Published in final edited form as:

Behav Brain Res. 2006 December 15; 175(2): 305–314.

Appetitive Sensitization by Amphetamine does not Reduce its Ability to Produce Conditioned Taste Aversion to Saccharin

John Scott-Railton, Gretchen Arnold, and Paul Vezina*

Department of Psychiatry, The University of Chicago, 5841 S. Maryland Ave., MC 3077, Chicago, IL 60637, USA

Abstract

Previous exposure to amphetamine attenuates its ability to induce conditioned taste aversion (CTA). Because amphetamine, unlike emetic agents like LiCl, possesses appetitive properties that sensitize when it is administered repeatedly, the present study assessed the contribution of sensitization to this US pre-exposure effect (US-PEE). It was found that not all sensitizing regimens of systemic amphetamine injections produce a US-PEE. In addition, previous exposure to amphetamine in the VTA, where it acts to induce sensitization but not CTA, did not produce a US-PEE. It is concluded that amphetamine sensitization alone does not modulate this drug's ability to produce CTA. Implications of these findings for anatomically based associative and non-associative models of CTA and the US-PEE are discussed.

Keywords

Amphetamine; Conditioned Taste Aversion; Sensitization; Ventral tegmental area; US-pre-exposure-effect

1. Introduction

Rats repeatedly exposed to a novel and otherwise palatable taste (e.g. saccharin, the Conditioned Stimulus; CS) followed by an agent that produces an unpleasant state (e.g. an emetic agent such as LiCl, the Unconditioned Stimulus; US) come to avoid the taste in subsequent presentations, a phenomenon called Conditioned Taste Aversion [CTA; e.g., 4]. This learned avoidance, and the ability of a substance to support it, is generally interpreted to reflect aversive conditioning dependent upon the unpleasant properties of the substance used as the US. Paradoxically, highly addictive psychoactive drugs such as amphetamine or morphine also support taste avoidance when paired with a palatable CS (often saccharin), this despite their ability to elicit clear appetitive effects [1,3,10,29]. Indeed, both CTA and drug reinforcing effects have been shown to occur in the same rats [e.g. 68].

While several differences between the taste aversions conditioned by emetic agents and those produced by psychoactive drugs have been identified [e.g. 58], a drug's ability to support CTA is frequently taken to indicate that it has aversive properties sufficient to support aversive conditioning but that these are usually masked by its behaviorally reinforcing properties [for discussion, see 26,33]. However, attempts to identify such aversive properties remain inconclusive [33].

In an alternative view, Grigson and colleagues [26,27,29,30] have proposed that it is the rewarding, rather than the aversive, properties of addictive drugs that support their ability to

*Correspondence: Paul Vezina, TEL: 773/702-2890, FAX: 773/702-0857, E-MAIL: pvezina@yoda.bsd.uchicago.edu

produce CTA. They have argued that a ‘reward contrast effect’ leads to decreased consumption of a less reinforcing CS when rats learn that it is to be followed by a much more reinforcing drug. While it is unclear why rats should forfeit a less reinforcing substance simply in anticipation of a greater reward, especially when the former also represents the daily allotment of water availability, as with a saccharin solution CS, it has been demonstrated that a sweeter substance can suppress ingestion of a less sweet substance that predicts it [9,18,19]. Such findings thus raise the possibility that the appetitive properties of addictive drugs may play a role in the development and expression of CTA.

Previous exposure to the LiCl US has long been known to reduce its ability to condition a taste aversion [for reviews see 50,54]. The mechanisms underlying this US-pre-exposure effect (US-PEE) remain under investigation. A number of reports have suggested a role for associative phenomena such as blocking, rather than non-associative habituation or tolerance in this process [5-7,40,50]. Previous exposure to psychostimulant drugs, including amphetamine, has also been shown to reduce its ability to condition a taste aversion [11,12,23,37,52-54], and blocking has similarly been proposed to underlie this effect [7]. This possibility notwithstanding, little is known of the aversive properties of amphetamine, of their susceptibility to plasticity, or of their contribution to CTAs. In addition, amphetamine is clearly different from LiCl in that it possesses powerful appetitive properties that are subject to both associative and non-associative forms of plasticity when the drug is administered repeatedly [59] as in the preexposure phase of US-PEE experiments. It is important, therefore, to determine whether these properties of amphetamine might contribute to CTAs, and whether the plasticity each is subject to relates to the US-PEE.

Two competing physiological changes known to develop following repeated drug exposure have been advanced as explanations for why previous exposure to amphetamine interferes with CTAs: tolerance and sensitization. The tolerance model proposes that an experience-dependent reduction in aversive properties might weaken the strength of a potential association between CS and US, thus attenuating the development of a CTA [2,13,22,54]. The sensitization model, meanwhile, proposes that repeated drug exposure sufficiently enhances the drug’s rewarding effects to overcome its aversive properties. Interestingly, such reward sensitization would also be expected to provide an even greater contrast in Grigson’s “reward contrast effect,” and thus be expected to lead to greater CTA [cf. 30].

Previous exposure to drugs like amphetamine in the ventral tegmental area (VTA), site of the cell bodies of ascending midbrain dopamine neurons, leads to heightened locomotor responding to subsequent systemic injections of the drug [8,32,35,47,67] by virtue of sensitization of the reactivity of these neurons [63,64]. This type of sensitization has also been reported to enhance the ability of drugs to support self-administration and to induce reinstatement [62,66; for a review, see 65].

