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Amphetamine-Induced Hypodipsia and its Implications for Conditioned Taste Aversion in Rats

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STOLERMAN, I. P. AND G. D. D'MELLO. *Amphetamine-induced hypodipsia and its implications for conditioned taste aversion in rats.* PHARMAC. BIOCHEM. BEHAV. 8(4) 333–338, 1978. – According to the conditioned anorexia hypothesis, conditioned taste aversions occur when flavour stimuli are classically conditioned to the anorexigenic or hypodipsic effects of drugs. The effects on water intake of a range of doses of amphetamine and of several related compounds have therefore been examined in an attempt to correlate their known potencies in taste aversion experiments with their hypodipsic potencies. (+)-Amphetamine was more potent than (–)-amphetamine in suppressing water intake but under similar experimental conditions, the isomers were equipotent in the conditioning of taste aversions. Methamphetamine and p-chloromethamphetamine were equipotent in suppressing water intake, but the latter was a more potent agent for conditioning taste aversions. Furthermore, fenfluramine produced taste aversions at doses well below those which suppressed water intake. It was concluded that the ability of the drugs to induce taste aversion was not related to their unconditioned, hypodipsic effects. However, it was confirmed that when drugs with different durations of action are compared for anorexic or hypodipsic potency, the outcome can be greatly influenced by the time over which measurements are made.

Anorexigenic drugs Water intake Conditioned taste aversion Amphetamine Fenfluramine Methamphetamine p-Chloromethamphetamine Chlorphentermine Cocaine

RATS can learn to reject distinctively flavoured solutions if their consumption precedes the administration of large doses of lithium or apomorphine [14,23]. Many hypotheses have been presented as to how drugs bring about such conditioned taste aversions (CTA), but no mechanism has been generally agreed [6]. According to the conditioned anorexia hypothesis, CTA occurs when flavour stimuli are classically conditioned to the anorexigenic or hypodipsic effects of drugs [8,18]. Such conditioning would be expected to decrease the intake of flavoured solutions, thus producing the effect typically seen in CTA experiments. This idea is attractive since it attempts to account for CTA in terms of a known behavioural effect and if correct, it would help to explain how many drugs which can serve as positive reinforcers in self-administration experiments also appear to have aversive properties in CTA procedures [2, 6, 17, 29]. In this paper, the terms anorexia and hypodipsia are used merely to describe behavioural change, with no implications intended as to specificity or mechanism.

Amphetamine may be regarded as the prototypical anorexigenic drug and it is indeed a very potent agent for inducing CTA [2, 5, 18]; furthermore, Carey and Goodall [8] have reported that the relative potencies of (+)- and (–)-amphetamine are similar in CTA and hypodipsia experiments. However, other work has provided little support for the conditioned anorexia hypothesis of CTA. Doses of X-radiation which induced CTA did not produce hypodipsia [9,14] whereas chlordiazepoxide, a drug which usually increases food intake, also induced clear CTA [6]. Doses of lithium, ammonium sulphate, arginine and glucose which produced similar degrees of anorexia induced varying intensities of CTA [20]. Conditioned anorexia seems therefore not to be tenable as an account of CTA in general, but it might be viable with regard to CTA induced by amphetamine.

We have previously compared the potencies of amphetamine, fenfluramine and several related compounds in a discriminative CTA procedure [3]. Aversive potency was more closely correlated with anorexigenic activity than with behavioural stimulation, thus providing tentative support for the conditioned anorexia hypothesis. However, estimates of anorexigenic potency were based on studies in other laboratories and for several reasons, the results were difficult to compare with our own. Firstly, most studies assessed mainly anorexigenic potency [11] rather than the more relevant, hypodipsic potency. Secondly, there is evidence that the simultaneous availability of food can influence the hypodipsic effects of amphetamine [13, 16, 22]. Finally, the relative potency and even the direction of effects of drugs on eating and drinking can be influenced by the times over which measurements are made [4, 15, 26]. It was therefore necessary to compare the results of our CTA studies with assessments of hypodipsia carried out under similar conditions and in the same strain of rat. The

present paper reports the results of such assessments, and discusses their implications for CTA.

METHOD

Animals

Male, hooded rats weighing 220–320 g and bred in the Department of Psychology, University of Birmingham were used throughout. The rats were housed individually in a room maintained at about 22°C and a regular light–dark cycle was imposed by fluorescent lighting (light from 08:00–20:00 hr).

