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Conditioned taste aversion from neostigmine or methyl-naloxonium in the nucleus accumbens

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ABSTRACT

Taylor, K.M. Mark, G.P. Hoebel, B.G. Conditioned taste aversion from neostigmine or methyl-naloxonium in the nucleus accumbens Physiol Behav 00(00):000–000, 2011. An opioid antagonist injected in the nucleus accumbens of a morphine-dependent rat will lower extracellular dopamine and release acetylcholine (ACh), as also seen in opiate withdrawal. It was hypothesized that raising extracellular ACh experimentally would be aversive as reflected by the induction of a conditioned taste aversion. Rats were implanted with cannulas aimed above the nucleus accumbens (NAc) for injection of the opiate antagonist methyl-naloxonium in morphine-dependent animals or neostigmine to increase ACh in drug naïve animals. Experiment 1 in addicted rats showed that local morphine withdrawal by local injection of methyl-naloxonium paired with the taste of saccharin induces a conditioned taste aversion. Experiment 2 in non-addicted rats demonstrated the same learned aversion after local administration of the cholinergic agonist neostigmine in the NAc. These results suggest that ACh released in the NAc during opiate withdrawal contributes to the dysphoric, aversive state characteristic of withdrawal. This accumbens system is implicated in the mechanism for generating the memory of an aversive event that is expressed as learned taste aversion.

1. Introduction

Opiate withdrawal has psychological as well as physical manifestations. To localize the source of these effects, researchers have injected opiate antagonists in various brain sites, suggesting different neural circuits mediating different aspects of drug withdrawal. Methyl-naloxonium (mNAX) is a naloxone derivative, which is less lipophilic and thus diffuses less when injected locally into brain tissue. When injected in the periaquaductal gray region of opiate-dependent rats, it causes somatic symptoms typical of morphine withdrawal, such as wet dog shakes and teeth chattering [1]. For these psychological symptoms, the amygdala and the nucleus accumbens are sites where local injection of mNAX caused a conditioned place aversion [2]. The nucleus accumbens (NAc) is the most sensitive site for generating this aversive state, without the physical symptoms associated with morphine withdrawal.

The NAc has been strongly implicated in the motivational aspects of drug administration, as well as potentially mediating some of the non-physical aversive aspect of opiate withdrawal. Opiates injected in the NAc are reinforcing, as shown by local self-injection of morphine or opioids [3,4]. Opioids in the NAc also elicit eating [5], as shown with the specific mμ-opioid receptor agonist [D-Ala^2,N-MePhe^4,Gly-ol]-enkephalin, (DAMGO) [6]. If opioid receptors in the accumbens generate incentive motivation and maintain eating, and local opiate blockade induces conditioned place aversion, then local mNAX should also cause a conditioned food aversion.

It is well established that an animal will avoid a flavor previously associated with illness or with an aversive internal state [7]. This robust effect has allowed us to measure the hedonic valence of a stimulus by pairing it with a novel flavor. If the animal later avoids that taste, we can infer that the stimulus is unpleasant or aversive to the animal. Drug withdrawal, nausea, and negative contrast all yield robust conditioned taste aversions (CTA) and are assumed to be aversive.

In the first experiment, the aversive nature of local morphine withdrawal was investigated to determine if local opioid antagonists in the NAc of animals after chronic morphine administration is sufficient to induce an aversive state as measured by CTA.

In addition to the behavioral effects of local morphine withdrawal, microdialysis evidence has suggested that systemic naloxone precipitated morphine withdrawal increases ACh in the NAc [8]. These results hint at a potential role of ACh in mediating the aversive effects of morphine withdrawal in the NAc. This possibility was tested

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directly by local injection of the cholinesterase inhibitor neostigmine into the NAc of drug-naïve rats to measure the aversive potential of a local cholinergic increase in the NAc.

Conditioned taste aversion is a powerful, long-lasting form of learning that is a clear sign of a prior aversive state, such as drug withdrawal, nausea or negative contrast [7,9–11]. The brain has evolved with neural circuits that readily link a flavor to nausea, so that animals learn to avoid poisonous food [12]. When the taste aversion is generated with naloxone, it is evident that the circuit uses opioids. This logic was used in the first experiment, which is designed to determine whether local blockade of accumbens opioid receptors causes a conditioned taste aversion. Methyl-naloxonium was tested in animals pretreated with morphine as a model of opiate dependence.

Methyl-naloxonium injected in the NAc of opiate-dependent animals releases Ach [8]. The source of ACh is local interneurons that control the accumbens output to motivational and motor systems [13,14]. Therefore, if mNAX induces a taste aversion and releases ACh, one can hypothesize that ACh might cause a taste aversion. Since ACh manipulations would bypass the opioid step, accumbens ACh should cause a taste aversion in naive rats. That was found as described in a preliminary report [15].

2. Method

2.1. Subjects and surgery

Sprague–Dawley male rats from the Princeton University Psychology Vivarium weighed between 350 and 400 g at the time of surgery. Animals were housed in an environmentally regulated room with lights on from 7:00 p.m. to 7:00 a.m. with ad libitum chow and tap water except where noted in the procedures. All test procedures were performed at least 2 h after dark onset.