The present experiments tested the hypothesis that sensitization of the incentive motivational effects of the psychostimulant amphetamine will change the ability of this drug to condition a taste aversion to saccharin. In Experiment 1, rats were exposed to regimens of systemic amphetamine injections known to induce sensitization, and the ability of amphetamine to condition a CTA was subsequently assessed. Because amphetamine, when injected systemically, most likely constitutes a US in the CTA paradigm (as evidenced by its ability to produce a CTA), such an experiment might confound the effects of amphetamine sensitization and US pre-exposure. Additional experiments were thus conducted to examine the effects of an amphetamine pre-exposure regimen that is capable of inducing sensitization but in which amphetamine does not function as a US for CTA. In Experiment 2, it was demonstrated that repeated exposure to amphetamine in the VTA constitutes such a regimen because these infusions failed to produce a CTA. In Experiment 3, rats were thus exposed to this sensitizing

regimen of VTA amphetamine microinjections before assaying the ability of systemically administered amphetamine to produce a CTA. Results unequivocally show that amphetamine sensitization does not interfere with the ability of the drug to condition taste aversions.

2. Experiment 1

Previous exposure to systemic amphetamine injections is known to interfere with its ability to condition taste aversions. The present experiment attempted to replicate and extend this finding by assessing the relationship between the intensity of previous amphetamine exposure regimens and the strength of the CTA subsequently produced by the drug. In one experiment, rats were exposed to 12 systemic amphetamine injections (1.5 mg/kg, IP) administered daily, a regimen similar to others known to reduce the ability of drugs like amphetamine to subsequently produce a CTA [11,22,24]. By way of comparison, different rats were exposed in a second experiment to a less intense regimen of amphetamine injections. A smaller number (5) of systemic amphetamine injections (1.5 mg/kg, IP) were administered with injections administered every third day. Importantly, both types of regimen are known to produce sensitization of amphetamine's locomotor and nucleus accumbens dopamine activating effects [44,64,69].

2.1. Subjects

Twenty-four male Sprague-Dawley rats (12 rats assigned to each experiment) weighing 250-275 g on arrival from Harlan Sprague-Dawley (Madison, WI) were used. All animals were individually housed in a reverse light-cycle room (12-h light: 12-h dark) with controlled temperature and humidity for the duration of the experiment.

2.2. Apparatus

Experiments were conducted in individual chambers, each constructed of stainless steel side walls and ceiling with clear plexiglass panels for the rear wall and front door and a tubular stainless steel floor (38 × 23 × 34 cm). Each chamber was housed in a sound-attenuating box.

2.3. Drugs

D-amphetamine sulfate (AMPH) was obtained from Sigma, Inc. (Saint Louis, MO). The amphetamine was dissolved in sterile saline (0.9% w/v). Doses refer to the weight of the salt.

2.4. Procedures

In the first experiment, rats were administered amphetamine (1.5 mg/kg, IP, $n/\text{group}=6$) or saline (1ml/kg, IP, $n/\text{group}=6$) once daily for 12 days. In the second experiment, rats were administered five injections of amphetamine (1.5 mg/kg, IP, $n/\text{group}=6$) or saline (1ml/kg, IP, $n/\text{group}=6$), one injection every third day. Following exposure to these regimens, all animals were placed on a progressive water restriction schedule leading in three days to 0.5-hour access to water per day. Rats remained on this water restriction schedule for the remaining 16 days of the experiment. On five of these days, rats were given access to a 0.1% saccharin solution (1g/l) in the 30-minute period in which they usually received water. On these saccharin days, the drinking period was immediately followed by an injection of amphetamine for all animals (1.5 mg/kg, IP). Volume of saccharin and water consumed was recorded every day for all animals.

2.5. Results and Discussion

In this experiment, pairing saccharin with amphetamine led, as expected, to a significant attenuation in saccharin consumption in rats previously exposed to saline, indicating the development of a CTA. Rats previously exposed to 12 daily injections of amphetamine did not

show this attenuation in saccharin consumption, indicating that they did not develop a CTA, in agreement with previous reports (Fig.1). Interestingly, previous exposure to 5 injections of amphetamine, one injection every third day, a regimen known to produce sensitization of this drug's locomotor, nucleus accumbens dopamine activating, and incentive motivational effects, was not sufficient to significantly protect rats against developing a CTA (Fig. 2).

12 previous injections.—ANOVA conducted on saccharin intake levels (on Sac+Amph days) observed in rats previously exposed to 12 amphetamine injections revealed a significant effect of groups [$F(1,10)=13.66$, $P<0.01$] and days [$F(4,40)=11.16$, $P<0.001$], but no group X day interaction. Post-hoc Scheffé comparisons showed that, compared to Day 1 intake, saccharin consumption was significantly lower as of Sac+Amph Day 2 in saline exposed rats ($P_s<0.01-0.001$), but no significant decrease in saccharin intake occurred in rats previously exposed to amphetamine. Additional Scheffé comparisons showed that saccharin intake was significantly lower in saline versus amphetamine-exposed rats, again as of the second Sac+Amph pairing day ($P_s<0.01$; Fig. 1, inset). T-test comparisons of saccharin intake on Sac+Amph days to water intake on the preceding day confirmed the progressive development of a CTA in rats previously exposed to saline ($P_s<0.01-0.001$) and confirmed that CTA did not occur in rats previously exposed to amphetamine. With the exception of the increase in intake observed on days 1-4, water consumption remained relatively stable on unpaired days.