Procedure

One week after the rats arrived in the laboratory, their access to water was restricted to 1 hr per day (10:00–11:00 hr). Distilled water was always used and it was presented in calibrated, glass tubes. All rats remained on this regimen for 2 weeks before the first day on which drugs were administered, and on all days between drug tests throughout the remainder of the experiment. Food was freely available at all times and tests with drugs took place on every fourth day. A different group of 7–8 rats was used for each drug.

On a test day, each rat was injected either with a dose of a drug or with isotonic saline. Thirty minutes later (i.e. at 10:00 hr for the first rat) distilled water was presented for 4 hr and readings of the amounts consumed were taken after 0.25, 1 and 4 hr. Water was not presented again until 10:00 hr on the next day. Each rat was tested in this way once after each drug dose and once after saline injection. The order in which the different injections were given was different for each rat, and was determined by a randomisation procedure.

There were 2 exceptions to the procedure described above. Firstly, cocaine was administered only 15 min before the 4 hr periods of access to water, in order to make some allowance for the known rapid onset and offset of its effects. Secondly, previous work with several drugs suggested that it would be necessary to administer them in high doses in order to suppress drinking completely. In order to minimise the carry-over of effects from one test day to another, especially with the halogen-substituted amphetamines whose actions can be extremely prolonged [10,24], the highest dose of all drugs except cocaine was given only on an additional test day after completion of the randomised series. The data from these additional tests were included in the graphical presentations of the results but not in the main statistical analyses.

Statistical Analyses

Single-factor or 2-factor analyses of variance for repeated measures were carried out, and then Dunnett's t-test for multiple comparisons were used to determine at which doses the drugs significantly reduced fluid intake [28]. Due to an error in injection, data for 1 rat were lost for 1 of the doses of methamphetamine. The procedure described by Snedecor and Cochran [25] was therefore used to complete the set of data so that analysis of variance was possible. The ED_{50} value for a drug was defined as the dose which would have been expected to reduce the mean fluid intake to 50% of its value after saline injection, and it was estimated by interpolation from the dose-response curve.

Drugs

All drugs were dissolved in isotonic saline and were injected intraperitoneally in volumes of 1 ml/kg. Doses were expressed as salts, which were as follows: (+)-amphetamine sulphate, Smith Kline and French; (−)-amphetamine sulphate, Menley and James; (±)-methamphetamine HCl, Sigma; (±)-p-chloromethamphetamine HCl, Regis; (±)-fenfluramine HCl, Servier; chlorphentermine HCl, Lundbeck; cocaine HCl, B. P. The doses were selected from studies of CTA and of anorexia [1, 3, 4, 8, 11, 21].

RESULTS

(+)- and (−)-Amphetamine

The findings for the 3, consecutive periods in which fluid intake was measured are considered in turn. Figure 1(A) shows dose-response curves for the hypodipsic effects of (+)- and (−)-amphetamine during the first 15 min of access to water. It can be seen that in sufficient doses, both isomers greatly reduced the mean water intake, $F(4,56) = 31.8$, $p<0.001$. The effect of (+)-amphetamine was greater than that of (−)-amphetamine, $F(4,56) = 13.0$, $p<0.001$ and ED_{50} values derived from the dose-response curves yielded a potency ratio of 3.4:1 for the two isomers (Table 1).

The water intake during the next 45 min was generally much lower, which merely reflects the satiating action of the water consumed previously. Figure 1(B) shows that the amphetamines continued to reduce water intake in a dose-related manner, $F(4,56) = 9.27$, $p<0.001$, and that there was little difference between the effects of the two isomers in this period, $F(4,56)<1$.

During the last 3 hr of the 4 hr period of access to water (Fig. 1(C)) the drug conditions showed significantly greater mean water intakes, $F(4,56) = 10.3$, $p<0.001$. The effect for (+)-amphetamine was significant at doses of 1 mg/kg, $t(28) = 2.90$, $p<0.05$ and 3.2 mg/kg, $t(28) = 5.09$, $p<0.01$, whereas the smallest dose of (−)-amphetamine after which water intake increased was 3.2 mg/kg, $t(28) = 2.96$, $p<0.05$. It should be noted that these increases in water intake are approximately proportional to, and merely compensate for, the water deficits incurred earlier.

