for 30 min in atmospheres maintained at either 80%, 60% or 0% nitrous oxide in oxygen (Gases supplied by British Oxygen Company). Gases were mixed by relative flow rate in conventional Boyle Apparatuses (British Oxygen Company). The initial combined flow rate of the gases through the chambers was high (10 l/min), to ensure that the air in the chambers was rapidly displaced by the relevant gas mixture. After 3 min, flow rate was reduced to 2.5 l/min, this rate being maintained throughout the rest of the gas period. Calibration studies with a Percent Oxygen Detector (Teledyne Analytic Instruments) indicated that with a flow rate of 10 l/min of pure oxygen, the chambers were saturated with 100% oxygen within 3 min of first turning on the oxygen, and that the chambers could be maintained at the 100% level throughout the 30 min period at the lower flow rate of 2.5 l/min. Since approximately 2 min were required to transport animals to the chambers, animals were inhaling the prescribed gas mixture within 5 min of the end of the saccharin intake session.

RESULTS

Figure 1 shows the results of Phase 1 of the study. The mean (±SE) amounts of 0.1% saccharin consumed by animals in each group are plotted as a function of the number of conditioning trials. Also shown are the baseline levels of water intake (mean ± SE) for each group recorded on Day 4 before any conditioning trials.

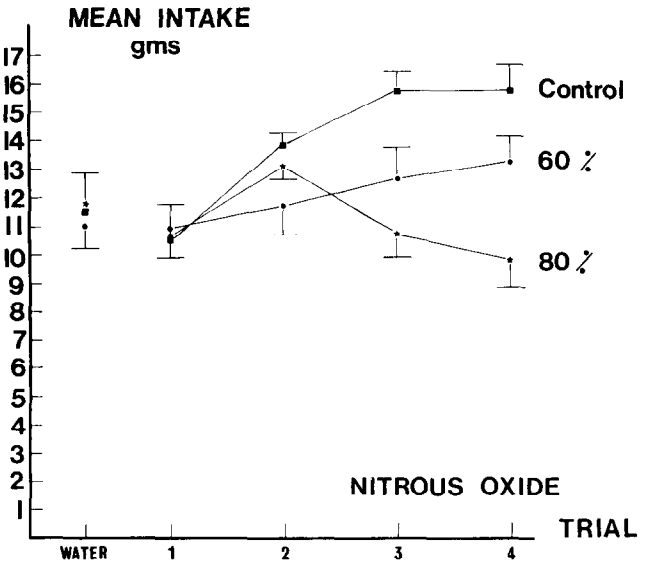

FIG. 1. Mean (±SE) amounts (gm) of 0.1% saccharin solution consumed by each group over repeated conditioning trials. Baseline levels of water intake are also shown. (Some SEs omitted for clarity.) Numbers on the right of the figure refer to nitrous oxide concentrations (percentages). Control animals received 100% oxygen.

Analysis of the Day 4 water intakes revealed that there were no differences between groups in basal fluid intake, $F(2,24)=0.18$; any subsequent group differences can consequently be attributed to the effects of aversive conditioning. On first access to saccharin (conditioning trial 1) animals drank marginally less fluid than on the preceding day, correlated $t(26)=2.65$, $p<0.02$, showing a characteristic neophobic response to a novel taste which dissipated with repeated exposure to the taste, as previously reported [11, 15–19]. The aversive effects of nitrous oxide were consequently assessed against a changing baseline of intake in control animals. Analysis of the saccharin intake data over trials 1–4 with a two-factor ANOVA with repeated measures over trials revealed significant group, $F(2,24)=5.56$, $p<0.025$, and trial, $F(3,72)=11.53$, $p<0.001$, effects, as well as a highly significant interaction, $F(6,72)=8.05$, $p<0.001$. Tukey (a) multiple comparison tests [40], using the appropriate MS error term from the overall ANOVA, indicated that there were no group differences in saccharin intake on trials 1 and 2 (HSD at $\alpha=0.05=4.39$). On trial 3 the animals receiving 80% nitrous oxide drank significantly less ($\alpha=0.05$)

than controls but did not differ significantly from the 60% group. Similarly, on trial 4, the 80% group differed significantly (HSD=5.64, at $\alpha=0.01$) from controls but not from the 60% animals, which did not differ significantly ($\alpha=0.05$) from controls on any trials. These data indicate that nitrous oxide induced a conditioned taste aversion to saccharin, but that this aversion was only detectable with the single bottle test at the 80% concentration after repeated conditioning trials. No significant aversion was detectable with the 60% concentration, although there was a nonsignificant tendency for animals treated with this dose to show an aversion relative to controls (Fig. 1).

