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Paradoxical simultaneous occurrence of amphetamine-induced conditioned taste aversion and conditioned place preference with the same single drug injection: A new "pre- and post-association" experimental paradigm

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ABSTRACT

The paradoxical phenomenon of co-existing physically aversive and psychologically rewarding effects of drugs is a crucial issue for drug addiction. The present study employed a new experimental paradigm to test whether the rewarding and aversive properties of amphetamine (AMPH) can exist simultaneously. Rats were given a 15 min period of exposure to saccharin injected with 0.15 M NaCl or 1.5 mg/kg AMPH and then were confined to one compartment of a test box for 30 min. After three paired and unpaired cycles, the aversive and rewarding effects were assessed. A reduction in consumption of the paired flavored solution provided evidence of avoidance while preference for the AMPH injection context provided evidence of rewarding effects. The present findings demonstrate that the development of AMPH-induced rewarding and aversive effects depends on the particular behavioral conditions and support both the task-dependent drug effects hypothesis and the reward comparison hypothesis. The formation of associations with stimuli that comes before (pre) *vs.* after (post) the unconditioned stimulus and the role of the dopaminergic system in such associations are discussed.

1. Introduction

Amphetamine (AMPH) is a psychostimulant that can elicit various rewarding and aversive effects (Huang and Hsiao, 2002, 2008; Young et al., 2005). The most common positive effects of AMPH injection are euphoria and alleviation of fatigue, although the strength of the euphoric effect is dose-dependent (Wyatt and Ziedonis, 1998). The drug's rewarding effects can become associated with external environmental cues, including the context in which the drug is administered—a phenomenon termed conditioned place preference (CPP) (Agustin-Pavon et al., 2007; Childs and de Wit, 2009; White and Hiroi, 1993). The aversive effects of AMPH include intoxication (e.g., hyperactivity, hypervigilance, and stereotyped behaviors), withdrawal effects (e.g., anxiety and agitation), psychosis, mood disorders, and sexual disorders (Barrett et al., 2005; Kitanaka et al., 2008). Gastrointestinal malaise is a particularly well studied aversive effect of AMPH (Bell et al., 1998; Di Chiara et al., 2004) that is also used to investigate the aversive properties of addictive drugs (Contreras et al., 2007; Goudie et al., 1982). When a tastant is associated with the gastrointestinal malaise of addictive drugs, the intake volume of the tastant would decrease. This decrease is generally seen as an aversive response and termed conditioned taste aversion (CTA) (Parker 1988, 1991).

Accumulating studies over the past 30 years have demonstrated that various drugs of abuse can induce opposite effects (reward and aversion) in distinct paradigms, such as CPP and CTA, including amphetamine (Cappell and LeBlanc, 1971; Carr and White, 1983), cocaine (Goudie et al., 1978; Spyraki et al., 1982), morphine (Blander et al., 1984; Farber et al., 1976), and ethanol (Lester et al., 1970; Reid et al., 1985). The paradoxical coexistence of addictive drugs' rewarding and aversive effects is critical to understanding their abuse (Goudie, 1979; Hunt and Amit, 1987). Attempts to explain addiction have included a physical dependence model (Childress et al., 1986; Darke et al., 2008) and a psychological dependence hypothesis (Laviola et al., 1994; Robinson and Berridge, 1993). Drug addicts may become physically dependent on a drug to avoid the drug's negative withdrawal symptoms (Solomon and Corbit, 1974; Srisurapanont et al., 2001b). Addiction may also be driven by craving for the abused drug's positive effects (so-called psychological dependence; Srisurapanont et al., 2001a).

Although the rewarding and aversive properties of drugs of abuse have been demonstrated in distinct behavioral paradigms, these

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paradoxical effects have not previously been demonstrated following the same single drug injection within an experiment. The present study examined whether a drug's rewarding and aversive properties can simultaneously exist under the influence of the same single AMPH injection within a single experiment. Rats were subjected to CPP and CTA tests following a single drug injection that was associated with both a context and a tastant.

2. Materials and methods

2.1. Subjects

Fifteen male Sprague–Dawley rats (weighing 310–380 g at the beginning of the experiment) were purchased from the National Laboratory for Animal Breeding and Research Center in Taipei, Taiwan. They were housed individually in suspended stainless steel home cages in a colony room kept at a constant temperature (approximately 20 ± 2 °C) with a 12 h/12 h light/dark cycle (lights on 6:00–18:00). Rats were fed standard rat chow and were provided water on a limited basis as described in the experimental procedure section below. All experiments were performed in compliance with the Animal Scientific Procedures Act of 1986 and received local ethics committee approval. Every effort was made to minimize animal suffering and to minimize the number of animals used.

