POSSIBILITY OF SPONTANEOUS DRUG ABUSE TESTED IN RAT

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SUMMARY

Given that a number of the techniques used to test drug abuse liability are not free from criticism, a series of oral free-choice experimental procedures was adopted. When simultaneously offered as alternatives to glucose using the classical polydipsic procedure, no preference for buprenorphine (0.025 mg/ml), morphine (0.5 mg/ml) or fentanyl (0.005 mg/ml) solutions was shown by premedicated rats. The same result was obtained when the two-bottle procedure was used for at least one month to offer etonitazene (10 μg/ml), buprenorphine (60 μg/ml), cocaine (300 μg/ml) and haloperidol (25 μg/ml) solutions as simultaneous alternatives to aspartame. This absence of preference was maintained even when the rats showed evident pharmacological effects and, in the case of the opiates, tolerance and withdrawal syndrome. However, when a gustatory marker (quinine) was introduced into one of the two solutions, preference was always shown for the other. Finally, in a conditioned taste aversion (CTA) test, etonitazene (5 or 40 μg/kg, i.p.) and haloperidol (0.5 or 2 mg/kg, i.p.) did not induce any reduction in saccharin consumption, while morphine (40 mg/kg) did. Pretreatment with naloxone (120 μg/kg, i.c.v.) did not antagonize morphine-induced CTA, while it did antagonize morphine-induced analgesia.

KEY WORDS: drug abuse; oral self-administration; conditioned taste aversion.

INTRODUCTION

Although on an anecdotal basis, it is often reported that many wild animals show a craving for vegetables capable of providing a pleasant intoxication [1]. Over the last 20 years, it has also been shown that primates and laboratory rodents seek and intravenously self-administer the majority of the drugs abused by humans, even if they are not made physically dependent [2]. With the exception of some hallucinogens, self-injecting behaviour agrees well with the abuse potential for

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humans of many classes of agents, although some classes (e.g. benzodiazepine anxiolytics) are equivocal. Making drugs orally available in animal research poses several problems, such as the intervention of gustatory factors and the relatively delayed production of reinforcing effects.

Nevertheless, given that, under various circumstances, humans prefer to take a number of drugs with abuse potential orally, investigation of the variables determining excessive drug ingestion is of interest. The schedule-induced polydipsia method [3] has been widely used to induce animals to ingest large volumes of opiates [4], ethanol [5], cocaine [6] and benzodiazepines [7]. A simple and more physiological drinking procedure in which animals are offered the drug in their home cage has also been tried, although its extensive use has been restricted to alcohol [8]. However, neither of these methods is free from the criticism that it involves a stress situation (confinement) and the constant availability of a drug without the possibility of choice. Voluntary development of a dependent state in laboratory animals with free drug availability and without any previous treatment has been only occasionally tried with opioids, but intensively used with alcohol [9]. Few and contradictory data exist concerning the consumption of narcotics in monkeys [10] and rats [11–13], and their demonstrated preference for drug solutions could be due to premedication or to the

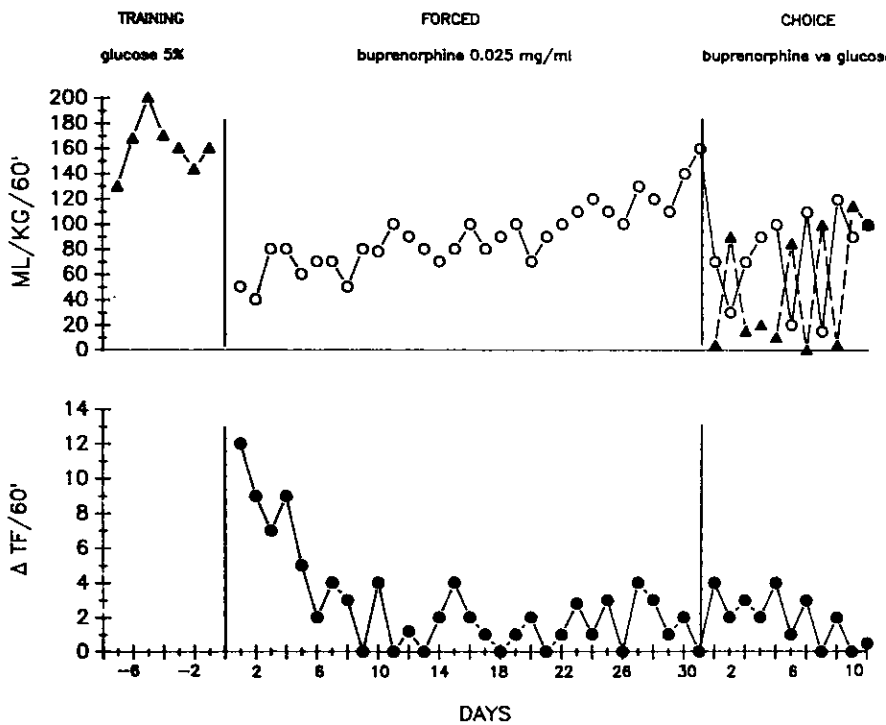

Fig. 1. Top: Fluid intake (ml/kg/60') of glucose (▲) and subsequently of BS (○) of one rat submitted to polydipsic regimen. Bottom: Analgesic effect (△ s) tested at 60'. From l the same parameters evaluated in a free-choice situation for 11 days.

type of schedule adopted. The aim of the present study was to investigate the critical factors affecting rat oral self-administration of addicting drugs in a free-choice paradigm: (i) the adopted schedule; (ii) palatability of the offered solutions; (iii) premedication; (iv) the relevance of the temporal dissociation between gustatory and pharmacological cues.

MATERIALS AND METHODS

Animals

Male Wistar rats, initially weighing 180–300 g lodged in a climatized room (humidity 50±5%; temperature 22±2°C; 12 h light) were individually housed in standard laboratory cages.