Figure 2 shows the results of the second phase of the study. The mean (±SE) saccharin preference scores in the two-bottle test are plotted as a function of the treatments administered.

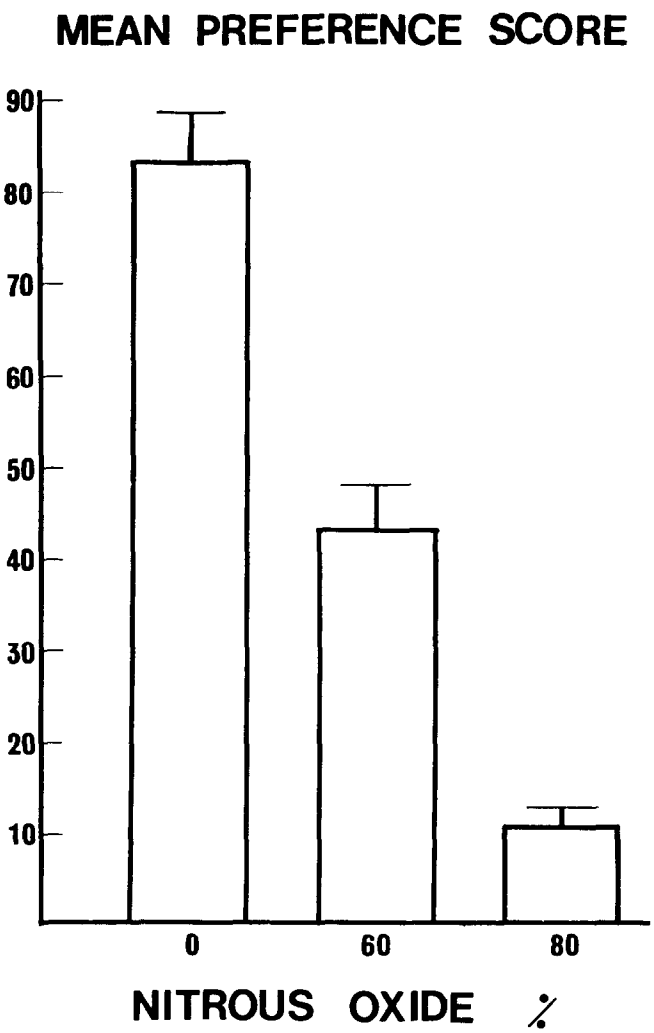

FIG. 2. Mean (±SE) percentage saccharin preference scores of animals in two-bottle preference test. Numbers along the abscissa refer to nitrous oxide concentrations (percentages).

These data were analysed by a single factor ANOVA of the percentage preference scores after application of an arc-sine transform, as recommended for percentage scores [40]. This analysis indicated that there was a highly significant group effect, $F(2,24)=63.23$, $p<0.001$. Subsequent Tukey (a) multiple comparison tests revealed that the 80% group differed significantly ($\alpha=0.01$) from both the 60% group and the 0% controls, whilst the 60% group also differed significantly ($\alpha=0.01$) from the controls. The two-bottle test was consequently more sensitive in detecting aversions than the one-bottle test, since the latter failed to detect a significant aversion at the 60% dose, and failed to convincingly illustrate the concentration-dependent nature of the aversion.

DISCUSSION

Nitrous oxide clearly induced a C.T.A. to saccharin which was concentration-dependent. However, the observed aversion was relatively weak. Even with the 80% concentration of nitrous oxide a C.T.A. was only detectable with the single bottle test after multiple conditioning trials, the degree of suppression of fluid intake being relatively limited. In contrast, studies with agents as diverse as ethanol, THC, amphetamines, fenfluramine, caffeine, lithium and chlordiazepoxide have reported almost complete suppression of fluid intake in one-bottle C.T.A. tests after repeated conditioning trials [3, 5, 6, 10, 15–18, 29]. It is possible that the observed C.T.A. was limited by the relatively short (30 min) duration of exposure to the gas in this experiment. Experiment 2 was designed to directly assess the role of duration of drug action in nitrous oxide-induced C.T.A.s and to determine whether or not stronger aversions could be conditioned with longer durations of exposure to nitrous oxide.