5 previous injections.—ANOVA conducted on saccharin intake levels (on Sac+Amph days) observed in rats previously exposed to 5 injections revealed only a significant effect of days [$F(4,40)=11.55$, $P<0.001$]. Post-hoc Scheffé comparisons showed that, compared to Day 1 intake, saccharin consumption was significantly lower as of Sac+Amph Day 4 in amphetamine exposed ($P_s<0.05-0.01$) and Day 3 in saline exposed rats ($P_s<0.05-0.01$). No significant difference between groups was observed at any time. T-test comparisons of saccharin intake on Sac+Amph days to water intake on the preceding day confirmed the progressive development of a CTA in rats previously exposed to amphetamine ($P_s<0.05-0.01$, days 1, 3-5) and saline ($P_s<0.05-0.01$, days 1-5). With the exception of the increase in intake observed on days 1-4, water consumption remained relatively stable on unpaired days.

These results are consistent with previous findings demonstrating a protective effect of previous amphetamine exposure on the development of CTA, and show that the effect is dependent on the intensity of the exposure regimen. Exposure to the more moderate regimen of amphetamine injections did not protect against the development of CTA. Considering that this exposure regimen has been repeatedly shown to produce robust and long-lasting sensitization of amphetamine's locomotor, nucleus accumbens dopamine activating, and incentive motivational effects [for references, see 65], these results argue that sensitization need not interfere with amphetamine's ability to condition taste aversions.

When administered systemically, as in the present experiment, amphetamine clearly constitutes a US in the CTA paradigm in that it produces significant CTA to saccharin. Thus, one explanation of the findings obtained is that previous exposure to amphetamine led to a US-PEE and that the more moderate regimen did not include a sufficient number of amphetamine injections to produce this effect. Additional experiments were thus conducted to identify a route of amphetamine injections that is capable of producing sensitization but in which amphetamine does not act as a US for CTA (Experiment 2) and then to use this injection route to re-assess directly whether amphetamine sensitization does in fact interfere with the drug's ability to condition taste aversions, this time, in a manner that does not confound amphetamine sensitization with US pre-exposure.

3. Experiment 2

Amphetamine is known to act in the VTA to initiate neuroadaptations that lead ultimately to the expression of sensitized responding to the drug. These neuroadaptations are initiated most likely by the increase in extracellular levels of dopamine by amphetamine in the VTA. One acute consequence of this somatodendritic dopamine release may be to activate inhibitory D2 dopamine autoreceptors and thereby decrease the synaptic release of dopamine in the nucleus accumbens where it promotes locomotion and drug self-administration. The present experiment assessed whether this potentially aversive effect of VTA amphetamine might be capable of producing a CTA when paired with saccharin, and thus whether amphetamine actions in this site constitute a US for CTA.

3.1. Subjects

Seventeen male Sprague-Dawley rats, procured and housed as described in Experiment 1, were used. Before any experimental procedures, all animals were surgically prepared with chronic bilateral guide cannulae aimed at the VTA. Following anesthesia with a mix of ketamine (100mg/kg, IP) and xylazine (6 mg/kg, IP), rats were mounted in a stereotaxic instrument with the incisor bar positioned 5.0 mm above the interaural line. Cannulae (22 gauge, Plastics One, Roanoke, VA) were then implanted bilaterally, aimed at the VTA [A/P, -3.6; L, ± 0.6 ; DV, -8.9 from bregma and skull; 46], and secured in place with skull screws and dental cement. Cannulae were angled at 16° to the vertical and positioned 1 mm above the final injection site. After surgery, 28 gauge obturators were placed in the guide cannulae and rats were returned to their home cages for a 7-10 day recovery period.

3.2. Apparatus

Experiments were conducted in individual chambers as described in Experiment 1.

3.3. Procedures

Unlike in Experiments 1 and 3, rats in this experiment were not subjected to previous exposure injections prior to CTA testing. Beginning 7-10 days after surgery, animals were placed on a progressive water restriction schedule as described in Experiment 1 and subsequently maintained on 0.5-hour water access per day for the remaining 18 days of the experiment. On five of these days, rats were given 0.1% saccharin solution. On these saccharin days, the drinking period was immediately followed by a microinjection of amphetamine (2.5 μ g/0.5 μ l/side) or saline (0.5 μ l/side) into the VTA. Microinjections were made with injection cannulae (28 gauge) connected to 1 μ l syringes (Hamilton, Reno, NV) via PE-20 tubing and inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μ l/side over 30 s. After 60 s, the injection cannulae were withdrawn and the obturators were replaced. Volume of saccharin and water consumed was recorded every day for all animals.

3.4. Histology

At the end of the experiment, rats were injected with sodium pentobarbital and perfused via an intracardiac infusion of a saline and 10% formalin solution. Coronal sections (40 μ m) were mounted onto gelatin-coated slides and subsequently stained with cresyl violet for verification of cannula tip placements. Only data obtained from animals with both cannula tips located in the VTA were retained for statistical analyses. These placements are illustrated in Fig. 3. Of the 17 rats prepared, three in the VTA amphetamine group were dropped because at least one of their cannula placements was found to be outside the VTA. The resulting *n*/group was therefore six for the amphetamine and eight for the saline conditions.

3.5. Results and Discussion

In this experiment, pairing saccharin with either VTA amphetamine or VTA saline failed to produce a significant attenuation in saccharin consumption (Fig. 4). These findings indicate that, under the present conditions, amphetamine actions in this site do not produce a CTA and thus do not constitute a US in this paradigm.

ANOVA conducted on saccharin intake levels (on Sac+Amph days) revealed only a significant effect of days [$F(4,48)=2.89$, $P<0.05$] indicative of a slight overall increase in saccharin consumption on paired days by both groups. Post-hoc Scheffé comparisons showed that, compared to Day 1 intake, saccharin consumption was never significantly lower in rats receiving VTA amphetamine or VTA saline microinjections. No significant difference between groups was observed at any time. T-test comparisons of saccharin intake on Sac+Amph days to water intake on the preceding day confirmed the lack of a significant decrease in consumption at any time. Water intake fluctuated throughout the course of the experiment resulting in an overall significant effect of days on unpaired days ($P<0.001$).