Procedure

Schedule-induced polydipsia. The body weights of three groups of four rats each, were reduced to 85% of their initial level over a 2-week period by limiting daily food rations. The animals were then kept at these weights for the duration of

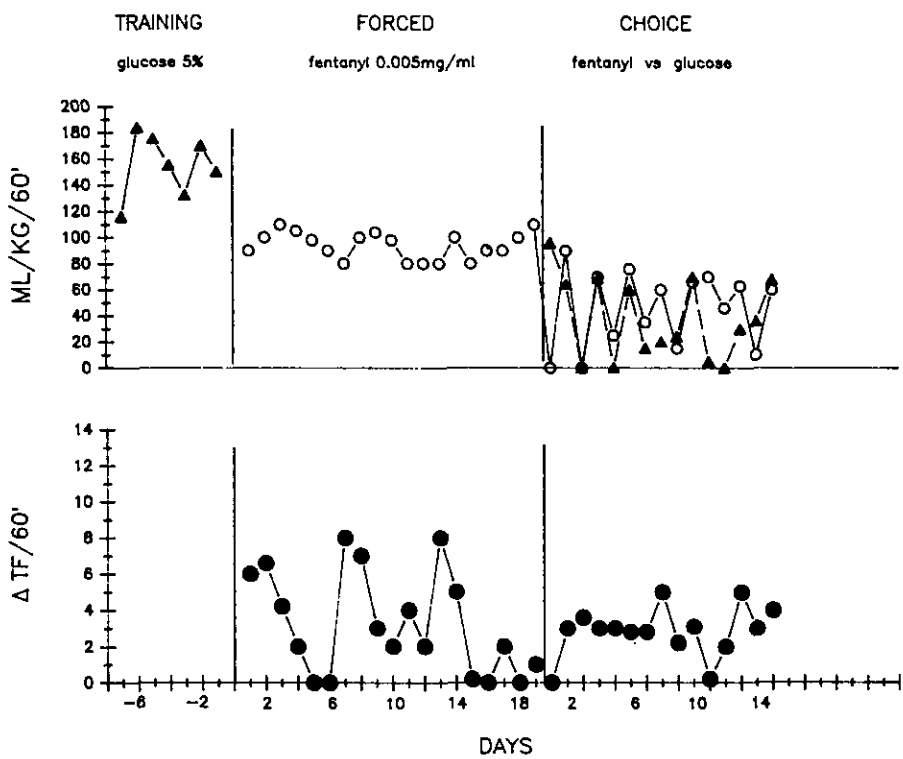

Fig. 2. Top: Fluid intake (ml/kg/60') of glucose (▲) and subsequently of FS (○) of one rat submitted to polydipsic regimen. Bottom: Analgesic effect (△ s) tested at 60'. From I the same parameters evaluated in a free-choice situation for 15 days.

the experiment. After their weights were stabilized, they were submitted to daily, 1-h, schedule-induced polydipsia according to our previously described technique [14]. After a period of training during which their glucose solution intake was stabilized, the animals were offered buprenorphine HCl (BS) (0.025 mg/ml), fentanyl citrate (FS) (5 μg/ml) or morphine HCl (MS) (0.5 mg/ml), all dissolved in glucose 5% (as sweetener). At the end of each daily session, recordings were made of fluid intake (ml/kg), analgesia [tested by the tail-flick method [15] and expressed as the difference (△ in s) between threshold latency and baseline values], catatonia (evaluated according to Dunstan *et al.* [16] with a maximum score of 6), and jumpings (according to Huang *et al.* [17]).

Two-bottle drinking test. The classic two-bottle technique, originally developed by Myers for alcohol [18], was used as previously described [19] in order to offer a free choice in a familiar environment without the use of premedication. Rats initially weighing 240–260 g, and water deprived for 23 h, were allowed to drink for only 60 min a day from two graduated bottles. Since, in previous experiments (unpublished data), we had tested increasing concentrations of BS (0.06–0.12–0.6 mg/ml) and ES (2.5–5–10–15–17.5 μg/ml) in a free-choice

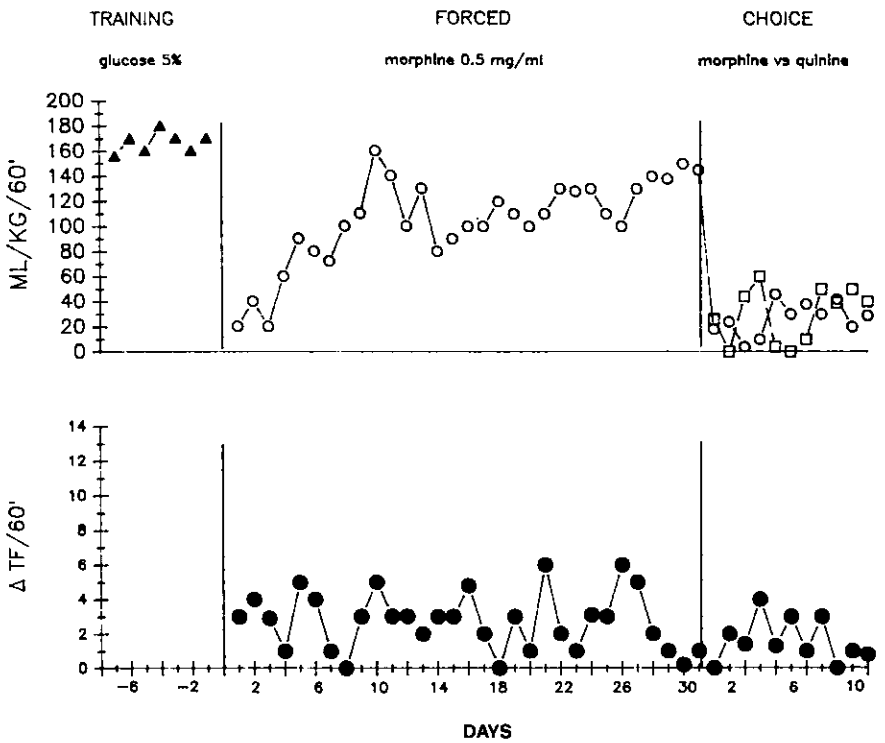

Fig. 3. Top: Fluid intake (ml/kg/60') of glucose (▲) and subsequently of MS (○) of one rat submitted to polydipsic regimen. Bottom: Analgesic effect (△ s) tested at 60'. From l the same parameters evaluated in a free-choice situation for 11 days. (□), Quinine 0.1 mg/ml dissolved in glucose 5%.