EXPERIMENT 2

METHOD

Animals

Seventy-two female albino rats derived from the same source as those in Experiment 1, and housed under the same conditions, were used. Their body weights varied between 250–320 g. Animals were allocated at random to 1 of 7 groups.

Procedure

Three groups (n=9) were subjected to aversive conditioning with nitrous oxide at a concentration of 70%, the durations of exposure to the gas mixture for the 3 groups being 0.5, 1 and 4 hr, respectively. Similarly, three further groups (n=9) were conditioned with 80% nitrous oxide for the same durations. A further group (n=18) acted as a control group which was exposed to 0% nitrous oxide (i.e., 100% oxygen) for a period of 4 hr. The study consequently effectively comprised a factorial design with two levels of nitrous oxide concentration (70% and 80%) and three levels of duration of exposure to nitrous oxide (0.5, 1 and 4 hr). In addition the control group exposed to 100% oxygen for 4 hr was included to control for any possible aversive effects of prolonged restriction within the chambers.

All animals were adapted for six days to a regime of water access for 30 min per day prior to conditioning. On the following day (Day 7) animals were given 30 min access to a 0.1% solution of sodium saccharin. This was followed by treatment with nitrous oxide in oxygen in the gas boxes described. The gas flow parameters were exactly as described for Experiment 1. For the next six days all animals received

access to water for 30 min per day. On Day 14 animals were tested for aversion to saccharin using the two-bottle test procedure described above, except that the duration of the test procedure was 60 rather than 45 min. Since only four animals could be trained and tested daily due to the limited number of gas boxes, the whole study was run over a period of six weeks. The nitrous oxide concentrations used in this study were based upon the earlier findings of Experiment 1. Since only weak aversions had been found with 60% nitrous oxide after repeated training trials, it was felt that it was unlikely that concentrations lower than 70% would induce C.T.A.s in the single trial procedure used in this study.

RESULTS

On first access to saccharin on Day 7 animals showed the expected neophobic response to saccharin, consuming less liquid than on the previous day, correlated $t(71)=3.26$, $p<0.01$. The neophobic response to saccharin shown by any individual animal could be quantified by expressing Day 7 saccharin intake as a percentage of Day 6 water intake. An ANOVA revealed that groups did not differ in their neophobic responses to saccharin, $F(6,65)=0.42$. Thus any group differences that were observed in the two-bottle preference test for saccharin could be attributed entirely to the effects of experimental treatments rather than to chance variations in basal neophobia which have been observed in some C.T.A. studies [4].

Figure 3 shows the mean (±SE) saccharin preference scores (percentages) in the two-bottle test of animals in each of the six experimental groups and in the control group.

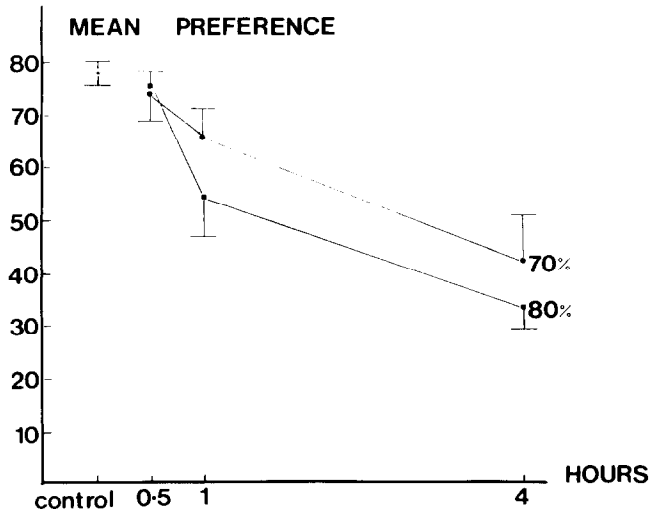

FIG. 3. Mean (±SE) percentage saccharin preferences in two-bottle test as a function of duration of exposure to nitrous oxide for times varying between 0.5 and 4 hr. Also shown are data for animals in a control group who were exposed to 100% oxygen for 4 hr.