2.2. Apparatuses

2.2.1. Lickometer

Solution consumption in the CTA task was measured via a one-bottle test using a lickometer. All CTA measurements were performed as described previously (Huang and Hsiao, 2002). The lickometer consisted of a wire-mesh cage, a white panel, and a 25 ml burette with 0.1 ml graduation. The burette was connected to the white panel and mounted in front of the wire-mesh cage. An electrical circuit was closed each time a rat's tongue made contact with the burette, allowing 60 nA of current to pass. The microelectrical signal was registered on a computer. The present study used only the consumed fluid volume data for analysis (Huang and Hsiao, 2008).

2.2.2. CPP box

The CPP behavioral analysis was conducted in a three-chamber, wooden, T-shaped apparatus consisting of two distinct, approximately square compartments ($45 \times 43 \times 43$ cm high) and a narrow intermediary shuttle compartment ($38 \times 18 \times 18$ cm high). The three chambers were separated by wood partitions. The two square preference compartments were accessible through the shuttle compartment, and each had a clear Plexiglas observation wall on one side of the box. One of the preference compartments was painted white with a wood-chip bedding floor, and the other was black with a wire-grid floor.

2.3. Behavioral training procedures

As summarized in Fig. 1, three experimental phases were conducted, including adaptation, conditioning, and testing (Fig. 1A). On Days 1–7 (adaptation phase), all rats were water-deprived for 23.5 h/day for 7 days in their home cages and provided a 30 min water access session in the afternoon, except when specific treatments were administered as described below. On the last 2 days (Days 6 and 7), each rat was given access to water for 15 min in a lickometer in the morning (30 min water access was given in the rats' home cages in the afternoon). Following exposure to the lickometer, rats were placed into the CPP apparatus without the partitions and allowed to explore for 10 min to permit familiarization.

On Days 8–13 (conditioning phase), the subjects were randomly assigned to two groups and treated with six concomitant schedules, including three drug-paired and three unpaired trials. During the conditioning phase, rats were subjected to drug-paired treatments on odd days and unpaired sessions on even days. On each of the three paired sessions, rats were offered 0.1% saccharin solution for 15 min and then injected intraperitoneally with normal saline (Saline group, $n = 7$) or AMPH (AMPH group, $n = 8$). The AMPH treatment served as the unconditioned stimulus (US) for both CTA and CPP.

For CPP training, half of the rats in each group were confined to each of the preference compartments for 30 min. On each of three unpaired sessions, both the AMPH and Saline groups were injected with saline immediately prior to being placed in the other compartment in which they had not been previously confined and were left in the compartment for 30 min. Exposures to the drug-paired and unpaired compartments in the AMPH and Saline groups were carried out in a counterbalanced fashion. During the conditioning phase, which involved three drug-paired sessions, consumption of the conditioned tastant was measured. Meanwhile, the rats were confined to one preference compartment to form a CPP. Behavioral sensitization was not assessed during the conditioning phase because the purpose of our study was not to examine AMPH-induced sensitization. Therefore, during the conditioning sessions, data from three CTA conditioning trials were collected, but no CPP data were collected.

2.4. Behavioral testing

Testing was conducted on Day 14 (Fig. 1B). Similar to the training schedule, the rats were subjected to the CPP test immediately after completing the CTA test trial. For CTA testing, rats were given free access to a 0.1% saccharin solution for 15 min. The volume of solution consumed during the test session was recorded. During the test trial, rats experienced the 0.1% saccharin solution for the fourth time.

For CPP testing, each rat was placed into the CPP apparatus without the wood partitions. The amount of time that rats spent in each compartment was monitored with stopwatches for a period of 15 min and recorded. A rat was considered to be within a compartment when any part its head and/or torso was within that compartment.

2.5. Drug preparation and administration

All three compounds used in this experiment were purchased from Sigma (St. Louis, MO). Sodium saccharin and sodium chloride were each dissolved in distilled water and prepared into the following final concentrations: 0.1% (w/v) saccharin solution and 0.15 M NaCl solution. D-amphetamine sulfate was dissolved in normal saline by stirring at room temperature. Rats in the AMPH group received 1.5 mg/kg of AMPH. This dose was based on a previous experiment (Campbell and Spear, 1999). Each injection was delivered in a volume of 4 ml/kg. All injections were intraperitoneal.

2.6. Statistical analysis

The intake volume of saccharin solution was measured as the CTA index, and the time spent in the paired *vs.* the unpaired side of the CPP apparatus served as the CPP index. The CTA response over four sessions was analyzed by a 2×4 mixed repeated-measures analysis of variance (ANOVA), with dose and session as factors. When appropriate, Tukey's Honestly Significant Difference (HSD) *post hoc* test was conducted. A p value less than 0.05 was considered significant in all cases. CPP was analyzed by a one-way ANOVA in which the mean time spent on the preferred side *vs.* non-preferred side was compared between the Saline and AMPH groups. The CPP ANOVA was followed by the Meehan and Schechter (1998) data analysis method.