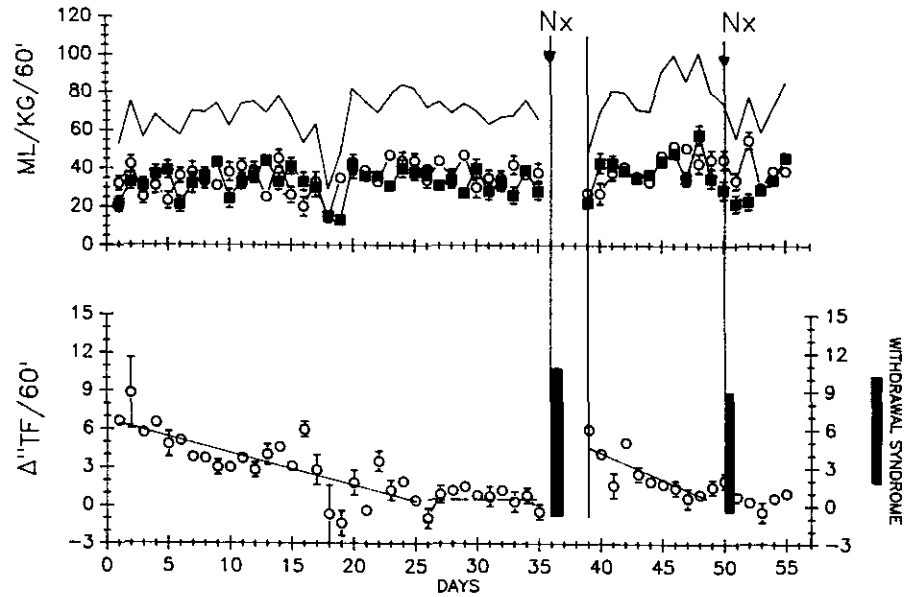

Fig. 4. Top: Mean volumes consumed in a free-choice situation (ml/kg/60') of BS (60 μg/ml) (○), aspartame (■) and both together (—). Bottom: analgesic effect (△ s). At ↓ naloxone (5 mg/kg, i.p.) administration followed by withdrawal syndrome score (N=12).

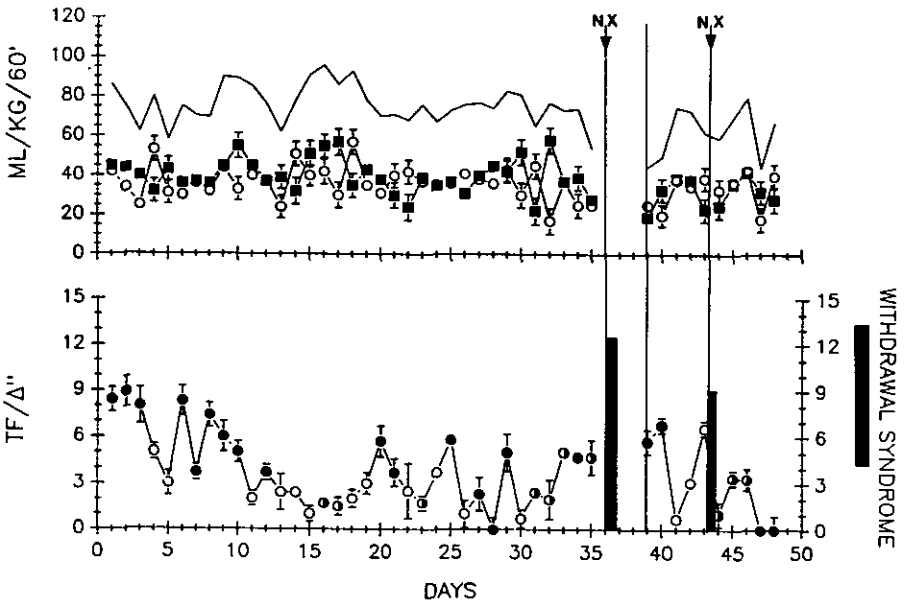

Fig. 5. Top: Mean volumes consumed in a free-choice situation (ml/kg/60') of ES (10 μg/ml) (○), aspartame (■) and both together (—). Bottom: analgesic effect (△ s) (○) associated with catatonia (●) or jumpings (◑). At ↓ naloxone (5 mg/kg, i.p.) administration followed by withdrawal syndrome score (N=12).

situation, in order to check palatability and behavioural modifications, we decided to use only the concentration best satisfying the above-mentioned requisites. In a first series of experiments, one bottle contained aspartame (0.18% in water) and the other contained etonitazene HCl (ES) 10 μg/ml (assumed as full μ-opiate agonist), BS 60 μg/ml or cocaine HCl (CS) 300 μg/ml dissolved in an aspartame solution; in a second series, to check whether the animals were unable to choose simply because the two offered solutions had the same taste, a gustatory marker was introduced. Thus, quinine sulphate 0.1 mg/ml (Q) was added to the bottle containing ES 10 μg/ml for one group of animals, and to the bottle containing aspartame for the other. Finally, a non-addicting but behaviourally active drug, haloperidol 25 μg/ml (HS), was offered simultaneously first with aspartame and then with a Q solution.

At the end of every drinking session, fluid intake (ml/kg), analgesia, catatonia and jumpings were evaluated as described above. Withdrawal syndrome was precipitated by naloxone (5 mg/kg, i.p.) and scored according to Grant and Redmond [20] with a maximum attainable score of 15. CS-induced behavioural stimulation was measured by evaluating sniffing, licking and rearing activities according to Roy *et al.* [21].