Rats were anesthetized with 25 mg/kg sodium pentobarbital supplemented with 20 mg/kg ketamine when necessary and implanted with guide cannulas aimed above the NAc. Bilateral 10 mm 22 gage stainless steel guide cannulas were positioned +10.2 mm anterior to the intraoral line ±1.2 mm lateral to midline and −4.0 below the skull surface, or for the STR at +10.4 mm anterior to bregma, ±3.0 mm lateral and −4.0 ventral. Injectors that were inserted later and extended 3 mm to reach the posterior NAc shell region [16]. Guide cannulas were kept patent with 26 gage stainless steel stylets. After surgery each animal was administered prophylactic penicillin in procaine suspension (100,000 IU i.m.; Pfizer) and allowed to recover at least one week before testing. Rats assigned to receive morphine were anesthetized with metofane four days after surgery, and three 75-mg morphine pellets (NIH) were placed subcutaneously between the scapulas.

2.2. Injection procedures

The injectors were constructed of a 3-ft piece of 150-μm OD fused silica tubing (Polymicro Technologies) that extended 3 mm from a 10 mm piece of protective 26-gage stainless steel tubing. The silica tubing went from the tip of the injector out the top of the stainless steel tubing and up through protective polyethylene tubing (PE 20) and a steel spring to a swiveling, counterbalance arm and a 10 μl gas tight syringe in an infusion pump (Harvard Apparatus, Model 675). This assembly allowed the animal complete freedom of movement within the 30 cm × 30 cm × 80 cm Plexiglas cage before, during and after the injection procedure [17]. For each injection, the stylets were removed; the injectors were lowered by hand into each guide cannula, and attached with a PE 50 collet. The rat was placed in the cage and allowed 2 min to habituate before the remote injection. Intra-accumbens injections were infused at a volume of 0.5 μl per side during 90 s. After a 2-min delay, the injectors were removed and the animal was returned to its home cage.

2.3. Histology

At the end of the behavioral testing each animal was given an overdose of sodium pentobarbital and transcardially perfused with physiological saline solution followed by 4% buffered formalin with intracranial injectors in place. Brains were extracted and stored in 4% formalin for a minimum of one week before being frozen and sectioned into 40 μm slices. Verification of injection site was made according to a stereotaxic atlas [16].

2.4. Taste aversion conditioning

Experiment 1 paired a saccharin flavor with mNAX 0.5 ug/0.5 ul/side in opiate-dependent rats, and Experiment 2 paired saccharin with neostigmine (2 ug/0.5 ul/side) in naive rats. First, each animal was trained to drink during a limited access period. Rats were water deprived in the home cage for 24 h, followed by 30 min unlimited access to tap water presented in two bottles with sipper tubes at the front of the home cage. The training procedure was repeated a day later, and the CTA procedure began 24 h after that.

Rats in the Paired-Saccharin group (n = 7) were given 2.5 mM saccharin to drink as the conditioned stimulus (CS), paired with the NAc injection of mNAX or neostigmine. The Paired-Ringer control group (n = 7) received saccharin taste as the CS paired with an injection of Ringer solution containing 1.2 mM CaCl2, 1.0 mM MgCl2, 142 mM NaCl, 3.9 mM KCl, 1.4 mM Na2HP04, and 0.3 mM NaH2PO4, degassed at pH 7.3. As a control for pseudo-conditioning due to handling, the Pseudo-Paired group (n = 7) received one of the NAc injections, mNAX or neostigmine, but paired with plain water in place of saccharin as the CS. For the neostigmine study (Experiment 2), an anatomical control (n = 7) was added by having a striatal injection paired with the taste of saccharin.

Training lasted four days. On days 1 and 3, rats in each group were presented with two bottles, both containing the taste CS, at the front of the home cage for 30 min (saccharin for Paired, Ringer and Striatum groups, or water for the Pseudo group). Injections in the NAc were made 10 min later. On days 2 and 4, rats received 30 min access to the other solution without intracranial injections (water for Paired, Ringer and Striatum groups, or saccharin for the Pseudo group). Thus each rat received two CS–US pairings.

CTA was assessed in a two-bottle choice test one day later (day 5). In CTA testing, animals were presented with two bottles for 30 min, one with 2.5 mM saccharin in tap water, and the other with plain tap water. Saccharin was placed on the preferred side, if a preference had been expressed, to bias the outcome against the expected result. Intake from each bottle was recorded as measure of taste preference or aversion.

3. Results

3.1. Experiment 1: methyl-naloxonium in the accumbens causes a learned taste aversion

Morphine-dependent rats receiving mNAX paired with saccharin developed a taste aversion to the flavor. Saccharin intake was calculated as a percentage of total fluid consumption, and the data were analyzed by 1-way ANOVA. A significant difference [$F(2,18) = 65.82$, $p < 0.0001$] was found and compared by Tukey–Kramer post hoc analysis. mNAX–saccharin paired animals drank significantly less saccharin than the control groups (Fig. 1: lower graph; $p < 0.05$). There were no differences in saccharin preference between the control groups. The total fluid intake from both bottles was the same for all groups (Fig. 1: upper graph). Histological examination indicated that injector tracks were within the borders of the NAc in all rats.