Pairing saccharin with VTA amphetamine failed to decrease saccharin consumption, indicating that the actions of amphetamine in this site do not contribute to the ability of this drug to produce CTA and thus do not serve as a US in the CTA paradigm. Interestingly, it was previously shown that repeatedly pairing VTA amphetamine microinjections with a specific environment leads to locomotor sensitization but fails to produce conditioned locomotion [67]. Taken together, these findings indicate that although amphetamine actions in the VTA do not constitute a US for associative forms of conditioning, they do initiate neuroadaptations that lead to long-lasting forms of non-associative plasticity like sensitization.

4. Experiment 3

This experiment used a regimen of amphetamine microinjections into the VTA that is known to produce sensitization of this drug's locomotor, dopamine activating, and incentive motivational effects [64,66] to directly assess, without the confound of US pre-exposure, whether this form of plasticity can interfere with the subsequent ability of systemically administered amphetamine to produce a CTA. The VTA is the site of action of amphetamine where neuroadaptations are initiated that lead to the expression of sensitization [see 65]. If the protective effect of previous exposure to the drug is linked to sensitization of amphetamine's appetitive properties, then previous exposure to VTA amphetamine should protect rats from developing a CTA.

4.1. Subjects

The subjects were 19 male Sprague-Dawley rats. They were procured, housed, and surgically prepared with chronic indwelling guide cannulae aimed at the VTA as described in Experiment 2.

4.2. Apparatus

Experiments were conducted in individual chambers as described in Experiment 1.

4.3. Procedures

Beginning 7-10 days after surgery, animals were administered a total of three microinjections corresponding to their exposure condition: amphetamine (2.5µg/0.5µl/side) or saline (0.5µl/side). Microinjections were made once every third day and were administered as described in Experiment 2. The dose and VTA amphetamine injection regimen were selected because together they have been shown to produce robust and long-lasting sensitization of the drug's locomotor, dopamine activating and incentive motivational effects [8,64,66].

Following exposure to the microinjection regimen described above, rats were placed on a progressive water restriction schedule as described in Experiment 1 and subsequently maintained on 0.5-hour water access per day for the remaining 18 days of the experiment. On five of these days, rats were given access to the 0.1% saccharin solution. Again, on these saccharin days, the drinking period was immediately followed by an injection of amphetamine for all animals (1.5 mg/kg, IP). Volume of saccharin and water consumed was recorded every day for all animals.

4.4. Histology

At the end of the experiment, coronal brain sections were obtained as described in Experiment 2 for verification of cannula tip placements. Only data obtained from animals with both cannula tips located in the VTA were retained for statistical analyses. These placements are illustrated in Fig. 5. Of the 19 rats prepared, six (3 in each exposure condition) were dropped from analysis because at least one of their cannula placements was found to be outside the VTA. The resulting *n*/group was therefore seven for the amphetamine and six for the saline exposure conditions.

4.5. Results and Discussion

In this experiment, pairing saccharin with amphetamine led, as expected, to a significant attenuation in saccharin consumption in rats previously exposed to VTA saline. Remarkably, rats previously exposed to VTA amphetamine also attenuated their saccharin consumption, indicating that this regimen of VTA amphetamine microinjections, known to produce sensitization of this drug's locomotor, dopamine activating and incentive motivational effects, was unable to block the decrease in saccharin consumption indicative of a CTA (Fig. 6).

ANOVA conducted on saccharin intake levels (on Sac+Amph days) revealed only a significant effect of days [$F(4,44)=23.97$, $P<0.001$]. Post-hoc Scheffé comparisons showed that, compared to Day 1 intake, saccharin consumption was significantly lower as of Sac+Amph Day 3 in both groups ($P_s<0.05-0.001$). No significant difference between groups was observed at any time. T-test comparisons of saccharin intake on Sac+Amph days to water intake on the preceding day confirmed the progressive development of a CTA in rats previously exposed to VTA amphetamine ($P_s<0.05-0.001$, days 2-5) and saline ($P_s<0.05-0.01$, days 3-5). Water intake fluctuated somewhat throughout the course of the experiment resulting in an overall significant effect of days on unpaired days ($P<0.001$).

As expected, pairing saccharin with amphetamine led to a significant attenuation in saccharin consumption in rats previously exposed to VTA saline. Importantly, this was also observed in VTA amphetamine exposed rats, indicating that sensitization, as dissociated from US pre-exposure, did not protect against the development of a CTA.

5. Discussion

Results from this series of experiments support the conclusion that appetitive sensitization by amphetamine does not interfere with the drug's ability to condition taste aversions. In Experiment 1, the ability of amphetamine to produce CTA was reduced in rats previously exposed to a sufficiently intense, but not a moderate, regimen of systemic amphetamine injections even though the latter is known to lead to robust and long-lasting sensitization by amphetamine. This finding was pursued first by establishing, in Experiment 2, that amphetamine actions in the VTA do not constitute a US for CTA and then by showing without the confound of previous US exposure, in Experiment 3, that VTA amphetamine sensitization does not interfere with the ability of this drug to subsequently condition taste aversion. These results are important because unlike emetic agents such as LiCl, drugs like amphetamine possess strong appetitive properties that are subject to associative and non-associative forms

of plasticity. The present findings show that appetitive sensitization, a non-associative form of plasticity produced when amphetamine is administered repeatedly, does not impact the ability of this drug to subsequently produce CTA.