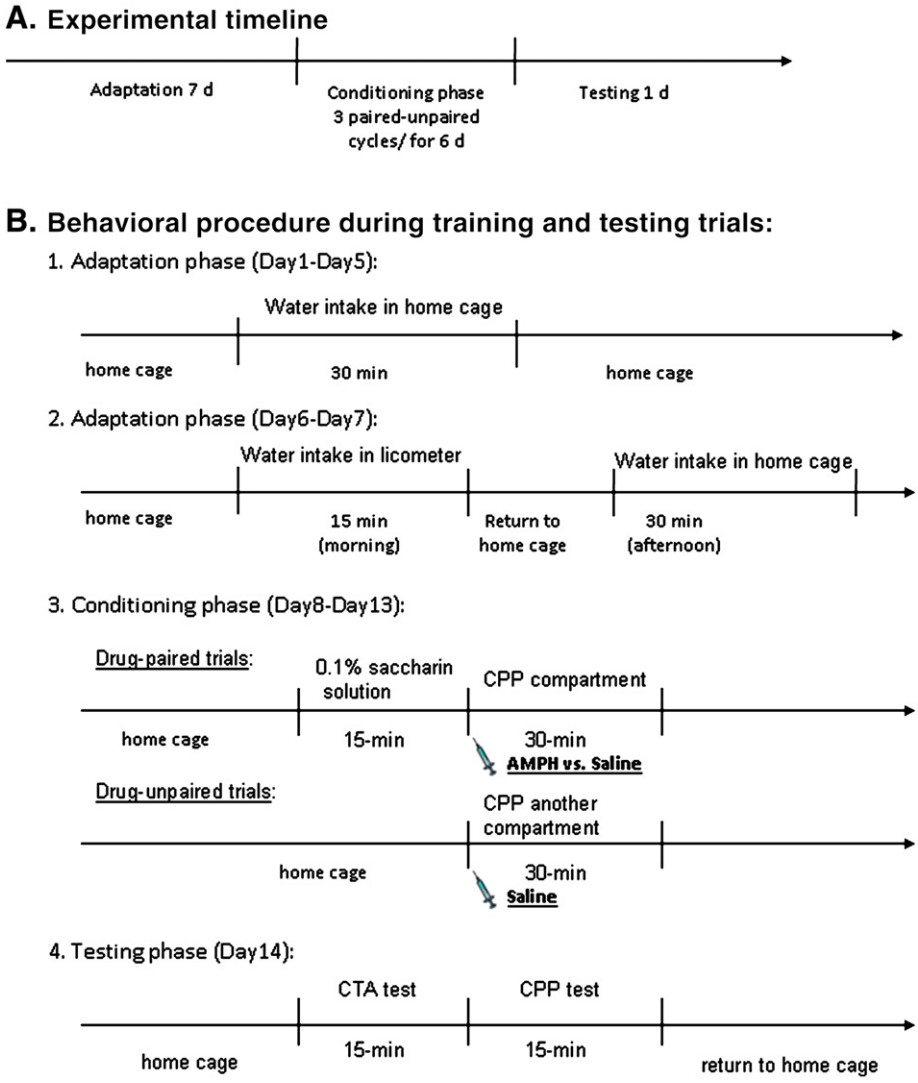

Fig. 1. Overview of experimental procedure. (A) The experimental timeline included three phases: adaptation phase for 7 days, conditioning phase consisting of three paired–unpaired cycles for 6 days, and testing phase for 1 day without drug injections. (B) The behavioral procedure during the training and testing trials is shown in detail.

3. Results

3.1. AMPH-induced CTA

Fig. 2 depicts mean (±SEM) intake volume of 0.1% saccharin solution by the Saline and AMPH groups during the four sessions in which the US-paired tastant was offered. A 2×4 two-way repeated-measures ANOVA revealed a main effect of group ($F_{1,13}=39.92$, $p<0.01$), an effect of conditioning session ($F_{3,39}=27.31$, $p<0.01$), and a significant group×session interaction ($F_{3,39}=9.67$, $p<0.01$). *Post hoc* analysis using Tukey's HSD test indicated that the saccharin solution intake of the AMPH group was suppressed during sessions 2, 3, and 4 compared with the Saline group ($ps<0.01$). Therefore, AMPH injections elicited a suppressive effect on saccharin solution consumption compared with Saline injections, and this suppression became gradually more pronounced over the four sessions.