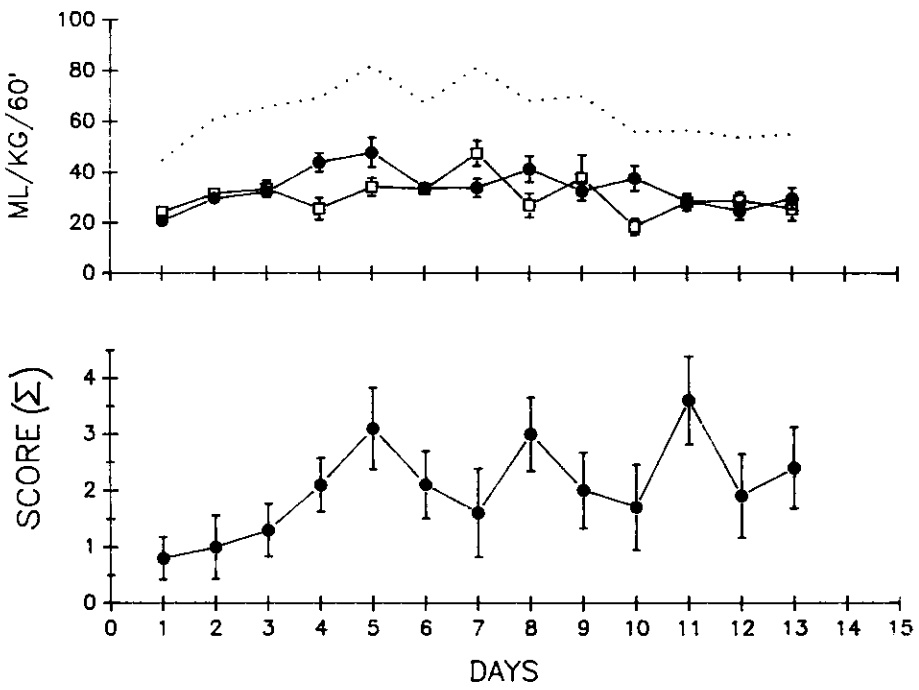

Fig. 6. Top: Mean volumes consumed in a free-choice situation (ml/kg/60') of CS (300 μg/ml) (●), aspartame (□) and both together (. . .). Bottom: Behavioural stimulation evaluated by sniffing, licking and rearing scored and expressed as Σ score (N=12).

Conditioned Taste Aversion (CTA) test. Since the relevance of the temporal dissociation between gustatory and pharmacological cues is well known [22], a conditioned taste aversion (CTA) test was carried out. The CTA test was performed according to Garcia *et al.* [22]. Rats, weighing 180–200 g were trained for 7 days to a 10 min period of access to water every 24 h. Subsequently, every third day, they received a 0.1% saccharin solution during the 10 min drinking session. Five min after the end of each saccharin session, the rats received an intra peritoneal (i.p.) injection of isotonic saline (5 ml/kg), ES (5 and 40 μg/kg), HS (0.5 and 2 mg/kg) or MS (40 mg/kg). Thirty min after the injection, analgesia and catatonia were scored as described above. These drinking sessions and subsequent drug administrations were repeated 10 times. Naloxone HCl 120 μg/kg was injected intracerebroventricularly (i.c.v.) 5 min before MS in a group of rats previously prepared for icv injection according to Altaffer [23].

Statistical analysis

All the values were expressed as mean±SEM and analysed by ANOVA test. Means of analgesic response(s) were analysed over the days using linear regression line.

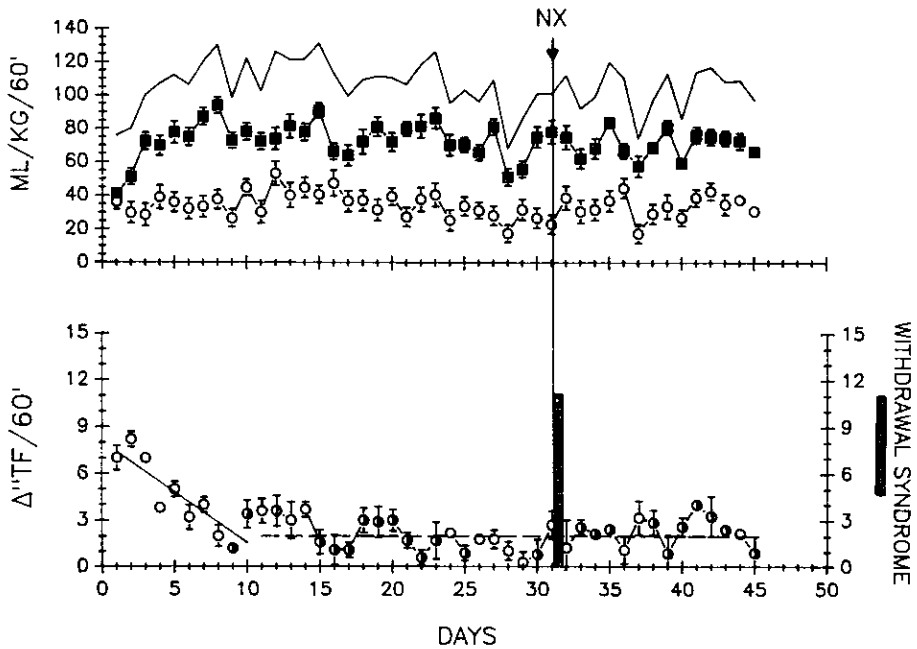

Fig. 7. Top: Drinking pattern of ES (10 μg/ml) containing Q (0.1 mg/ml) (○), simultaneously offered with aspartame (■) during 60' daily sessions in a free-choice situation. Bottom: analgesic effect (△ s) (○) associated with jumpings (◑). At ↓ naloxone (5 mg/kg, i.p.) administration followed by withdrawal syndrome (N=12).

RESULTS

Schedule-induced polydipsia. As shown in Fig. 1, when BS was added to the glucose after training, fluid intake decreased for the first few days and then progressively increased. This dramatic drop was probably due to the sedative effects elicited by the drug. The consumption progressively increased when the strong analgesic effect was submitted to tolerance. Thirty days after premedication, when a choice between BS and a glucose solution was offered, no significant preference (ANOVA) was shown over a period of 11 days. Similar results were obtained in FS and MS premedicated rats (Figs 2 and 3). For MS, the initial drop in intake was probably associated with the aversive taste of the solution. For this reason, the analgesic effect of MS was generally less evident and more variable than that of BS.