Different views of drug-induced CTA have posited different substrates for this effect. It has, for example, been suggested that drugs like amphetamine produce CTA by virtue of their aversive properties and their support of aversion conditioning [for discussion, see 33,58]. In addition, it has been proposed that previous exposure to amphetamine reduces its ability to produce CTA (the US-PEE) by sensitizing its rewarding effects sufficiently so that these overcome its aversive effects [20,21]. The findings obtained in the present experiments do not support this view. First, pairing VTA amphetamine with saccharin did not condition a taste aversion. Amphetamine acts in this site to increase extracellular levels of dopamine, leading in part to activation of local D2 dopamine autoreceptors that could decrease dopamine release in the nucleus accumbens. The present findings indicate that this effect is either not sufficiently aversive to produce CTA or that such aversive properties of amphetamine in fact do not contribute to its ability to condition taste aversion. Second, previous exposure to sensitizing regimens of systemic or VTA amphetamine injections did not reduce its ability to subsequently produce CTA, indicating that sensitization of its rewarding or incentive motivational effects, presumably sufficient to overcome its aversive effects, does not decrease its ability to condition taste aversion.

Alternatively, it has also been suggested that the rewarding properties of drugs can support their ability to produce CTA via a 'reward contrast effect' by which consumption of a less reinforcing CS decreases as rats learn that it is to be followed by a more reinforcing drug. Sensitization of the rewarding properties of the drug would provide an even greater contrast and thus would be expected to subsequently produce greater CTA [30]. No evidence was obtained in the present experiments for enhanced CTA following exposure to sensitizing regimens of either systemic or VTA amphetamine injections, suggesting that a drug's rewarding effects or sensitization of these effects does not impact its ability to produce CTA. It can be argued that the sensitizing regimens of drug injections used in the present experiments, even though sufficient to produce sensitization of amphetamine's locomotor [8,47], nucleus accumbens dopamine [63,64] and incentive motivational effects [66], were not robust enough to produce the expected enhancement in CTA. On the contrary, previous exposure to regimens in which the number, dose or frequency of drug injections is superior is known to reduce the ability of drugs like amphetamine to produce CTA [Fig. 1;11,22,24].

A number of reports support the view that an associative mechanism such as blocking may underlie the US-PEE. According to this view, the formation of associations between the US and context and/or injection cues impedes the subsequent formation of an association between the CS and the US in the CTA paradigm. Although this view has been evoked mostly to explain the US-PEE produced by pre-exposure to emetic agents like LiCl [e.g., 5,6,55], blocking has also been suggested to underlie the US-PEE observed when amphetamine is the US [7]. Indeed, earlier reports showed that diminishing the associative strength of amphetamine injection cues reduced the US-PEE produced by the drug [14,48]. Consistent with these findings, previous exposure to amphetamine in the VTA, where its actions do not constitute a US for associative forms of learning [Experiment 2; 67], did not produce a US-PEE in Experiment 3. In Experiment 1, the finding that 12, but not five, amphetamine pre-exposure injections were sufficient to prevent CTA, can be accounted for, according to the blocking interpretation, by arguing that the latter regimen was not sufficiently intense to allow for the formation of associations between context/injection cues and amphetamine. However, this identical regimen has been shown to lead not only to amphetamine sensitization but also to conditioned locomotion and conditioned inhibition of the expression of amphetamine sensitization [e.g., 59,60]. It is not entirely clear, therefore, why this pre-exposure regimen did not produce a US-

PEE in the present experiments. In addition, little is known of the aversive properties of amphetamine that would provide the US for blocking in the above associative view of the US-PEE.

It is likely that the manner in which drugs condition taste aversions and the manner in which previous exposure to drugs diminishes their ability to do so involves multiple, complex mechanisms. For example, drugs like amphetamine have multiple actions in multiple brain regions. It is conceivable that some of these actions could mediate different aspects of the CTA process and that the regions in which they occur and the information these regions exchange with other sites determine the nature of their contribution. While there is general agreement that dopamine projections to the nucleus accumbens mediate the appetitive and reinforcing properties of psychostimulant drugs [65], dopamine has also been proposed to act in this site to facilitate pavlovian learning including CTA learning [16,17] even though amphetamine infusions into the nucleus accumbens do not produce CTA [15]. These findings, together with others showing that the conditioning of taste aversion by amphetamine is attenuated by systemically administered dopamine receptor antagonists [31,38], suggest that amphetamine might also act at other dopaminergic sites to produce CTA under appropriate environmental conditions. Interestingly, infusing amphetamine into regions subjacent to the area postrema, but not other sites like the nucleus accumbens, medial prefrontal cortex, caudate and amygdala, has been reported to produce CTA [15; see also 49]. The regions subjacent to the area postrema and interconnected with it, namely the nucleus of the solitary tract and the dorsal motor nucleus of the vagus, receive input from the sensory vagus and taste input from the tongue [41,61] and contain dopaminergic processes [34,39]. The area postrema and vagal afferent fibers also make axonal connections with the parabrachial nucleus [43,57], a site identified as playing a critical role in the acquisition of CTAs produced by a broad range of visceral US-CS pairings, possibly by mediating the US-CS association [25,28,42,51,56,70]. This convergence of visceral, taste, and dopaminergic inputs accessible by psychostimulants positions these regions well for mediating amphetamine-induced CTA. As suggested for the anorectic effect of amphetamine [36], tolerance of this drug's aversive properties may stem from its decreased ability to increase extracellular dopamine levels in these regions following previous administration in a sufficiently robust regimen. It is also conceivable that associative processes can be enlisted by amphetamine actions in such a circuit that can impact the conditioning of taste aversions.