A two factor ANOVA for independent groups applied to the arc-sine transformations of preference scores for animals in the six experimental groups revealed that there was a highly significant effect of duration of exposure, $F(2,48)=18.37$, $p<0.001$. However, neither the concentration effect, $F(1,48)=1.45$, nor the concentration×duration interaction, $F(3,64)=0.49$ was significant. Control animals treated with oxygen for 4 hr did not differ significantly from animals treated with either 70% or 80% nitrous oxide for 30 min, $t(25)=0.60$ and 0.65, respectively. However, these animals did differ significantly from those treated with nitrous oxide for 4 hr, $t(25)=3.95$ and 8.87 for 70% and 80% groups respectively, $p<0.001$ in both cases. The critical aversive event was therefore exposure to nitrous oxide, rather than any effect of prolonged restriction within the gas administration chamber.

These data indicate clearly that, in confirmation of the results of Experiment 1, nitrous oxide induced a C.T.A. to 0.1% saccharin. Furthermore the strength of the conditioned aversion was directly related to the duration of exposure to the gas.

GENERAL DISCUSSION

The data indicate that nitrous oxide at concentrations between 60% and 80% is effective in inducing C.T.A. in a concentration and duration related fashion. However, in neither study reported in this paper were the observed conditioned aversions very marked. In Experiment 1, with a short duration of exposure to the gas, significant aversions were only obtained after repeated conditioning trials and were then only very clearly demonstrated in the sensitive two-bottle test. In Experiment 2, with a sensitive two-bottle test, marked aversions were only noted with very long exposures to the drug. Nitrous oxide is clearly a relatively weak agent in inducing conditioned aversions. The weak aversive potency of the drug may simply be a reflection of the fact that in animals the behavioural effects of nitrous oxide are relatively weak [1, 9, 42] due presumably to the low solubility of the drug in plasma. Alternatively, it may be possible to link the weak aversive potency of the gas with the similar weak potency of morphine [30], since nitrous oxide is known to act on endogenous opiate systems [1], which have been implicated in the mediation of drug-induced C.T.A. [30]. Such conclusions must, of course, remain speculative in the absence of definitive empirical data.

The major finding of the work reported here was that nitrous oxide-induced C.T.A. was duration dependent. However, it is clear the duration of drug action is not the only factor determining aversive conditioning. In Experiment 1, in which duration of exposure was held constant, the *number* of conditioning trials was clearly an important determinant of potency of conditioning. Similarly, a comparison between the results of the two-bottle tests for animals treated with 80% nitrous oxide for 30 min in Experiments 1 and 2 reveals that in Experiment 1, after four taste-drug pairings, saccharin preference had declined to about 10% (Fig. 2) whereas in Experiment 2 a *single* taste-drug pairing (of the same concentration and duration) reduced saccharin preference to only about 55% (Fig. 3). It is clear that the number of aversive conditioning trials is also a critical determinant of degree of aversive conditioning. Similar findings to these have been noted with a variety of other drugs [5, 6, 10, 12, 18, 19, 30].

The finding that duration of drug action is an important determinant of potency of aversive conditioning complements other evidence which has indirectly implicated duration of drug action as a critical variable. Cappell and Le Blanc [5,6] originally hypothesised that duration of action was a critical factor on the basis of their findings with cocaine, which failed to induce a C.T.A. at very high doses, an effect which was attributed to the drug's very short half-

life. Other investigators *have* succeeded in conditioning aversions with cocaine [3,19], although it is clear that the drug is a remarkably weak aversive agent since very high doses were required to induce relatively weak C.T.A.s. The low aversive potency of cocaine has been attributed to its short duration of action by all groups working with the compound, although no direct evidence linking these phenomena has been presented. More convincing support for the duration of action hypothesis has been provided by Stolerman and D'Mello [32] who noted that the aversive potencies of a series of psychostimulant drugs were correlated with their durations of action. We have previously reported that prolonging drug action by inhibition of metabolism is effective in potentiating amphetamine's aversive effects [18], although these findings are confounded by possible effects of inhibition of metabolism on peak plasma levels of the drug. C.T.A.s have been found to be more potent when the drug is administered IP than IV [7], an effect which has been attributed to the drug having a shorter duration of action when injected IV [5, 6, 33]. Whilst these observations *indirectly* support the hypothesis that duration of drug action is an important determinant of aversive conditioning, these studies all appear to be confounded to a greater or lesser extent by other variables. The data reported above provide more definitive support for the duration of action hypothesis.