Two-bottle drinking test. The daily intake of BS induced an evident analgesic effect linearly related to the first 25 days. Despite the elicited analgesia, the rats did not show any significant preference for either BS or aspartame over 35 days. This lack of preference was present both after full tolerance to analgesia and 3 days or 1 h after naloxone-precipitated withdrawal syndrome (Fig. 4). The same results were obtained when ES was offered in a free-choice situation (Fig. 5), although once again there was evidence of the existence of pharmacological effects (analgesia, catatonia and jumpings). With CS, no significant preference

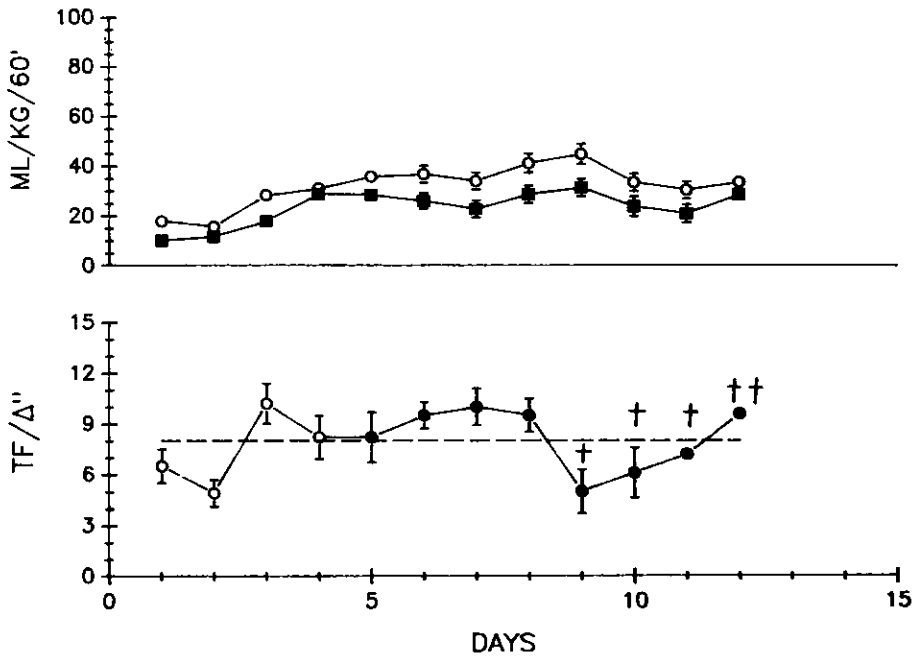

Fig. 8. Top: Drinking pattern of ES (10 μg/ml) (○) simultaneously offered with aspartame containing Q (0.1 mg/ml) (■). Bottom: analgesic effect (Δ s) (○) associated with catatonia (●). † represents one rat death (N=12).

was evident for at least 13 days (Fig. 6), in spite of the presence of typical excitatory behaviour characterized by more frequent sniffing, licking and rearing.

When Q was added to ES, the rats exhibited a significant preference (ANOVA) for aspartame over 45 days (Fig. 7). The small amount of ES consumed had a strong analgesic effect which showed tolerance with the onset of jumpings and physical dependence elicited by the administration of naloxone (5 mg/kg, i.p.) on the day 31. On the contrary, when Q was added to aspartame, a significant preference for ES was elicited, leading to very strong analgesia and catatonia, and followed by a high percentage of deaths (Fig. 8). As shown in Fig. 9, the rats were unable to choose between aspartame and HS over 25 days, despite the fact that HS led to severe catatonia. They showed a significant preference for HS (despite the catatonia) only when Q was added to aspartame over 7 days.

CTA test. As shown in Fig. 10, ES did not induce any significant CTA, the volume of ingested saccharin remaining unchanged both at the analgesic and catatonic dose of 40 μg/kg, and at the behaviourally inactive dose of 5 μg/kg. The same results were obtained with HS: neither the weak (0.5 mg/kg) nor the strong catatonic dose (2 mg/kg) led to any reduction in saccharin consumption (Fig. 11). As expected, MS elicited both CTA and analgesia. Pretreatment with i.c.v.-

