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Research report

Conditioned saccharin avoidance induced by infusion of amphetamine in the nucleus accumbens shell and morphine in the ventral tegmental area: Behavioral and biochemical study

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HIGHLIGHTS

- Intra-NAc shell amphetamine and intra-VTA morphine induced CSA at doses known to induce CPP.
- Intra-VTA morphine increases DA selectively in the NAc shell.
- Drug-CSA is related to stimulation of DA transmission in the NAc shell.
- Our results are consistent with a role of drug reward in morphine and amphetamine CSA.

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ABSTRACT

Drugs of abuse possess the seemingly paradoxical property of conditioning rats to avoid from drinking a saccharin solution that had been predictively paired with their systemic administration (conditioned saccharin avoidance, CSA). CSA is dependent upon an intact dopamine (DA) transmission but the locus, central or peripheral, and eventually the brain area from which this effect originates and its relationship with the rewarding properties of the drug is debated. In order to clarify this issue we tested the ability of amphetamine and morphine to induce CSA after infusion at the same dose-range and in the same areas from which these drugs induce conditioned place preference (CPP). Drugs were infused intracerebrally immediately after saccharin drinking in two acquisition trials and CSA was tested on a two bottle saccharin/water choice. Amphetamine (10 and 20 μg/0.5 μl) induced CSA after infusion in the NAc shell but was ineffective in the NAc core. Morphine (0.5 and 1 μg/0.5 μl) induced CSA from the VTA at both doses tested. Amphetamine (20 μg/0.5 μl) and morphine (1 μg/0.5 μl) failed to induce CSA after infusion 1.2 mm dorsal the NAc shell and the VTA respectively. Finally, morphine (1 μg/0.5 μl), infused in the VTA, elicited a selective increase in dialysate DA in the NAc shell. These results indicate that drugs of abuse induce CSA from the same intracerebral sites and at the same doses at which they induce CPP. These observations are consistent with the existence of a strong relationship between CSA and drug reward related to their ability to stimulate DA transmission in the NAc shell.

1. Introduction

Most, if not all, drugs of abuse act as unconditioned stimuli (US) to induce avoidance of a saccharin solution (conditioned stimulus, CS) predictively paired with their parenteral administration (conditioned saccharin avoidance, CSA) [1–5]. In spite of the robustness and reproducibility of drug CSA, its motivational nature and neural substrate is debated [4,6]. Initially, drug CSA has been viewed as an expression of the aversive properties of drugs of abuse and as

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a case of conditioned taste aversion (CTA) induced by agents that cause visceral illness such as irradiation, lithium and apomorphine [7–10].

However, it was later shown that in drug CSA the taste CS does not induce aversive taste reactions (e.g., gaping), as it does in lithium CTA [11,12]. Drug CSA, in contrast to lithium CTA, is also affected by changes in the incentive value of the taste (CS) [13–15] and is impaired by bilateral lesions of the gustatory thalamus [16,17].

A clue to the mechanism of drug CSA is provided by the finding that saccharin is avoided also when paired to a highly palatable sucrose solution (US) [18,19]. Since drugs of abuse are powerful rewards, it has been thought that drugs of abuse induce CSA by a mechanism similar to that of sucrose. Accordingly, saccharin-drug pairing trials, would result, on a subsequent saccharin preference test, in a comparison between saccharin and the drug as reward and in devaluation of the lesser reward (saccharin) in anticipation of the stronger one (drug) [4]. Consistent with a devaluation of the taste (CS), saccharin-drug pairing blunts the ability of saccharin to activate DA transmission in the nucleus accumbens (NAc) shell [20]. Also consistent with the reward comparison hypothesis, drug CSA, like drug conditioned place preference (CPP), is impaired by systemic administration of DA D_1 receptor antagonists [21–24]. This effect is time-related but in a way different from lithium CTA. Thus, in lithium CTA the critical DA-dependent phase corresponds to consolidation of memory of the saccharin taste. In contrast, in drug CSA the critical DA-dependent phase coincides with the time-course of action of the conditioning drug [21–24]. These differences between drug CSA and lithium CTA have been interpreted to indicate that drug CSA is dependent on the DA-mediated hedonic properties of the drug (US), while in lithium CTA DA is necessary for consolidation of the taste memory trace (CS) [23].