Acknowledgments

This research was supported by NIH grant DA09397.

References

- [1]. Bechara A, van der Kooy D. Opposite motivational effects of endogenous opioids in brain and periphery. *Nature* 1985;314:533–34. [PubMed: 2986002]
- [2]. Berman RF, Cannon DS. The effect of prior ethanol experience on ethanol-induced saccharin aversions. *Physiol Behav* 1974;12:1041–44. [PubMed: 4832445]
- [3]. Blanchard DC, Blanchard RJ. Cocaine potentiates defensive behaviors related to fear and anxiety. *Neurosci Biobehav Rev* 1999;23:981–91. [PubMed: 10580312]
- [4]. Breslin PA, Spector AC, Grill HJ. A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium chloride pairings: A unidimensional account of palatability. *Behav Neurosci* 1992;106:820–36. [PubMed: 1332732]
- [5]. de Brugada I, Gonzalez F, Candido A. Repeated administration of LiCl produces an unconditioned stimulus preexposure effect in backward excitatory CTA but not habituation of the unconditioned increment in neophobia. *Behav Processes* 2003a;60:227–33. [PubMed: 12505176]
- [6]. de Brugada I, Gonzalez F, Candido A. The role of injection cues in the associative control of the US pre-exposure effect in flavour aversion learning. *Quart J Exp Psych Bull* 2003b;56:241–52.

- [7]. de Brugada I, Hall G, Symonds M. The US-preexposure effect in lithium-induced flavoraversion conditioning is a consequence of blocking by injection cues. *J Exp Psych: Animal Behav Processes* 2004;30:58–66.
- [8]. Cadot M, Bijiou Y, Stinus L. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. *Neuroscience* 1995;65:385–95. [PubMed: 7777156]
- [9]. Capaldi ED, Sheffer JD. Contrast and reinforcement in consumption. *Learning and Motivation* 1992;23:63–79.
- [10]. Cappell H, LeBlanc AE. Conditioned aversion to saccharin by single administrations of mescaline and d-amphetamine. *Psychopharmacologia* 1971;22:352–56. [PubMed: 5133436]
- [11]. Cappell H, LeBlanc AE. Conditioned aversion by amphetamine: rates of acquisition and loss of the attenuating effects of prior exposure. *Psychopharmacologia* 1975;43:157–62. [PubMed: 1187950]
- [12]. Cappell H, LeBlanc AE. Parametric investigations of the effects of prior exposure to amphetamine and morphine on conditioned gustatory aversion. *Psychopharmacology* 1977;51:265–71. [PubMed: 403542]
- [13]. Cappell H, LeBlanc AE, Herling S. Modification of the punishing effects of psychoactive drugs in rats by previous drug experience. *J Comp Physiol Psych* 1975;89:347–56.
- [14]. Cappell H, Poulos CX. Associative factors in drug pretreatment effects on gustatory conditioning: Cross-drug effects. *Psychopharmacology* 1979;64:209–13. [PubMed: 115043]
- [15]. Carr GD, White NM. Anatomical disassociation of amphetamine's rewarding and aversive effects: An intracranial microinjection study. *Psychopharmacology* 1986;89:340–46. [PubMed: 3088661]
- [16]. Fenu S, Bassareo V, Di Chiara G. A role for dopamine D1 receptors of the nucleus accumbens shell in conditioned taste aversion learning. *J Neurosci* 2001;21:6897–904. [PubMed: 11517277]
- [17]. Fenu S, Di Chiara G. Facilitation of conditioned taste aversion learning by systemic amphetamine: Role of nucleus accumbens shell dopamine D1 receptors. *Eur J Neurosci* 2003;18:2025–30. [PubMed: 1462235]
- [18]. Flaherty CF, Checke S. Anticipation of incentive gain. *Animal Learning Behav* 1982;10:177–82.
- [19]. Flaherty CF, Turovsky J, Krauss KL. Relative hedonic value modulates anticipatory contrast. *Physiol Behav* 1994;55:1047–54. [PubMed: 8047570]
- [20]. Gaiardi M, Bartoletti M, Bacchi A, Gubellini C, Costa M, Babbini M. Role of repeated exposure to morphine in determining its affective properties: place and taste conditioning studies in rats. *Psychopharmacology* 1991;103:183–86. [PubMed: 2027919]
- [21]. Gaiardi M, Bartoletti M, Gubellini C, Bacchi A, Babbini M. Modulation of the stimulus effects of morphine by d-amphetamine. *Pharmacol Biochem Behav* 1998;59:249–53. [PubMed: 9443562]
- [22]. Goudie AJ, Taylor M, Atherton H. Effects of prior drug experience on the establishment of taste aversions in rats. *Pharmacol Biochem Behav* 1975;3:947–52. [PubMed: 1208640]
- [23]. Goudie AJ, Thornton EW. Effects of drug experience on drug induced conditioned taste aversions: studies with amphetamine and fenfluramine. *Psychopharmacologia* 1975;44:77–82. [PubMed: 1239032]
- [24]. Goudie AJ, Thornton EW, Wheller TJ. Drug pretreatment effects in drug induced taste aversions: Effects of dose and duration of pretreatment. *Pharmacol Biochem Behav* 1976;4:629–33. [PubMed: 951441]
- [25]. Grancha ML, Navarro M, Cubero I, Thiele TE, Bernstein IL. Induction of a brainstem correlate of conditioned taste aversion expression: role of the pontine parabrachial nucleus. *Behav Brain Res* 2002;131:205–9. [PubMed: 11844587]
- [26]. Grigson PS. Conditioned taste aversions and drugs of abuse: a reinterpretation. *Behav Neurosci* 1997;111:129–36. [PubMed: 9109631]
- [27]. Grigson PS. Drugs of abuse and reward comparison: A brief review. *Appetite* 2000;35:89–91. [PubMed: 10896765]
- [28]. Grigson PS, Shimura T, Reilly S, Norgren R. Ibotenic acid lesions of the parabrachial nucleus and conditioned taste aversion: Further evidence for an associative deficit in rats. *Behav Neurosci* 1998;112:160–71. [PubMed: 9517824]