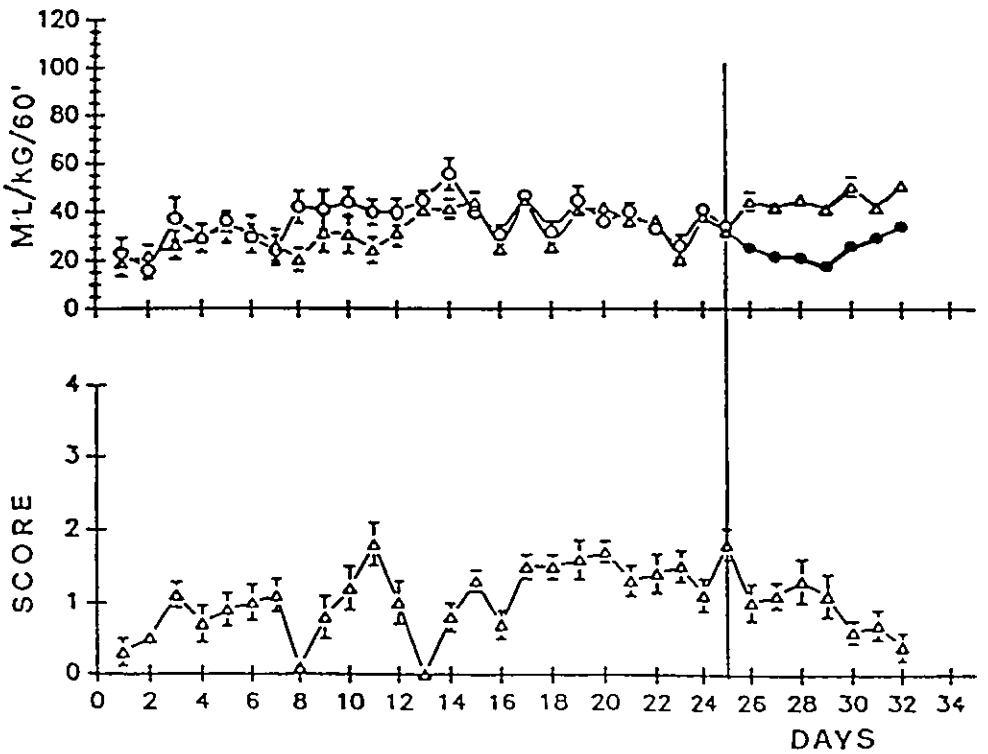

Fig. 9. Top: Drinking pattern of HS (25 μg/ml) (△) simultaneously offered with aspartame (○); at l with aspartame containing Q (0.1 mg/ml) (●). Bottom: Mean catatonic score (N=10).

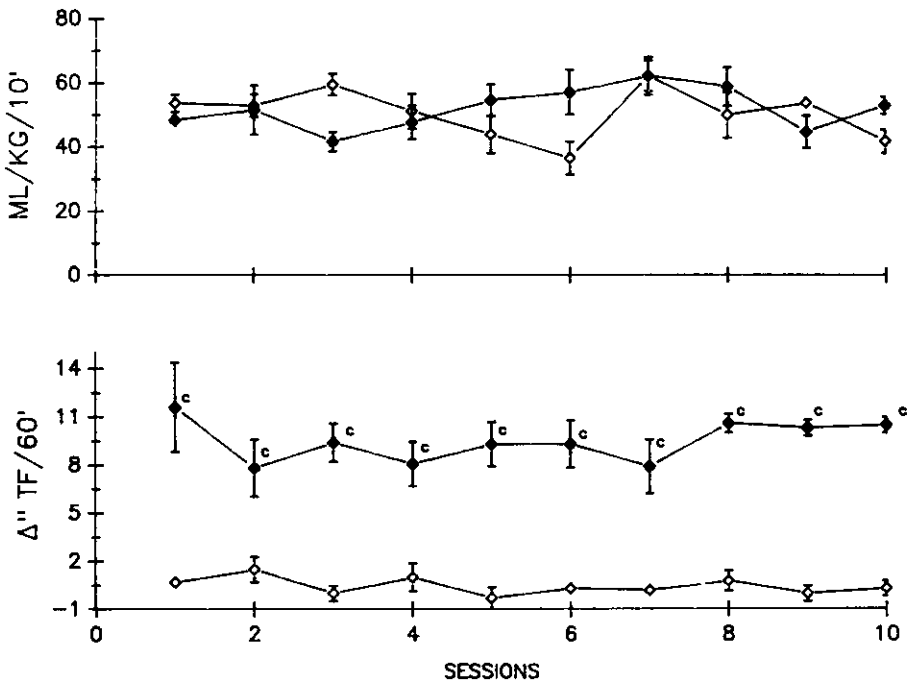

Fig. 10. Top: Mean saccharin intake in 10 min over 10 sessions of CTA, after i.p. administration of ES 5 (◇) or 40 μg/kg (◆). Bottom: analgesic effect (△ s) after i.p. administration of ES 5 (◇) or 40 μg/kg (◆). c, catatonia, (N=10).

administered naloxone HCl antagonized only analgesia and not the MS-induced CTA (Fig. 12).

DISCUSSION

Our free-choice results agree well with those recently reported by Falk in relation to a study using schedule-induced polydipsia [24]. He found that no preference was shown for cocaine concurrently offered with water except when a sweetening compound was added to the drug solution. This suggests that the development of preference was due to an association of CS with the highly acceptable taste of the compound solution rather than to any learned appreciation of pharmacological effects.

In our experiment, it was only the introduction of a bitter gustatory marker (quinine) which led to a preference being shown for the less aversive tasting solution, regardless of the evoked pharmacological effect. Moreover, the use of a constant and behaviourally active concentration did not seem to influence the choice. In fact, a previous (unpublished) study using increasing concentrations of BS and ES did not show any evident preference despite the induction of tolerance and dependence.

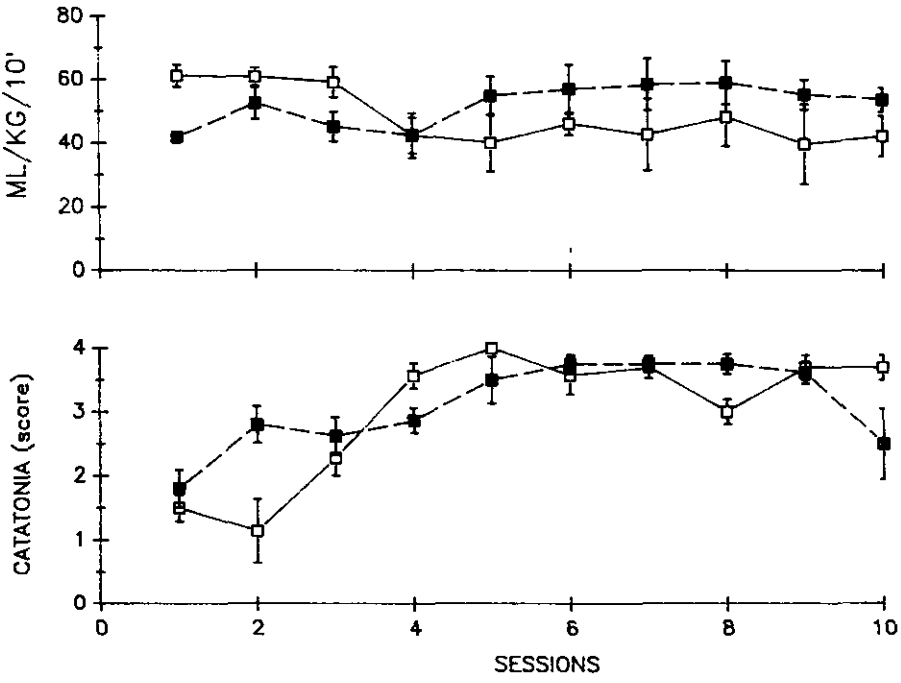

Fig. 11. Top: Mean saccharin intake in 10 min over 10 sessions of CTA, after i.p. administration of HS 0.5 (□) or 2 mg/kg (■). Bottom: Catatonic effect after i.p. administration of HS 0.5 (□) or 2 mg/kg (■) (N=10).