3.2. AMPH-induced CPP

During the habituation sessions, rats spent similar amounts of time in the drug-paired and unpaired compartments ($p>0.05$), indicating no initial compartment bias. Fig. 3 presents the group mean (±SEM) time spent on the preferred side and non-preferred side during the test session. One-way ANOVA revealed a main effect of the injection (AMPH *vs.* Saline) on time spent in the paired compartment ($F_{1,13}=4.98$, $p<0.05$; Fig. 3A). No effect of injection (AMPH *vs.* Saline) was observed on time spent in the unpaired compartment ($F_{1,13}=0.28$, $p>0.05$;

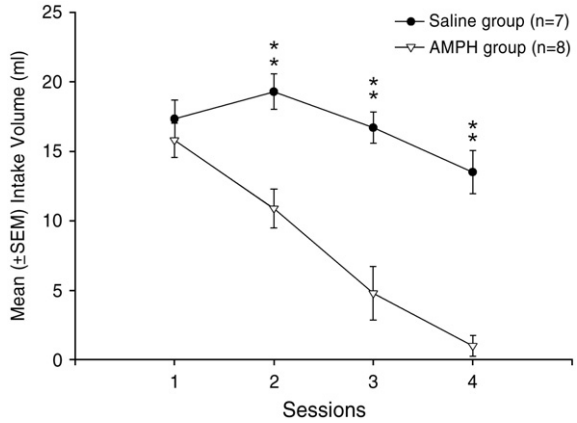

Fig. 2. AMPH acts as an aversive US in CTA. Mean (±SEM) intake volume of saccharin solution in rats injected with Saline ($n=7$) and AMPH ($n=8$) for four sessions. Rats were given a 0.1% saccharin solution in the lickometer for 15 min and then injected with 0.15 M NaCl or 1.5 mg/kg AMPH during the conditioning sessions (sessions 1–3). $^{**}p<0.01$ *vs.* Saline group. AMPH, amphetamine.

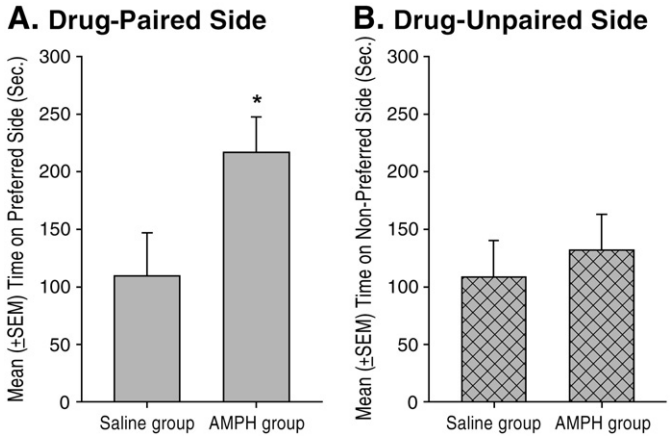

Fig. 3. AMPH acts as a rewarding US in CPP. Mean (±SEM) time spent on (A) the drug-paired side and (B) the unpaired side during the test session in the Saline and AMPH groups. Notice that no actual US pairing occurred for the Saline control group. Thus, the "paired" compartment for the Saline group represents the day when the other (experimental) group received an AMPH injection. $^{*}p<0.05$ *vs.* Saline group. AMPH, amphetamine.

Fig. 3B). Thus, AMPH injection elicited preference for the drug-paired side but did not affect the time spent on the unpaired side.

4. Discussion

The present results revealed the presence of simultaneous associations formed with both a tastant CS and a contextual CS before and after a single AMPH injection, demonstrating a "pre- and post-association" experimental paradigm. Both aversive and rewarding effects of AMPH were demonstrated simultaneously as rats exhibited a reduction in consumption of an AMPH-paired solution in the CTA task and spent more time in an AMPH-associated compartment.

4.1. Conditioned stimuli

Garcia and Koelling (1966) asserted, "stimuli are selected as cues dependent upon the nature of the subsequent reinforcers" (p. 123) in which the distinct properties of conditioned stimuli differentially predispose them to associate with appropriate unconditioned stimuli (Garcia and Koelling, 1966). For example, taste stimuli (e.g., flavored solutions) are selectively associated with intestinal reactions (malaise), whereas external stimuli (e.g., lights, tones) are apt to associate with externally painful reactions (e.g., shock). A large body of evidence has demonstrated similar results in various experimental paradigms (Garcia and Ervin, 1968; Garcia et al., 1968, 1970, 1974; Rusiniak et al., 1982). Di Chiara et al. (2004) showed that psychostimulant drug-induced CTA learning was abolished by antagonism of dopamine D_1 and/or D_2 receptor antagonists and that a conditioned instrumental response could be enhanced by intracranial self-administration of a mixed $D_{1/2}$ receptor agonist into the shell region of the nucleus accumbens. Di Chiara's findings suggested that paradoxical aversive and rewarding effects could be observed in distinct behavioral paradigms (Di Chiara et al., 2004). Our present findings demonstrated that an internal response to AMPH injection could associate with *both* a taste stimulus and an external stimulus (context), consistent with Di Chiara's findings.