Some observations however, are difficult to fit in the reward comparison hypothesis. For example, it has been reported that in the same animal, changes in drug CSA do not parallel changes in CPP induced by repeated pre-exposure to the conditioning drug [25,26]. In view of these inconsistencies, alternative hypotheses have been proposed. Thus, it has been speculated that the internal state induced by drugs of abuse and the resulting disruption of homeostasis generates a fear signal related to the attribution of potentially poisonous properties to the novel taste [5]. This hypothesis however is not incompatible with a role of drug reward if one assumes that it is the ethologically weird association between drug reward and saccharin, rather than drug reward per se, that determines the fear state. Thus, the incongruous sweet taste/drug reward association would act to attribute putative poisonous properties, sufficient for avoiding it, to the food upon which the rewarding drug is contingent.

Another complication arises from inconsistencies over the site of origin, intracerebral versus peripheral, of drug CSA. Some evidence indicates that the aversive properties of morphine originate from the periphery. Thus, inactivation or blockade by methylnaltrexone of the peripheral opiate receptors abolishes morphine taste aversion [27,28] while, the peripheral opioid agonist loperamide induces CSA [29]. However intracerebroventricular infusion of the μ agonist [D-Ala^2, *N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO) induced CSA in Lewis and Fischer rats [30].

We reasoned that if drug CSA, resulting either from reward comparison or conditioned fear, is dependent upon the peculiar nature of drug reward, it should share with drug reward its central origin, as in the case, for example, of CPP, a paradigm widely utilized as an expression of the rewarding properties of drugs of abuse [31].

Therefore we tested the ability of amphetamine infused in the NAc shell and in the core and of morphine in the ventral tegmental area (VTA) to induce CSA. Drug doses were selected in the range known to induce CPP [31–35].

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Harlan, San Pietro al Natisone, Udine, Italy) weighing 275–300 g were housed in group of six per cage with standard food and water ad libitum, for at least one week in the central animal room, under controlled environmental conditions: constant temperature (23 °C), humidity (60%) and a 12 h light/dark cycle (light from 8 a.m. to 8 p.m.). After this period rats were single housed in the behavioral test room at the same controlled environmental conditions. All experiments were performed in their home cage and carried out during daylight hours (starting 10 a.m.). In all CSA experiments rats had access to fluid (0.1% saccharin or water depending on the stage of the experiment) for 20 min daily starting the day before the beginning of experimental procedures and throughout its entire duration. The use of such a fluid restriction protocol is common in CTA studies involving drinking from bottles and is justified by the need to ensure reproducible drinking during a given time interval [36–40]. Animals drank fluid from two special bottles (50 ml capacity), placed inside the home cage by metallic supports.

All experimental procedures met the guidelines and protocols approved by the European Community (2010/63/UE L 276 20/10/2010) and by the Ethical Commission for Animal Care and Use at the University of Cagliari. All efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery

Rats were anaesthetized with chloral hydrate (320 mg/kg i.p.), placed in a stereotaxic apparatus and bilaterally implanted with stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) in the NAc shell, NAc core and VTA (*NAc shell*: coordinates A +1.8 mm, L ±1.1 mm from bregma, V −7.5 mm from dura; *NAc core*: A +1.6 mm, L ±1.8 mm from bregma, V −7.2 mm from dura; *VTA*: A −5.3 mm, L ±0.9 mm from bregma, V −8.0 mm from dura), according to Paxinos and Watson [41]. Guide cannulae were fixed with glasionomeric cement (Glass Ionomer Cement, CX-Plus, Ilic, Milan, Italy) 5 mm above the aimed site of injection to prevent the injected solution from leaking out of the cannulas. In order to perform microdialysis study, a separate group of rats were bilaterally implanted with guide cannulae (as previously reported) in the VTA, and with microdialysis probe guide cannulae in the NAc shell (coordinates: AP +1.8 mm, ML ±1.1, from bregma, V −5.8 mm from dura) or in the NAc core (AP +1.6 mm, ML ±1.8 mm, from bregma, V −5.5 mm from dura), according to Paxinos and Watson [41]. From the day after surgery, rats were handled daily, patency of the cannula was checked and administered with gentamicin (50 mg/kg) for 6 days. After 1 week recovery rats were divided in different experimental groups in order to start with CSA or microdialysis experiments.

2.3. Conditioned saccharin avoidance protocol

The experiments were performed for 8 days and consisted of three phases: training, conditioning (CSA acquisition) and test (CSA expression).

2.3.1. Phase 1: training

Following 24 h of water deprivation, all rats were given 20-min access to water daily for 5 consecutive days and the intake was recorded for each rat.

2.3.2. Phase 2: conditioning (CSA acquisition)

The conditioning phase lasted 2 days. In this phase, all rats were given access to a novel saccharin solution (0.1% in tap water)