- [29]. Grigson PS, Twining RC, Carelli RM. Heroin-induced suppression of saccharin intake in water-deprived and water-replete rats. *Pharmacology Biochemistry and Behavior* 2000;66:603–608.
- [30]. Grigson PS, Wheeler RA, Wheeler DS, Ballard SM. Chronic morphine treatment exaggerates the suppressive effects of sucrose and cocaine, but not lithium chloride, on saccharin intake in Sprague-Dawley rats. *Behav Neurosci* 2001;115:403–16. [PubMed: 11345965]
- [31]. Grupp LA. Effects of pimozide on the acquisition, maintenance, and extinction of an amphetamine-induced taste aversion. *Psychopharmacology* 1977;53:235–42. [PubMed: 408851]
- [32]. Hooks MS, Jones GH, Liem BJ, Justice JB Jr. Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine infusions. *Pharmacol Biochem Behav* 1992;43:815–23. [PubMed: 1448477]
- [33]. Hunt T, Amit Z. Conditioned taste aversion induced by self-administered drugs: paradox revisited. *Neurosci Biobehav Rev* 1987;11:107–30. [PubMed: 3554039]
- [34]. Kalai M, Fuxe K, Goldstein M, Harfstrand A, Agnati LF, Coyle JT. Evidence for the existence of putative dopamine-, adrenaline- and noradrenalin-containing vagal motor neurons in the brainstem of the rat. *Neurosci Lett* 1984;50:57–62. [PubMed: 6149507]
- [35]. Kalivas PW, Weber B. Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. *J Pharmacol Exp Ther* 1988;245:1095–1102. [PubMed: 3385638]
- [36]. Kuo DY, Cheng JT. Role of cerebral dopamine but not plasma insulin, leptin and glucocorticoid in the development of tolerance to the anorectic effect of amphetamine. *Neurosci Res* 2002;44:63–69. [PubMed: 12204294]
- [37]. LeBlanc AE, Cappell H. Attenuation of punishing effects of morphine and amphetamine by chronic prior treatment. *J Comp Physiol Psych* 1974;87:691–98.
- [38]. Lin HQ, McGregor IS, Atrens DM, Christie MJ, Jackson DM. Contrasting effects of dopaminergic blockade on MDMA and d-amphetamine conditioned taste aversions. *Pharmacol Biochem Behav* 1994;47:369–74. [PubMed: 7908448]
- [39]. Maley BE. Immunohistochemical localization of neuropeptides and neurotransmitters in the nucleus solitarius. *Chemical Senses* 1996;21:367–76. [PubMed: 8670716]
- [40]. Meyer U, Chang de LT, Feldon J, Yee BK. Expression of the CS- and US-pre-exposure effects in the conditioned taste aversion paradigm and their abolition following systemic amphetamine treatment in C57BL/6J mice. *Neuropsychopharmacology* 2004;29:2140–48. [PubMed: 15238994]
- [41]. Morest DK. Experimental study of the projections of the nucleus of the tractus solitarius and the area postrema in the cat. *J Comp Neurol* 1967;130:277–300. [PubMed: 6059369]
- [42]. Mungarndee SS, Baldwin BA, Chindadoungratana C, Kotchabhakdi N. Hypothalamic and zona incerta neurons in sheep, initially only responding to the sight of food, also respond to the sight of water after intracerebroventricular injection of hypertonic saline or angiotensin II. *Brain Res* 2002;925:204–12. [PubMed: 11792369]
- [43]. Norgren R. Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 1978;3:207–18. [PubMed: 733004]
- [44]. Paulson RE, Robinson TE. Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: A microdialysis study in behaving rats. *Synapse* 1995;19:56–65. [PubMed: 7709344]
- [45]. Paxinos, G.; Watson, C. *Rat Brain in Stereotaxic Coordinates*. Academic Press; New York: 1997.
- [46]. Pellegrino, LJ.; Pellegrino, AS.; Cushman, AJ. *A Stereotaxic Atlas of the Rat Brain*. Plenum Press; New York: 1979.
- [47]. Perugini M, Vezina P. Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. *J Pharmacol Exp Ther* 1994;270:690–6. [PubMed: 8071860]
- [48]. Poulos CX, Cappell H. An associative analysis of pretreatment effects in gustatory conditioning by amphetamine. *Psychopharmacology* 1979;64:201–7. [PubMed: 115042]
- [49]. Rabin BM, Hunt WA. Interaction of haloperidol and area postrema lesions in the disruption of amphetamine-induced conditioned taste aversion learning in rats. *Pharmacol Biochem Behav* 1989;33:847–51. [PubMed: 2616604]