4.2. Withdrawal and relapse

AMPH aversion is generally attributed to withdrawal symptoms (Barrett et al., 2005; Childress et al., 1986; Kitanaka et al., 2008). The aversive effects of AMPH have been shown to differ by sex and to be affected by early maternal separation (Roma et al., 2008). Given that a small number of acute AMPH injections were given in the present study, the aversion cannot be attributed to chronic treatment effects. Thus, our findings do not provide support for the view that the aversive properties of AMPH are attributable to withdrawal symptoms following chronic exposure. In contrast, the presently observed aversion may be attributable to (*i*) disruption of neuroendocrine homeostasis (i.e., the hypothalamic–pituitary–adrenal [HPA] axis), which is hypothesized to elicit a hedonic effect, and/or (*ii*) an altered state of neuroadaptation, which may produce acute withdrawal symptoms, including anxiety, mood dysregulation, and somatic symptoms (Koob and Le Moal, 2008; Weiss et al., 2001). Indeed, acute drug injections have been demonstrated to increase levels of corticotropin-releasing factor (a constituent of the HPA axis) within the central nucleus of the amygdala, thereby inducing stress-like symptoms and anxiety-like responses (Koob and Le Moal, 2008). Therefore, a brain stress response activated by acute excessive drug intake may mitigate a drug's rewarding effects. The combination of a reward deficit (aversive effect) and the occurrence of reward may enhance vulnerability to relapse during treatment (Weiss et al., 2001). This acute aversive property, which may manifest as anxiety or visceral discomfort, may serve as the US in CTA while other rewarding properties are associated with external environmental stimuli.

4.3. Paradoxical existence of rewarding and aversive effects: factors in behavioral phenomena

Growing data suggest that drugs of abuse can elicit rewarding and aversive properties and opposite effects have appeared in different paradigms such as with ethanol (Cunningham, 1979), morphine (White et al., 1977), cocaine (Hunt et al., 1985), apomorphine (Wise et al., 1976), and AMPH (Turenne et al., 1996). Regarding the aversive effects of AMPH, one is hypothesized to be attributable to visceral discomfort or malaise (Contreras et al., 2007). This aversive effect is contingent with a tastant such that animals reduce their intake of this tastant and show CTA (Parker, 1995). A microinjection study demonstrated that when using tetrodotoxin to block parabrachial nuclei, AMPH- as well as LiCl-induced CTA was attenuated (Bielavska and Bures, 1994). Thus, parabrachial nuclei may be a common neural substrate for AMPH's aversive malaise effect and LiCl-induced nausea. Moreover, a critical study demonstrated that the tachykinin NK1 antagonist GR205171, which inhibits emesis, blocked apomorphine- and AMPH-induced CTA (McAllister and Pratt, 1998), suggesting that the aversive property of AMPH-induced CTA may be attributable to gastrointestinal malaise.

However, several possibilities may explain why different types of conditioning responses to a drug can develop. The propensity for rewarding *vs.* aversive effects may depend on individual differences and dosage. Kunin et al. (2001) separated rats into high and low AMPH self-administration responders and examined the rewarding and aversive effects of AMPH using a locomotor activity test and a CTA task, respectively. The high responders showed more sensitivity to the drug in the locomotor activity test but less sensitivity in the CTA task compared with the low responders. AMPH self-administration produced either a rewarding or aversive effect depending on the behavioral paradigm (Kunin et al., 2001). The propensity for AMPH to produce aversive *vs.* rewarding effects may also be dose-dependent. Moderate to high doses of AMPH have been shown to produce place preference, and low doses have been shown to produce place aversion (Cabib et al., 1996).

Moreover, the route and schedule of administration may influence the propensity for drugs to become associated with multiple stimuli. Subcutaneous and intraperitoneal delivery of cocaine selectively produced CTA and CPP, respectively, but not *vice versa* (Mayer and Parker, 1993). Meanwhile, temporal factors may be crucial in enabling a drug's rewarding properties to dissociate from its aversive properties such that short and intermediate time periods (5 and 120 min) may enable place preference to develop and flavor avoidance to be revealed,

while a longer time period (240 min) may fail to produce a dissociation between context and tastant (Sherman et al., 1980).