Methamphetamine and p-Chloromethamphetamine

During the first 15 min of the 4 hr tests, both methamphetamine and p-chloromethamphetamine reduced the mean water intake in a dose-related manner, $F(4,55) = 22.5$, $p<0.001$. Figure 2(A), and the ratio of the ED_{50} values (1.1:1), show that there was virtually no difference between the potency of the 2 drugs in this part of the experiment. The results were similar during the period 15–60 min into the 4 hr tests; both drugs continued to reduce water intake, $F(4,55) = 13.3$, $p<0.001$, and their effects did not differ appreciably.

In the final 3 hr of the 4 hr periods of access to water, there was a clear and significant difference between the methamphetamine and p-chloromethamphetamine conditions, $F(4,55) = 7.56$, $p<0.001$. Figure 2(C) shows that methamphetamine at a dose of 3.2 mg/kg was followed by an increased mean water intake, $t(27) = 6.41$, $p<0.01$, whereas the same dose of p-chloromethamphetamine gave rise to no appreciable effect, $t(28) = 1.23$. Water intake after the administration of methamphetamine at a dose of 10 mg/kg was also much greater than that after the same

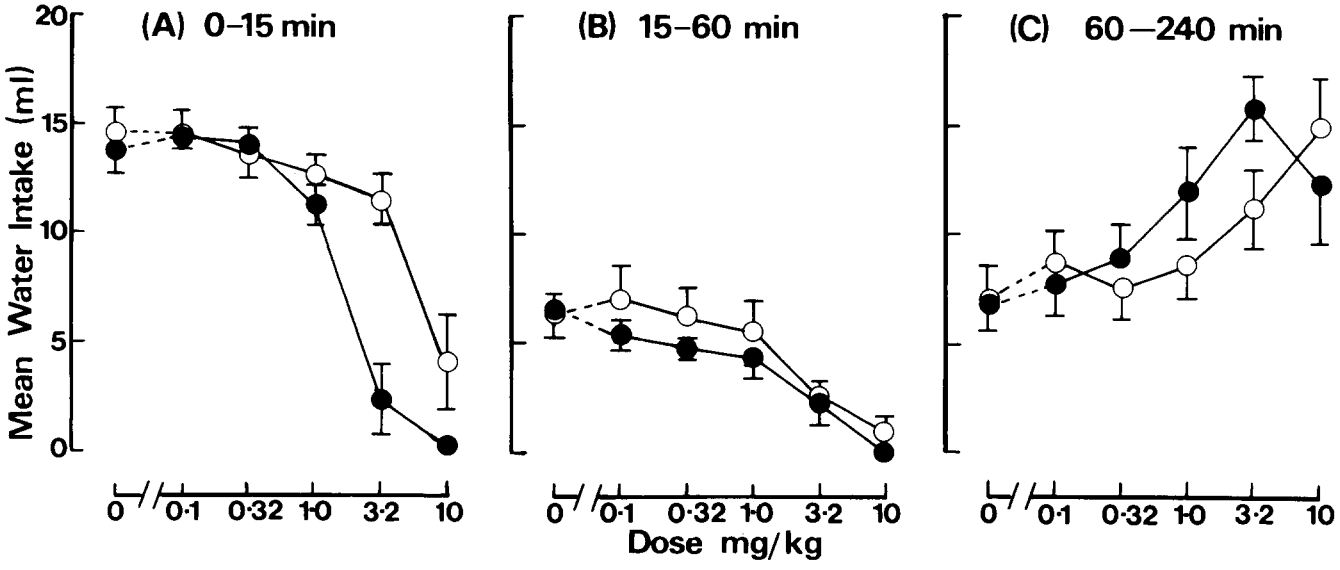

FIG. 1. Mean water intake of rats (n = 8) during 3 consecutive periods beginning 30 min after IP injections of either (+)-amphetamine (•) or (−)-amphetamine (○). Both isomers initially suppressed drinking, although (+)-amphetamine was clearly the more potent (A). Subsequently, there was a compensating increase in fluid intake (C). Vertical bars indicate 1 SE on each side of means (overlapping bars have been omitted for clarity).

dose of p-chloromethamphetamine, $t(14) = 4.71, p<0.001$. Thus, the rats compensated for the deficit in water intake brought about by the initial effect of methamphetamine, but p-chloromethamphetamine continued to depress drinking throughout much of the 4 hr test period. As a consequence of this difference in duration of action, the relative ED_{50} values for the 2 drugs depended on the time over which measurements were made (Table 1); the longer the period of measurement, the greater was the relative potency of the long acting, halogen-substituted compound.