However, before accepting the hypothesis uncritically, it is important to assess whether or not the duration of exposure effect on C.T.A. could be explained in some other way. A basic assumption behind the work outlined here is that, after an initial period of equilibration, the "effective dose" and duration of drug action are independent during the administration of nitrous oxide. The data available on the pharmacokinetics of nitrous oxide supports this assumption [14, 21, 22, 39]. Kety *et al.* [22] reported that the blood/brain partition coefficient for nitrous oxide both in vivo and in vitro reached a peak within 10 min of administration and did not change over a subsequent 2 hr period of continuous administration of the gas. Furthermore, since there is evidence for the development of acute (i.e., within-session) tolerance to the effects of nitrous oxide *after* equilibration in humans [39] and in rats [1,9], a test of the duration of action hypothesis using nitrous oxide may be considered to be a relatively conservative one. It seems unlikely that the reported effects of duration of exposure to nitrous oxide could be explained in terms of an increase in "effective dose" over the 4 hr period of administration since the blood/brain equilibration time for nitrous oxide is generally considered to be within 10–15 min [22]. Since an increase in potency of aversive conditioning was observed over a 4 hr period (Fig. 3) it seems unlikely that this could be explained in terms of a continuous increase in the level of saturation of brain tissue (i.e., increased "effective dose"). Furthermore, the absence of any effect of concentration of nitrous oxide reported in Experiment 2 indicates that, at least within the relatively narrow dose range studied, duration was a more important determinant of potency of aversive conditioning than dosage, further suggesting that that observed duration effect can not be accounted for simply in terms of an increase in "effective dose" with time. However, it *is* clear from the concentration-dependent nature of the aversion reported in Experiment 1, that over a wider concentration range a duration by concentration interaction would probably have been observed. These considerations suggest that potency of aversive conditioning is related to the duration of drug action, an effect originally suggested by Cappell and Le Blanc [5,6].

The fact that duration of drug action appears to be one of the critical determinants of the aversive potency of a drug may allow a resolution of the paradoxical nature of C.T.A. studies with self-administered drugs. It is possible that different temporal components of the *same* injection procedure may have different types of reinforcing effect. Rapidly induced "state changes" [23] of relatively short duration may be reinforcing, whereas longer duration "state changes" may prove to be aversive. Now if this hypothesis of temporal determinants of hedonic effects of drugs of abuse is to be consistent with data demonstrating that injections of drug in the same animals in the same experimental sessions can have both reinforcing and C.T.A.-inducing effects, [24, 38, 41] it is necessary to explain why the short duration drug-induced "state changes" that are hypothesised to mediate reinforcement should become associated only with operant responses such as lever pressing or maze running, whereas longer duration drug-induced "state changes" that are hypothesised to be aversive should become selectively associated with tastes. To account for these findings one may appeal to the principal of "selective associations" [13]. Testa [33–35] has recently demonstrated that similarity between the temporal parameters of the CS and the UCS markedly facilitates Pavlovian avoidance conditioning. Since learning theorists have often postulated that taste-related CSs are of very long duration [2, 25, 28], it is to be expected that C.T.A.s will be most potent when aversions are induced by UCSs of long duration rather than those of short duration [33–35]. "Selective association" between the aversive properties of the drug UCS and taste CS may develop because of the temporal similarity between the taste and the aversive drug effect. On the other hand, stimuli of short duration associated with operant responses may become selectively associated with the short duration, rapid onset effects of the drug which are hypothesized to mediate reinforcement.

In summary, the data presented suggest that duration of drug action may be a critical determinant of whether or not a particular drug-induced state is either predominantly aversive or reinforcing. Virtually *all* drug-induced states may have both reinforcing and aversive components since the vast majority of psychoactive drugs so far tested have been shown to induce both self-administration [8] and C.T.A. [5, 6, 12]. Due to the effects of stimulus similarity in determining potency of conditioning [33–35] the self-administration paradigm may be uniquely adapted to detecting reinforcing properties of drugs whilst the C.T.A. paradigm may be uniquely adapted to detecting aversive drug effects.

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