4.4. Paradoxical existence of rewarding and aversive effects: physiological mechanisms

The paradoxical coexistence of addictive drug-induced reward and avoidance may be mediated by physiological mechanisms involving specific neurotransmitter receptors and brain areas (Di Chiara et al., 2004). Different neurotransmitter binding sites within specific brain regions and different receptor subtypes could differentiate between a drug's rewarding and aversive effects (Lin et al., 1994). For example, dopamine D_2 receptors have been shown to mediate the reinforcing effects of AMPH, while both D_1 and D_2 receptors have been shown to modulate CTA (Hoffman and Beninger, 1988). Additionally, some evidence indicates that different brain sites may mediate the rewarding and aversive effects of drugs of abuse (Bassareo et al., 2002). Dopamine receptor subtypes may differentially influence CTA learning. For example, Fenu et al. (2001) injected dopamine D_1 (SCH23390) and $D_{2/3}$ (raclopride) receptor antagonists into the nucleus accumbens and found that only the D_1 antagonist disrupted LiCl-induced CTA learning (Fenu et al., 2001). In terms of anatomical localization, the nucleus accumbens has been implicated in the rewarding effects that support CPP, and the area postrema has been implicated in the aversive properties that produce CTA (Carr and White, 1986; Van der Kooy et al., 1983).

Limited evidence suggests that the rewarding and aversive effects could be controlled by a unified physiological mechanism (Carlezon and Thomas, 2009). Interestingly, the brain dopamine system, particularly within the nucleus accumbens, has been implicated in mediating both motivationally rewarding and aversive effects (Young et al., 2005). For example, the D_2 receptor antagonist haloperidol attenuated AMPH-induced tastant suppression (a rewarding task) and CTA (Huang and Hsiao, 2002). Subcutaneous administration of the D_1 antagonist SCH39166 has been shown to disrupt association of a tastant with a US regardless of whether the US was a rewarding drug (morphine) or an aversive drug (LiCl) (Fenu et al., 2009). Therefore, we suggest that AMPH acts through the dopaminergic system to simultaneously control motivational valence in both the rewarding (CPP) and aversive (CTA) responses.

4.5. Comparing the experimental procedures of addictive drug-induced paradoxical reward and aversion: a new "pre- and post-association" experimental paradigm

To assess the nature of the paradoxical effect, numerous investigators have manipulated various factors, such as route of administration (Mayer and Parker, 1993), timing (Sherman et al., 1980), dosage (Lin et al., 1994), and apparatus (White et al., 1977). Nevertheless, few prior studies have precisely demonstrated these paradoxical effects in a single experiment with a single injection (for drug self-administration *vs.* CTA, see Wise et al., 1976; CPP *vs.* CTA, see Carr and White, 1986). Particularly, White et al. (1977) showed similar data for morphine. They found that rats ran down an alley, ate in a goal box, and received a morphine injection. Over several days, running speeds increased and food consumption decreased, thus demonstrating the rewarding and aversive effects of a drug in a single experiment with a single injection. Moreover, there is the closest to showing that drugs of abuse could simultaneously elicit paradoxical effects (Reicher and Holman, 1977). In their study, rats were exposed to a CPP compartment with access to a flavored solution in a single bottle 20 min after being injected with AMPH. The next day, rats were injected with saline and exposed to the other CPP compartment with access to a different flavored solution. After 20 such pairings, rats preferred the AMPH-associated compartment. A separate CTA test conducted in the rat's home cage using a two-bottle technique revealed a strong aversion to the AMPH-associated tastant. Thus, Reicher and Holman (1977) concluded that AMPH injections could induce paradoxically aversive and rewarding effects.

Although Reicher and Holman's (1977) experimental design eliminated some disadvantages of traditional procedures, several shortcomings remained. First, the use of different CTA training and testing locations made the associated cues indefinable. Second, they used a single-bottle (no choice) procedure during training but a two-bottle (choice) procedure during testing. Therefore, the testing may not actually show simultaneous effects because of different bottle contexts. Third, their use of non-drug and drug testing hindered differentiation between pharmacological and associative effects. Accordingly, AMPH's pharmacological anorexic effect likely interfered with the drinking behavior necessary to produce "CTA-like" effects. Finally, because of procedural constraints, the rats required more training sessions (nearly 20 trials) to produce a stable result. Therefore, the experimental paradigm presented by Reicher and Holman (1977) is not ideal for showing the paradoxical coexistence of addictive drug-induced reward and avoidance.

The present experimental design—a "pre- and post-association" paradigm—had a critical advantage with regard to timing in which the saccharin solution was presented prior to drug treatment, and the place cue was presented after treatment. The paradigm employed a single location and a one-bottle (no choice) procedure during both training and testing sessions to isolate the cues that were associated with the responses. Saccharin intake during conditioning and testing in our design occurred prior to drug treatment and thus occurred in the absence of any drug response, making the distinct identification of pharmacological and associative effects possible. No interference of the AMPH anorexic effect was found in this design.