Fenfluramine, Chlorphentermine and Cocaine

Figure 3 shows dose-response curves for the effects of these drugs on water intake during the first 15 min of the 4 hr drinking periods. When administered in moderate doses, both fenfluramine and chlorphentermine suppressed drinking, $p<0.001$ in both cases. The effects of cocaine were much weaker; even at the very high dose of 36 mg/kg, cocaine only reduced the mean water intake by 34%, $t(28) = 3.09, p<0.05$. Doses of cocaine larger than 36 mg/kg were

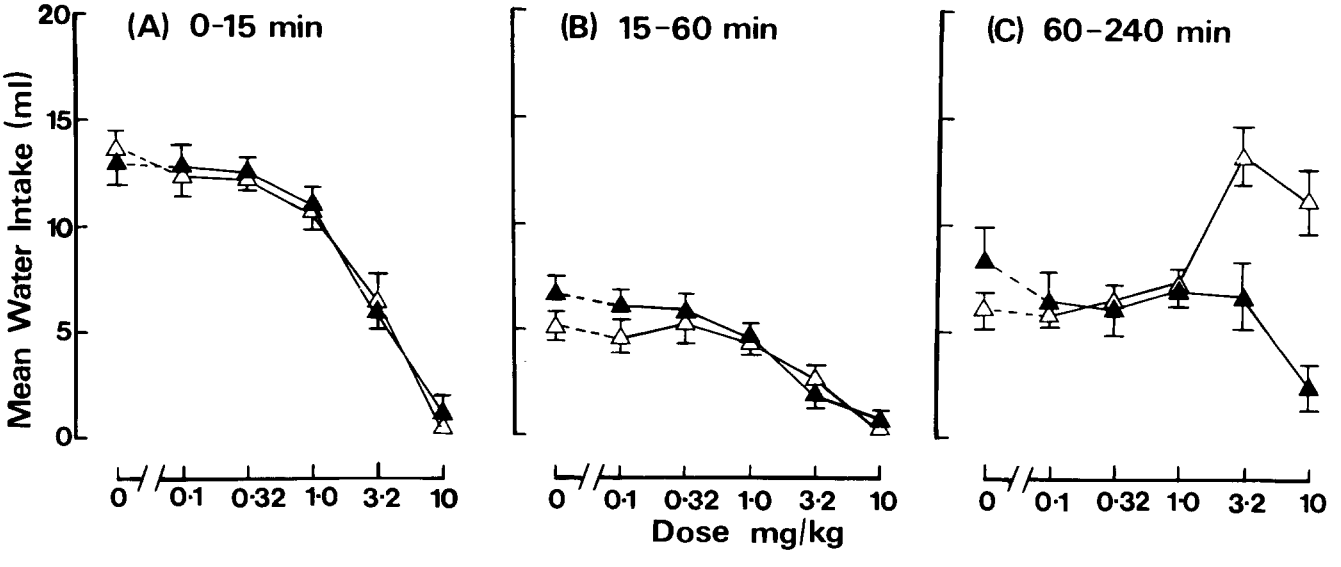

FIG. 2. Mean water intake of rats (n = 8) during 3 consecutive periods beginning 30 min after injection of either methamphetamine (△) or p-chloromethamphetamine (▲). Both drugs were initially equipotent in suppressing drinking (A) and the effects of the high doses of p-chloromethamphetamine persisted throughout the 4 hr drinking sessions, whereas compensatory increases in fluid intake occurred after the larger doses of methamphetamine (C). Vertical bars indicate 1 SE on each side of mean.