This lack of the development of a clear preference for drug solutions over vehicles under concurrent presentation conditions contrasts with previous findings obtained with cocaine in monkeys [25], and with morphine [26, 27] and ethanol [28, 5] in rats. Although no satisfactory explanation of our results can yet be offered, it is possible that the reinforcing function of the drug depends upon its schedule of availability. For example, it has been difficult to demonstrate the reinforcing efficacy of continuously available intravenous nicotine [29], but when schedules permitting only intermittent availability are used, nicotine quite clearly functions as a powerful reinforcing agent.

Furthermore, the introduction of conditions such as chronic pain, stress, isolated or crowded housing, or modified social rank can positively interfere with drug abuse consumption.

Another possible explanation is the inability of the animals to distinguish the central pharmacological effect of the adopted drug. In this respect, the CTA findings are contradictory: the flavour cue paired with morphine led to a conditioned aversion; when paired with etonitazene and haloperidol, it did not. Bechara and Van der Kooy [30] explained the opposite motivational effects they found using a Conditioned Place Preference paradigm (CPP) by suggesting that endogenous and exogenous opioids acting on peripheral receptors (especially in the gut) produce aversion or nausea, whereas those mainly acting on central brain

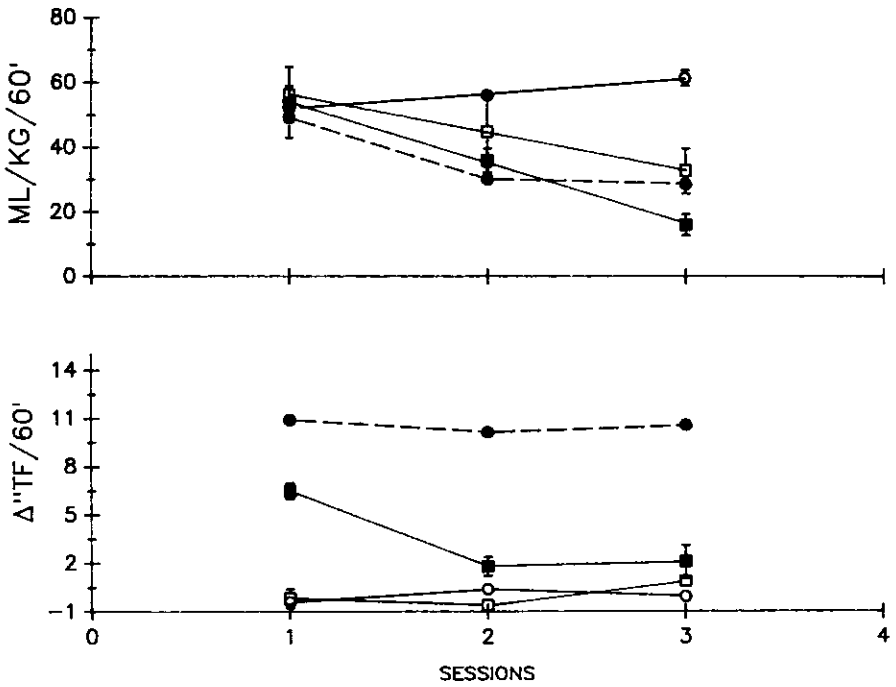

Fig. 12. Top: Mean saccharin intake in 10 min over 3 sessions of CTA, after administration of saline i.c.v.+saline i.p. (○); naloxone 120 μg/kg i.c.v.+saline i.p. (□); saline i.c.v.+MS 40 mg/kg i.p. (●); naloxone 120 μg/kg i.c.v.+MS 40 mg/kg i.p. (■). Bottom: Analgesic effect (Δ s) after administration of saline i.c.v.+saline i.p. (○); naloxone 120 μg/kg i.c.v.+saline i.p. (□); saline i.c.v.+MS 40 mg/kg i.p. (●); naloxone 120 μg/kg i.c.v.+MS 40 mg/kg i.p. (■).

receptors have positive reinforcing or euphoric effects. Using the same test, it has more recently been shown that etonitazene produces its reinforcing properties only in a limited range of doses [31], an inverted U-shaped function being obtained which is similar to that of buprenorphine [32]. As shown by Spyraky, haloperidol (which was not refused in our free-choice experiments) also fails to induce CTA (probably because of a lack of peripheral involvement) but does induce CPP [33].

On the other hand, it has been shown that i.c.v.-injected morphine does not elicit CTA [34] and the lack of antagonism found by us using peripherally administered morphine plus i.c.v. naloxone further supports the relevance of CTA peripheral component. In conclusion, the methods of using the relative free intake level of two fluids presented in a concurrent choice as indicators of their respective potential as reinforcing agents may have limited utility depending mainly on palatability. In fact this factor seems to overcome the intrinsic reinforcing properties of a drug. Also, the CTA test is not free from criticism: the obtained contradictory findings with morphine and etonitazene, suggest that among addicting drugs only those having a peripheral component are able to induce CTA.