4.6. The task-dependent drug effects hypothesis

The present results appear to support our task-dependent drug effects hypothesis (Huang and Hsiao, 2008). We posit that a drug can produce multiple effects (an assertion consistent with a widely accepted pharmacological principle) and that the determination of its effects depends on the particular behavioral conditions in which it is delivered, regardless of whether the paradigm involves reward or avoidance. Consistent with the findings of Garcia and Ervin (1968), the present study demonstrated that the rewarding properties of a drug of abuse can readily associate with external environmental cues, and the aversive properties of the same drug become associated with an internal tastant stimulus. The two simultaneously conditioned stimuli (i.e., tastant and context) may become preferentially associated with different effects of the drug. Our ability to demonstrate both associations indicates that CTA is an appropriate paradigm for examining the aversive nature of abused drugs (Hunt and Amit, 1987), and CPP is an appropriate paradigm for examining the rewarding nature of abused drugs (Itzhak and Martin, 2002).

4.7. Rewarding or aversive properties: what kind of an effect does AMPH administration have on CTA?

Nevertheless, the present results showing that rats spent more time in the drug-paired compartment (i.e. CPP) and simultaneously avoided drug-paired taste (i.e. CTA) may be equally consistent with the task-dependent drug effects hypothesis (Huang and Hsiao, 2008) and the reward comparison hypothesis (Grigson, 1997), suggesting that both CTA and CPP may be attributable to the appetitive properties of AMPH. Based on the reward comparison explanation, the CPP effect is caused by the rewarding effect of AMPH, and the reduction of tastant intake is attributable to comparisons between the rewarding tastant and the later rewarding AMPH. The rewarding effect of AMPH, therefore, outweighs the rewarding effect of the tastant. Thus, rats suppress tastant intake after AMPH injection. Consistent with this interpretation, one report suggests that the dopamine system is involved at different stages of the

association in LiCl-induced CTA compared with abused drug-induced taste avoidance (Fenu et al., 2009). Moreover, the involvement of the dopamine system is shown with different receptor subtypes (e.g., D_1 and D_2 receptors) (Fenu et al., 2001), time points (such as short, moderate, and long) of drug administration before and after the taste (Fenu et al., 2005), and neural substrates (Bassareo et al., 2002). Although the provided data are so diverse, they support the hypothesis that the abused drug-induced taste avoidance results from the hedonic effects of dopamine. Thus, the theory that CTA is caused by the rewarding or aversive properties of abused drugs should be scrutinized.

4.8. Responses to Grigson's comments on the task-dependent drug effects hypothesis

Recently, we performed three experiments that argued against Grigson's reward comparison hypothesis (Grigson, 2008). Grigson then responded to our task-dependent drug effects hypothesis (Grigson, 2008). Regarding our data from Experiment 1 in the prior study (Huang and Hsiao, 2008), we examined AMPH- and LiCl-induced suppression of saccharin intake. A three-way saccharin × dose × trials ANOVA indicated that AMPH- and LiCl-induced suppression of saccharin intake did not differ. However, Grigson (2008) argued that (*i*) our data did not show the first trial in Figs. 1 and 2, (*ii*) the statistical analysis did not use a *post hoc* test, and (*iii*) a significant three-way interaction was not found in the LiCl experiment.

Normally, the first trial data are usually viewed as baseline. Because the first trial data might reveal large variations due to individual differences or other uncontrolled variables, significant differences might appear among groups. Thus, the first trial data would be transferred into a new formulation (Fenu et al., 2001; Huang and Hsiao, 2002) and were not shown in the figures. Experiment 1 tested the rewarding AMPH- and aversive LiCl-induced suppression of saccharin intake, with the purpose of comparing the patterns of saccharin suppression between AMPH and LiCl. Thus, the key point is the main analysis of three-way saccharin × doses × trials ANOVA and not the *post hoc* tests or their three-way interaction. We believe that if the rewarding AMPH suppression of saccharin intake is really different from LiCl, then the overall pattern would be shown in a three-way ANOVA.

Grigson's argument against our Experiment 2 (Huang and Hsiao, 2008) was the reverse conditioning when the stronger amphetamine reward occurred prior to the weaker saccharin solution reward in terms of simultaneous or successive negative contrast (Flaherty and Rowan, 1986). Thus, Grigson emphasized that the results of Experiment 2 were suitable for the reward comparison hypothesis but did not mention the reverse contrast style in the original Grigson (1997) reward comparison hypothesis. Grigson has not mentioned the reverse style between the two reward contrasts since Grigson (1997) proposed the reward comparison hypothesis. However, Grigson makes an analogy between the concept of Flaherty and Rowan's (1986) simultaneous or successive negative contrast and the reward comparison viewpoint. Whether different schedules of administration for saccharin solutions and amphetamine serving as the first reward could get the identical effect and demonstrate the suppression effect in terms of simultaneous or successive negative contrast (Flaherty and Rowan, 1986) should be investigated in further studies. The reward comparison hypothesis needs to be modified and tested with reverse schedules with different doses and saccharin concentrations.

