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Orexin A in the ventral tegmental area enhances saccharin-induced conditioned flavor preference: The role of D1 receptors in central nucleus of amygdala

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ABSTRACT

In industrialized societies, food intake is largely determined by its hedonic characteristics, which can be modified by our experience *via* taste learning. In this learning, the hedonic value of a neutral flavor changes after its association with a motivationally significant stimulus. Experiment 1 analyzes the effect of orexin administration (53 and 107 ng) in the ventral tegmental area (VTA) on hedonic intake through acquisition of a *flavor-taste* preference and a *flavor-taste* aversion. Accordingly, animals underwent four one-bottle acquisition sessions with unilateral application of orexin-A or saline in the VTA at 10 min before a 15-min flavor intake period. Preference and aversion were tested by a two-bottle test containing the flavors used for CS+ and CS−. Results indicate that intra-VTA orexin strengthens *flavor-taste* conditioned flavor preference (CFP) by saccharin but does not facilitate *flavor-taste* aversion induced by association of a neutral flavor with the unpalatable taste of quinine. Experiment 2 examines the acquisition of a *flavor-taste* preference after co-administration of an effective dose of orexin-A in the VTA and of D1-like dopamine receptor antagonist SCH23390 (6 and 12 nmol) in the central nucleus of the amygdala (CeA). SCH23390 impedes the CFP strengthening observed after intra-VTA orexin administration, indicating that this effect may be mediated by dopaminergic receptors in the CeA. These data suggest that the simultaneous presentation of a flavor and a hedonically positive taste may be detected by orexinergic neurons that activate dopamine-releasing neurons of the VTA, thereby reinforcing the positive signals required to develop a taste preference.

1. Introduction

Food products that we choose for daily consumption largely comprise those that we have learned to prefer in terms of their post-intake (oral and post-oral) consequences. Four types of food learning have been proposed as a function of the positive or negative consequences of the intake: flavor-taste preference, flavor-nutrient learning, flavor-toxin learning, and flavor-taste aversion learning [1]. *Flavor-taste* learning appears to play a critical role in modeling human dietary preferences, given that the intake of foods is largely determined by their hedonic characteristics in industrialized societies [2,3]. Learning that increases the hedonic valence of a flavor (*flavor-taste* learning) can be distinguished from flavor-nutrient learning, which does not necessarily depend on the hedonic value of the associated taste [3]. A *flavor-taste* preference is established in the laboratory by associating a flavor with the sweet taste of saccharin. Given that saccharin has no nutritious properties, *flavor-taste* preference would be exclusively based on hedonic taste mechanisms and can therefore be used to analyze purely hedonic intake, regardless of the nutritional value of the ingested product. In fact, independent anatomical pathways have been described for the taste quality and nutritional actions of sugar [4] and for *flavor-nutrient* and *flavor-taste* learnings [5,6]. Study of the neuroanatomical and neurochemical bases of *flavor-taste* preference learning may help to identify and treat overeating, especially the over-consumption of tasty or sweet foods considered responsible for some types of obesity [7].

Various authors [8,9] have addressed the neuropharmacology of flavor preference learning, studying the participation of dopaminergic systems [10,11], opiates [12,13], and *N*-methyl-D-aspartate (NMDA) receptors [14,15,16]. Sclafani and coworkers highlighted the role of dopamine (DA), mainly via D1-like receptors, in the acquisition but not expression of sugar-conditioned flavor preferences (CFPs) [5,6,17]. They also verified, using a sham-feeding preparation or fructose as the unconditioned stimulus, that naltrexone has no effect on the acquisition or expression of flavor preference conditioning using a sweet taste, i.e.,

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on learning in which orosensory stimulation (sweet taste) prevails over post-intake information [12,13]. According to the above authors, the acquisition and/or expression of food preferences may require interaction between DA and other neurotransmission systems [9,18].

Orexin/hypocretin neuropeptides [19,20] have been related to numerous functions, especially those that mediate reward and motivated behaviors [21–26]. Orexinergic neurons of the lateral hypothalamus (LH) send excitatory projections to the VTA, which is part of the brain reward system; therefore, orexin may regulate emotional and motivational aspects of intake through interaction with the dopaminergic mesolimbic system [27,28]. Thus, it has been demonstrated that the self-administration of positive reinforcers, such as fatty and tasty foods (chocolate), selectively enhance the plasticity of NMDA receptors mediated by orexin A in the VTA, and that the motivation to obtain these tasty foods is reduced in rats treated with the orexin-1 receptor (OX_1R) antagonist SB-334867-A [29]. Orexin was reported to promote the overconsumption of sweet and pleasant tastes such as saccharin [30], while the systemic injection of orexin-1 receptor antagonist SB-334867-A was found to reduce saccharin intake 50 min later [31,32]. The orexinergic system also appears to participate in the establishment of associations between rewards (natural [e.g. food] or drugs of abuse) and relevant stimuli in conditioned place preference (CPP) [28,33]. Thus, a correlation was found between the degree of activation of LH orexinergic neurons and the learned preference for a place associated with food [27]. Orexin also appears to be important in other appetitive learnings, such as cue-food conditioning [34,35] or *flavor-taste* preferences. Specifically, previous studies in our laboratory demonstrated that administration of orexin-1 receptor antagonist SB-334867-A impedes the acquisition of saccharin-induced CFP but does not block LiCl-induced flavor aversion learning (FAL) [36,37]. According to the above data, orexin may be relevant in flavor learning that has positive but not negative post-intake consequences. Alternatively, orexin may play a role in flavor-taste learning that is based on the hedonic signals of stimuli rather than on orosensory-viscerosensory associations, in which post-intake nutritional consequences determine the learning of a food preference or aversion. With this background, the aim was to establish the role of orexin in flavor-taste learning induced by the association of a neutral flavor with orosensory stimuli that have affective value or palatability, determining whether orexin participates in the same manner in flavor learning based on innately preferred (sweet) tastes as in that based on innately avoided (bitter) tastes. The specific objective of the present study was to examine the effect of intra-VTA orexin administration on the acquisition of saccharin-induced flavor preference and quinine-induced flavor aversions.