REFERENCES

1. Siegel RK. *Intoxication: life in pursuit of artificial paradise.* New York: EP Dutton, 1989.
2. Brady JV, Fischman MW. Assessment of drugs for dependence potential and abuse liability: an overview. In: Seiden LS, Balster RL, eds. *Behavioral Pharmacology: the current status.* New York: AR Liss Inc., 1985: 361–82.
3. Falk, JL. Production of polydipsia in normal rats by an intermittent food schedule. *Science* 1961; **133:** 195–6.
4. Leander JD, McMillan DE, Harris LS. Schedule-induced oral narcotic self-administration: acute and chronic effects. *J Pharmacol Exp Ther* 1975; **195:** 279–87.
5. Tang M, Falk JL. Ethanol dependence as a determinant of fluid preference. *Pharmacol Biochem Behav* 1977; **7:** 471–4.
6. Tang M, Falk JL. Oral self-administration of cocaine: chronic excessive intake by schedule induction. *Pharmacol Biochem Behav* 1987; **28:** 517–19.
7. Falk JL, Tang M. Schedule induction of drug intake: differential responsiveness to agents with abuse potential. *J Pharmacol Exp Ther* 1989; **249:** 143–8.
8. Myers RD. Changes in learning, extinction and fluid preferences as a function of chronic alcohol consumption in rats. *J Comp Physiol Psychol* 1961; **54:** 510–16.
9. Mardones JR, Segovia-Riquelme N, Hederra AD, Alcaino FG. Effect of some self-selection conditions on the voluntary alcohol intake of rats. *Quart J Stud Alc* 1955; **16:** 425–37.
10. Claghorn JL, Ordy JM, Nagy A. Spontaneous opiate addiction in rhesus monkeys. *Science* 1965; **149:** 440–2.
11. Kumar R, Steinberg H, Stolerman IP. How rats can become dependent on morphine in the course of relieving another need. In: Steinberg H, ed. *Scientific basis of drug dependence.* Churchill Ltd., 1969; 209–20.
12. Khavary KA, Peters TC, Baity PL. Voluntary morphine ingestion, morphine dependence, and recovery from withdrawal signs. *Pharmacol Biochem Behav* 1975; **3:** 1093–6.
13. Chernov HI, Barbaz BS, Bosher RL, Feist MN. Age and lack of handling as factors in the consumption of an etonitazene solution by naive rats. *Arch Int Pharmacodyn* 1972; **195:** 231–9.
14. Sala M, Biagetti R, Braida D, Maggioni A, Gori E. La polidipsia da condizionamento aggiuntivo o da angiotensina centralmente somministrata quale modello di dipendenza da oppiacei. Atti I° Congresso CNR, Sottoprogetto Tossicodipendenze. 1985: 141–50.
15. D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J Pharmacol Exp Ther* 1941; **72:** 74–9.
16. Dunstan R, Broekkamp CL, Lloid KG. Involvement of caudate nucleus, amygdala or reticular formation in neuroleptic and narcotic catalepsy. *Pharmacol Biochem Behav* 1981; **14:** 169–74.
17. Huang JT, Yano I, Takemori AE. Effect of various central biogenic amine-modifiers and ambient temperature on the naloxone-induced jumping in morphine-dependent mice. In: Adler MR, Manara L, and Samanin R, eds. *Factors affecting the action of narcotics.* New York: Raven Press, 1978: 495–509.
18. Myers AK. Alcohol choice in wistar and G-4 rats as a function of environmental temperature and alcohol concentration. *J Comp Physiol Psychol* 1962; **55:** 606–9.
19. Sala M, Braida D, Leone MP, Calcaterra P, Gori E. Oral opiate intake in a free-choice procedure in the rat. *Pharmacol Res* 1989; **21:** 67–8.
20. Grant S, Redmond DE. Clonidine suppresses methylxanthine induced quasi-morphine withdrawal syndrome. *Pharmacol Biochem Behav* 1982; **17:** 655–8.
21. Roy SN, Bhattacharyya AK, Pradhan S, Pradhan SN. Behavioural and neurochemical effects of repeated administration of cocaine in rats. *Neuropharmacology* 1978; **17:** 559–64.
22. Garcia J, Hankins G, Rusiniak KW. Behavioral regulation of the milieu interne in man and rat. *Science* 1974; **185:** 824–31.
23. Altaffer FB, De Balbian V, Hals J, Long CJ, D'Ençarnacao PA. A simple and

inexpensive cannula technique for chemical stimulation of the brain. *Physiol Behav* 1970; **5:** 119.
24. Falk JL, Vigorito M, Tang M, Lau CE. Schedule-induced cocaine drinking: Choice between cocaine and vehicle. *Pharmacol Biochem Behav* 1990; **35:** 187–93.
25. Johanson CE, Schuster CR. A choice procedure for drug reinforcers: cocaine and methylphenidate in the rhesus monkey. *J Pharmacol Exp Ther* 1975; **193:** 676–88.
26. Kumar R, Steinberg H, Stolerman IP. Inducing a preference for morphine in rats without premedication. *Nature* 1968; **218:** 564–5.
27. Khavari KA, Risner ME. Opiate dependence produced by *ad libitum* drinking of morphine in water, saline, and sucrose vehicles. *Psychopharmacologia* 1973; **30:** 291–302.
28. Samson HH, Falk JL. Alteration of fluid preference in ethanol dependent animals. *J Pharmacol Exp Ther* 1974; **190:** 365–76.
29. Goldberg SR, Henningfield JE. Reinforcing effects of nicotine in humans and experimental animals responding under intermittent schedules of IV drug injection. *Pharmacol Biochem Behav* 1988; **30:** 227–34.
30. Bechara A, Van der Kooy D. Kappa receptors mediate the peripheral aversive effects of opiates. *Pharmacol Biochem Behav* 1987; **28:** 227–33.
31. Sala M, Braida D, Calcaterra P, Leone MP, Gori E. Dose-dependent conditioned place preference produced by etonitazene and morphine. *Eur J Pharm* 1992; **217:** 37–41.
32. Brown EE, Finlay JM, Wong JTF, Damsma G, Fibiger HC. Behavioral and neurochemical interactions between cocaine and buprenorphine: implications for the pharmacotherapy of cocaine abuse. *J Pharmacol Exp Ther* 1991; **256:** 119–26.
33. Spyraky C, Fibiger HC, Phillips AG. Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. *Brain Res* 1982; **253:** 195–203.
34. Hunt T, Amit Z, Switzman L, Sinyor D. An aversive naloxone-morphine interaction in rats. *Neurosci Lett* 1983; **35:** 311–15.