TABLE 1

HYPODIPSIC POTENCIES OF AMPHETAMINE AND RELATED COMPOUNDS (ED_{50} VALUES, MG/KG)

Drug	n	Time of Measurement within 4 hr Sessions		
		0–15 min	0–1 hr	0–4 hr
(+)-Amphetamine	8	1.8	1.8	8.5
(–)-Amphetamine	8	6.2	5.0	(33)
Methamphetamine	8	2.9	3.1	9.4
Cl-methamphetamine*	8	3.2	2.5	3.6
Fenfluramine*	7	6.6	5.6	6.6
Chlorphentermine*	7	13.3	9.6	16.7
Cocaine†	8	(11.2)	(20.0)	(36)

ED_{50} values are doses (IP) of salts required to suppress water intake by 50%. The various drugs are grouped according to duration of action, in order to show how this factor combines with time of measurement to influence apparent potency. (* = very long-acting; † = short-acting). The ED_{50} value for (–)-amphetamine at 0–4 hr was estimated by extrapolation of the dose-response curve since the largest dose tested only reduced water intake by 30%. Cocaine did not reduce water intake by more than 34% at any time, and therefore ED_{75} values were calculated for it.

not used because they induced transient convulsions during preliminary tests.

During the remainder of the 4 hr test period, the results for fenfluramine and chlorphentermine continued to resemble those for p-chloromethamphetamine (Fig. 2). Thus, water intake was reduced during the 15–60 min period ($p<0.01$ for both drugs) and there were no compensatory increases during the subsequent 3 hr. Cocaine differed from all the amphetamines tested in the sense that it had no significant effect on fluid intake during the 15–60 min period, $F(4,28) = 2.01$. However, there was a weak, compensatory facilitation of drinking in the final 3 hr; during this period, the mean water intake after cocaine (36 mg/kg) was 14.8 ml, as compared with only 9.1 ml after saline injection, $t(28) = 2.75$, $p<0.05$. Complete tables of means and significance levels for all drugs and time intervals may be obtained from the authors.

DISCUSSION

A series of 7 compounds including amphetamine and fenfluramine has been studied in rats for potency in suppressing water intake (this paper) and for inducing CTA [3]. The results show, on the one hand, that drugs which are equipotent in CTA can differ in hypodipsic potency and, on the other hand, that drugs with similar hypodipsic potencies can differ in CTA. It will be argued that this double dissociation is incompatible with the conditioned anorexia hypothesis of CTA, although it should be noted that the experiments do not address the question of whether other behavioural or physiological effects of amphetamine can be conditioned to flavour stimuli.

The results have confirmed that the hypodipsic potency of (+)-amphetamine is at least 3 times that of (–)-amphetamine [8]. Studies of anorexia have yielded similar results [1]. However, under conditions very similar to those used in the studies of hypodipsia, and in rats of the same sex, strain and weight [3], the 2 isomers were essentially equipotent in CTA (potency ratio = 1.2:1). An earlier report showed that (+)-amphetamine was more effective than (–)-amphetamine in CTA [8], but we have presented evidence elsewhere that this finding was probably due to a difference in time-course of action rather than in potency [3].

Cox and Maickel [11] have found little difference between the anorexigenic potencies of methamphetamine and p-chloromethamphetamine and our finding that the hypodipsic potencies of these drugs were similar (i.e. 1.1:1) is therefore not surprising. Table 1 further confirms that relative potency appears to depend on the time over which measurements are made; this is a finding which can be expected whenever different durations of drug action interact with rats' tendency to compensate for earlier, drug induced deficits in food or water intake [4, 15, 19, 26].