2. Experiment 1

2.1. Methods

2.1.1. Animals

Thirty-four male Wistar rats weighing 230–250 g on arrival at the laboratory were provided by Charles River (France). They were randomly distributed into three experimental groups and individually housed in methacrylate cages (21.5 cm × 46.5 cm × 14.5 cm) that served as training chambers during the experiment. All rats were acclimated to their housing facility for a minimum of 5 days before undergoing surgery, and environmental enrichment was provided. The room was maintained at a constant temperature of 21 °C under a 12:12 light-dark cycle, with lights on at 07:30. Food and water were available ad libitum except when otherwise reported. All procedures were carried out in accordance with guidelines established by the European Union (2010/63/UE) and Spanish Royal Law 23/2013 and were approved by the Ethical Committee for Animal Research of the University of Granada. All possible efforts were made to minimize animal suffering and the number of animals used.

2.1.2. Surgery

Rats were deeply anesthetized with a mixture of ketamine (86 mg/kg) and xylazine (12.9 mg/kg), positioned in a stereotaxic device (Digital Lab Standard Stoelting. Wooddale, IL), and unilaterally implanted with a cannula (Plastic One, 26-gauge stainless-steel guide) in the VTA. Stereotaxic coordinates were determined from the rat brain atlas of Paxinos and Watson [38] as follows: AP: 4.92 mm posterior to the bregma, L: 0.9 mm from midline, V: 8.3 mm below the skull surface. The incisor bar was placed 3.3 mm below the interaural line. The guide cannula was secured to the skull with two screws and dental cement and closed with a dummy cannula. After the surgery, all animals received an intramuscular injection of penicillin (0.1 cc, Penilevel Level, Spain) and an analgesic (buprenorphine, 60 μg/kg, Sigma-Aldrich, Spain) followed by a recovery period of at least 10 days with food and water available ad libitum. During this period, the rats were handled daily, and the dummy cannula was also removed and replaced.

2.1.3. Microinjection procedure

Two doses (53 and 107 ng/0.3 μl) of orexin-A (Sigma-Aldrich, Spain) dissolved in physiologic saline were utilized. We based our selection of effective orexin doses (53 and 107 ng/0.3 μl) on the results of a specific study on the impact of orexin-A in the VTA on acquisitive tasks [39]. Orexin-A and saline (control group) were administered through a guide cannula using a 33G injection needle connected by polyethylene tubing to a 5.0-μL Hamilton micro-syringe driven by an infusion pump (KD Scientific Inc., Holliston, MA). The injection needle was inserted 1 mm beyond the tip of the guide cannula, and a total injection volume of 0.3 μl was infused over a period of 60 s. After each infusion, the injector remained in place for 60 s to allow diffusion of the solution into tissue and to minimize reflux along the injection track.

2.1.4. Experimental procedures

2.1.4.1. Flavor preference learning induced by saccharin. Animals were adapted to water deprivation schedule by offering them water for 15 min/day for three days, using two inverted graduated cylinders (20 ml, 1-ml gradation) with sipper spouts extending into the cage that were located centrally on its front. Liquid consumption and food intake were measured daily and bodyweight every two days throughout the experiment. There was a 30-min period of re-hydration in the afternoon. Food pellets were removed before the start of each drinking session, and all animals received 40 g of food at 30 min after its end. After this three-day adaptation period, animals underwent four one-bottle acquisition sessions (eight days) to develop CFP. Orexin-A or saline (control group) were unilaterally administered in the VTA at 10 min before a 15-min flavor intake period. The CS+ was 0.15% saccharin solution flavored with 0.05% (w/w) non-sweet cherry or grape flavor (Kool-Aid, General Foods, White Plains, NY), and the CS− was the same flavor diluted with water. The position of the tubes was counterbalanced by following a double alternation sequence (LRRL) to prevent a side preference. Preferences were tested on days 9 and 10 by means of two-bottle tests containing the CS+ and CS− flavors in counterbalanced left-right positions; the animals were drug-free in these choice tests, and both flavors were diluted in water without saccharin.

2.1.4.2. Flavor aversion learning (FAL) induced by quinine. The same animals used in the *flavor-taste* learning were used in the FAL procedure; however, because some of these animals lost their cannulas at some time in the procedure, the final sample comprised a control group of 10, low-dose group of 6, and high-dose group of 11 animals. Upon completion of the flavor preference tests, the rats were allowed a period of 1 week with food and water ad libitum before the restriction schedule was re-imposed. After two days of preliminary training when water was available for 15 min/day from two tubes, rats underwent one-bottle sessions to acquire a conditioned flavor aversion induced by quinine. Thus, *flavor-taste* aversion was established by