According to Flaherty and Rowan's (1986) contrast theory for contrast effects in the consumption of gustatory solutions, three situations exist. First, the successive negative contrast paradigm involves animals first encountering a higher saccharin solution reward prior to a lower one, thus reducing the rewarding effect of the first solution. Second, the simultaneous contrast procedure involves animals rapidly and repeatedly being exposed to two different concentrations of saccharin solutions. Third, the anticipatory contrast procedure involves the first saccharin solution reward being outweighed by the second one. Later, animals encounter the first saccharin solution, thus decreasing the intake volume. The reward comparison hypothesis (Grigson, 1997) basically depends on the concept of Flaherty's anticipatory contrast theory. However, the reverse style does not follow the experimental procedure of anticipatory contrast. Instead, the reverse style is seemingly similar to the procedure of successive negative contrast. Nevertheless, Flaherty and Rowan (1986) found that successive negative contrast did not occur under all comparison conditions. To illustrate, Flaherty suggested that when the concentrations of the saccharin solution shift from 0.15% to 0.075% or 0.05%, successive negative contrast occurs. However, when concentrations shift from 0.15% to 0.1% or 0.125%, successive negative contrast does not occur. Thus, the reverse style of reward comparison follows successive negative contrast, consistent with Grigson's assertion, and the reward comparison hypothesis also has limitations (Flaherty and Rowan, 1986). These discrepancies and consistencies should be scrutinized in future studies.

Grigson criticized the findings in Experiment 3 (Huang and Hsiao, 2008) showing that the saccharin suppression effect under a combined injection of AMPH and LiCl is stronger than a single amphetamine or LiCl injection. Grigson cited a series of studies by Riley's group (Etkind et al., 1998; Grakalic and Riley, 2002) and found that few data support our results from Experiment 3 (Busse et al., 2005). For example, a 0.5 g/kg dose of alcohol injected simultaneously with 20, 30, and 40 mg/kg doses of cocaine did not appear to exert an additive effect relative to cocaine alone-induced taste aversion (Busse et al., 2005). However, two other Riley studies did demonstrate an addictive effect (Etkind et al., 1998; Grakalic and Riley, 2002) and support our previous data (Huang and Hsiao, 2008). However, these two supporting studies used a relatively weaker dose of alcohol (0.56 g/kg) and cocaine (25 mg/kg). Thus, the inconsistent data of Busse et al. (2005), which did not demonstrate an additive effect on saccharin suppression, may be attributable to a floor effect.

Grigson (2008) manipulated a complex "successive contrast" saccharin–morphine–saccharin/sucrose procedure to investigate whether an additive effect would be revealed. However, this study used a very complex experimental procedure which questions why their group did not utilize a simple CS–US association test paradigm to test the hypothesis or simply employ the procedure from Grigson (1997). Indeed, behavioral results (such as the saccharin suppression effect) might be affected by the US drug treatment, dose and concentration of the CS and US, property of the CS and US, time interval between CS and US pairings, and duration of CS and US presentation. However, Grigson used a very short duration for the first saccharin solution intake and subsequent saccharin intake (each for 3 min). Moreover, Grigson injected the US-like agent morphine between these two saccharin solutions. Thus, the first saccharin may contrast with the US-like agent morphine, and then the second saccharin presentation might contrast with the previous US-like agent morphine. Thus, many confounding factors may be found in the series of contrasts. Unclear is which saccharin suppression effect is caused by which contrast situation. We suggest that direct and simple procedures are employed to address these issues.

5. Conclusion

The present results demonstrate that the brain dopamine system may mediate a common physiological mechanism related to AMPH-induced rewarding and aversive paradoxical effects. A new "pre- and post-association" experimental paradigm is presented in which opposing rewarding and aversive effects of AMPH can occur concomitantly under the influence of the same single drug injection within an experiment. The present data are consistent with both the task-dependent drug effects hypothesis (Huang and Hsiao, 2008) and reward comparison hypothesis (Grigson, 1997).

Grigson (2008) commented on our task-dependent drug effects hypothesis (Grigson, 2008), but the explanations for CTA in behavioral phenomena are seemingly not able to dismiss the task-dependent viewpoint. Furthermore, dissociating these two hypotheses might require further pharmacological and anatomical studies rather than only behavioral studies.

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