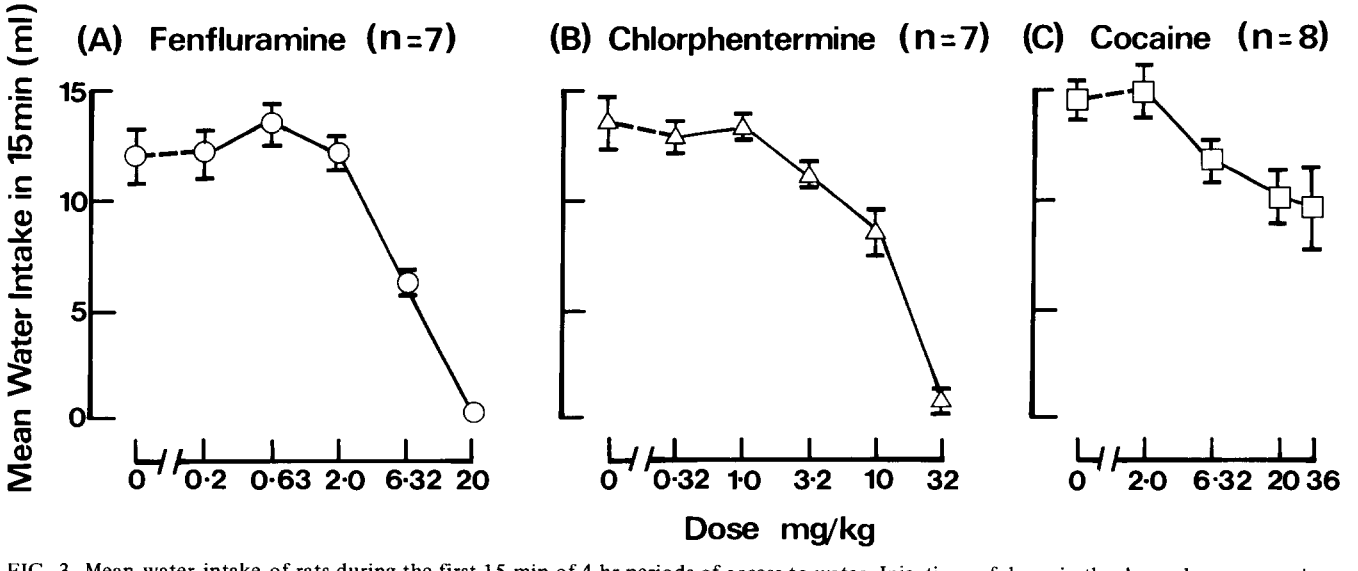

FIG. 3. Mean water intake of rats during the first 15 min of 4 hr periods of access to water. Injections of drugs in the doses shown were given either 30 min (fenfluramine, chlorphentermine) or 15 min (cocaine) before making the water available.

Nevertheless p-chloromethamphetamine was twice as potent as methamphetamine in CTA experiments with 15 min periods of access to flavoured solutions [3], precisely the conditions used to show that the same 2 drugs were equipotent as hypodipsic agents (Table 1).

Fenfluramine was less potent than (+)-amphetamine as a hypodipsic agent, but was approximately equipotent in CTA [3]. Furthermore, fenfluramine at 2 mg/kg induced very strong CTA after only a single conditioning trial, but this dose of fenfluramine was totally lacking in hypodipsic activity (Fig. 3(A)). This dissociation between the doses needed to induce CTA or hypodipsia was greater with fenfluramine than with any of the other drugs tested, but such dissociations were found in nearly all cases. Even (+)-amphetamine, one of the more effective agents for producing hypodipsia, induced some degree of CTA at a dose of 0.1 mg/kg, which was about one tenth of the threshold dose for hypodipsia. However, it should be noted that tests with only chlorphentermine and cocaine would have suggested a correlation between hypodipsia and CTA: the ED_{50} values for both the hypodipsic and the CTA effects of chlorphentermine were about 8 times those for (+)-amphetamine, whereas cocaine was only weakly active in both situations (to an extent that ED_{50} determinations were not feasible).

Other lines of research have also yielded evidence which appears inconsistent with the conditioned anorexia hypothesis of CTA. Treatment with α-methyl-p-tyrosine blocked both amphetamine-induced anorexia and the acquisition of CTA with amphetamine [1, 7, 17], but failed to attenuate an aversion previously established with amphetamine [7]. Furthermore, the effects of presenting flavours previously paired with amphetamine (1 mg/kg) were uniformly depressant on fixed-interval and fixed-ratio schedules of operant responding, whereas the same dose of amphetamine itself produced the well-known, mixed pattern of facilitation and depression [12,27]. Thus, the conditioned response to the flavour differed from the response to the drug. With regard to CTA induced by agents other than amphetamine, evidence incompatible with the conditioned anorexia hypothesis has been summarised in the introduction [5, 9, 20].

The present studies were carried out to test the validity of a single idea, conditioned anorexia, and they do little to support or refute any of the alternative accounts of CTA [6]. However, the results do add to the evidence that the longer the duration of action of a drug, the greater its efficacy in CTA [6]. The studies of hypodipsia confirmed, under relevant conditions, that cocaine (a weak agent in CTA) has a short duration of action whereas the halogen-substituted amphetamines (generally very potent in CTA) have long-lasting effects [10,24]. Whether this possible relationship might provide further clues as to how drugs bring about CTA remains to be determined.

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