- [50]. Randich A, LoLordo VM. Associative and nonassociative theories of the UCS preexposure phenomenon: implications for Pavlovian conditioning. *Psych Bull* 1979;86:523–48.
- [51]. Reilly S, Grigson PS, Norgren R. Parabrachial nucleus lesions and conditioned taste aversion: Evidence supporting an associative deficit. *Behav Neurosci* 1993;107:1005–17. [PubMed: 8136054]
- [52]. Riley AL, Diamond HF. The effects of cocaine preexposure on the acquisition of cocaine-induced taste aversions. *Pharmacol Biochem Behav* 1998;60:739–45. [PubMed: 9678659]
- [53]. Riley AL, Simpson GR. Cocaine pre-exposure fails to sensitize the acquisition of cocaine-induced taste aversions. *Pharmacol Biochem Behav* 1999;63:193–199. [PubMed: 10371647]
- [54]. Riley, AL.; Simpson, GR. The attenuating effects of drug preexposure on taste aversion conditioning: Generality, experimental parameters, underlying mechanisms and implications for drug use and abuse. In: Mowrer, RR.; Klein, SB., editors. *Contemporary learning theory*. 2nd edition. Lawrence Erlbaum Associates; Hillsdale, NJ: 2001.
- [55]. Rudy JW, Iwens J, Best PJ. Pairing novel exteroceptive cues and illness reduces illness-induced taste aversions. *J Exp Psych: Animal Behav Processes* 1977;3:14–25.
- [56]. Sakai N, Yamamoto T. Role of the medial and lateral parabrachial nucleus in acquisition and retention of conditioned taste aversion in rats. *Behav Brain Res* 1998;93:63–70. [PubMed: 9659987]
- [57]. Saper, CB. Hypothalamus. In: Paxinos, G.; Mai, JK., editors. *The Human Nervous System*. Academic Press; London: 2004.
- [58]. Stewart J, Eikelboom R. Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. *Pharmacol Biochem Behav* 1978;9:639–45. [PubMed: 733852]
- [59]. Stewart, J.; Vezina, P. Conditioning and behavioral sensitization. In: Kalivas, PW.; Barnes, CD., editors. *Sensitization in the nervous system*. Telford Press; Caldwell, NJ: 1988.
- [60]. Stewart J, Vezina P. Extinction procedures abolish conditioned stimulus control but spare sensitized responding to amphetamine. *Behav Pharmacol* 1991;2:65–71. [PubMed: 11224049]
- [61]. Sumal KK, Blessing WW, Joh TH, Reis DJ, Pickel VM. Synaptic interaction of vagal afferents and catecholaminergic neurons in the rat nucleus tractus solitarius. *Brain Res* 1983;277:31–40. [PubMed: 6139145]
- [62]. Suto N, Tanabe LM, Austin JD, Creekmore E, Pham CT, Vezina P. Previous exposure to psychomotor stimulants enhances the reinstatement of cocaine seeking by nucleus accumbens AMPA. *Neuropsychopharmacology* 2004;29:2149–59. [PubMed: 15266353]
- [63]. Vezina P. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: An in vivo microdialysis study in the rat. *Brain Res* 1993;605:332–7. [PubMed: 8386970]
- [64]. Vezina P. D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. *J Neurosci* 1996;16:2411–20. [PubMed: 8601820]
- [65]. Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci Biobehav Rev* 2004;27:827–39. [PubMed: 15019432]
- [66]. Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N. Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. *J Neurosci* 2002;22:4654–62. [PubMed: 12040071]
- [67]. Vezina P, Stewart J. Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res* 1990;516:99–106. [PubMed: 2364286]
- [68]. Wise RA, Yokel RA, de Wit H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science* 1976;191:1273–5. [PubMed: 1257748]
- [69]. Wolf ME, White FJ, Nassar R, Brooderson RJ, Khansa MR. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J Pharmacol Exp Ther* 1993;264:249–55. [PubMed: 8093727]
- [70]. Yamamoto T, Fujimoto Y, Shimura T, Sakai N. Conditioned taste aversion in rats with excitotoxic brain lesions. *Neurosci Res* 1995;22:31–49. [PubMed: 7792081]

Figure 1.

Previous exposure to 12 IP injections of amphetamine blocked its ability to produce CTA. Data are shown as group mean ($\pm$ SEM) ml liquid consumed on saccharin+amphetamine (Sac +Amph) paired and unpaired days. Only water was available on unpaired days. *Inset:* Group mean ($\pm$ SEM) ml saccharin consumed on each of the 5 paired days. **, significant difference between groups revealed by post-hoc Scheffé comparisons following ANOVA.

Figure 2. Previous exposure to 5 IP injections of amphetamine spared its ability to produce CTA. Data are shown as in Figure 1.

Figure 3.

Location of the injection cannula tips in the VTA of rats included in the data analyses for Experiment 2. Line drawings are from [45]. Numbers to the right indicate distance (mm) from bregma. Symbols indicate group affiliation: filled circles, VTA amphetamine exposed (n=6), open circles, VTA saline exposed (n=8).

Figure 4.

VTA amphetamine failed to produce CTA when repeatedly paired with saccharin. Data are shown as group mean ($\pm$ SEM) ml liquid consumed on Sac+Amph paired and unpaired days. Only water was available on unpaired days. *Inset:* Group mean ($\pm$ SEM) ml saccharin consumed on each of the 5 paired days.

Figure 5. Location of the injection cannula tips in the VTA of rats included in the data analyses for Experiment 3. Line drawings are from [45]. Numbers to the right indicate distance (mm) from bregma. Symbols indicate group affiliation: filled circles, VTA amphetamine exposed (n=7), open circles, VTA saline exposed (n=6).

Figure 6.

Previous exposure to VTA amphetamine in a sensitizing regimen failed to interfere with amphetamine's ability to evoke a CTA when administered systemically in Sac+Amph pairings. Data are shown as in Figure 1.