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Research report

Functional brain networks underlying latent inhibition of conditioned disgust in rats

Patricia Gasalla[a,b,*], Azucena Begega[a], Alberto Soto[a], Dominic Michael Dwyer[b], Matías López[a]

[a] Department of Psychology, University of Oviedo, Oviedo, Spain
[b] School of Psychology, Cardiff University, UK

HIGHLIGHTS

- Nonreinforced exposure attenuates the establishment of nausea-induced conditioned disgust.
- Decreased CO activity in the PBN, VTA, BLA, BNET, and PFC associated with taste-nausea parings.
- Exaggerated PBN metabolic activity decrease associated with latent inhibition in CTA.
- Novel activation patterns of brain networks involved in the processing of the hedonic value of flavors were described.
- Accumbens-amygdala interaction plays a role in processing taste hedonic value.

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ABSTRACT

The present experiment examined the neuronal networks involved in the latent inhibition of conditioned disgust by measuring brain oxidative metabolism. Rats were given nonreinforced intraoral (IO) exposure to saccharin (exposed groups) or water (non-exposed groups) followed by a conditioning trial in which the animals received an infusion of saccharin paired (or unpaired) with LiCl. On testing, taste reactivity responses displayed by the rats during the infusion of the saccharin were examined. Behavioral data showed that preexposure to saccharin attenuated the development of LiCl-induced conditioned disgust reactions, indicating that the effects of taste aversion on hedonic taste reactivity had been reduced. With respect to cumulative oxidative metabolic activity across the whole study period, the parabrachial nucleus was the only single region examined which showed differential activity between groups which received saccharin-LiCl pairings with and without prior non-reinforced saccharin exposure, suggesting a key role in the effects of latent inhibition of taste aversion learning. In addition, many functional connections between brain regions were revealed through correlational analysis of metabolic activity, in particular an accumbens-amygdala interaction that may be involved in both positive and negative hedonic responses.

1. Introduction

Nausea and vomiting resulting from chemotherapy treatments for cancer are reported as the most distressing side-effect that can cause patients to discontinue treatment [1,2]. In addition to the effects directly induced by the drug-induced nausea, patients often develop anticipatory nausea and vomiting to contextual stimuli related to the hospital environment, and effect that can be explained in terms of classical conditioning [3,4]. The cues that constitute the clinic (smell, sounds, and even the staff) can become associated with the nausea-inducing treatment and, as a consequence of the formation of such association, these stimuli may become able to evoke a response (the conditioned response, CR), that is, nausea and vomiting [5]. Theoretical analysis of the condi-

Abbreviations: ATP, adenosine triphosphate; BLA, amygdala, basolateral nucleus; CeA, amygdala, central nucleus; BNST, bed nucleus of stria terminalis; Cg, cingulate cortex; CS, conditioned stimulus; CO, cytochrome c oxidase; IL, infralimbic cortex; IC, insular cortex; IO, intraoral; LiCl, lithium chloride; mPFC, medial prefrontal cortex; NAcb, nucleus accumbens; PBN, parabrachial nucleus; PrL, prelimbic cortex; NaCl, sodium chloride; TR, taste reactivity; US, unconditioned stimulus; VTA, ventral tegmental area; VPL, ventralis posterolateral thalamic nucleus; VPM, ventralis posteromedial thalamic nucleus.

* Corresponding author at: School of Psychology, Cardiff University, Tower Building, Park place, Cardiff, CF10 3AT, UK.
E-mail address: gasallacantop@cardiff.ac.uk (P. Gasalla).

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tioning processes involved in the acquisition of context aversions in animals suggests possible behavioral strategies that might be used in the alleviation of anticipatory nausea and vomiting [6,7]. In particular, one effective way to attenuate anticipatory nausea is preventing the formation of context-nausea association by giving extensive prior exposure to the contextual cues, a conditioning phenomenon referred as the latent inhibition effect [8,9].

The initial goal of the current study was to examine the brain regions involved in latent inhibition of nausea-induced conditioned disgust. In the taste aversion paradigm, the latent inhibition effect is defined as a retardation of conditioning due to extensive prior exposure to the flavor that is to be paired with a drug with emetic properties (e.g., lithium chloride, LiCl). Therefore, the contribution of flavor novelty/familiarity in the development of conditioned disgust can be examined with this paradigm. Whilst the latent inhibition effect has been extensively evaluated using voluntary fluid ingestion, the current study aims to evaluate this effect by measuring conditioned disgust reactions elicited by a nausea-paired flavor. Flavors can be administered directly into the ratís mouth through a cannula implanted in the oral cavity. This procedure allows the orofacial reactions displayed by the rats during the intraoral infusion of the fluid to be recorded and analyzed, a method termed the taste reactivity (TR) test [10]. The TR method measures changes in the hedonic value of a flavor due to aversive visceral experience. In particular, rodents display conditioned gaping reactions (a disgust reaction selective to nausea) during re-exposure to the conditioned flavor. TR is a particularly suitable procedure for measuring conditioned taste aversion because it is selective to disgust, in contrast to decreases in the voluntary intake of the flavor which may simply reflect taste avoidance without a change in hedonic responses [11–13]. In recent studies of our laboratory using taste reactivity and licking analysis methods we found that flavor preexposure attenuates the effects of taste aversion learning on both flavor consumption and taste palatability [14]. Based upon these findings, in the present study we explored the neural networks involved in latent inhibition of conditioned disgust by measuring changes in metabolic activity through cytochrome c oxidative (CO) histochemistry in brain regions generally accepted as being involved in conditioned taste aversion. These brain regions may be divided into two systems, one related to the processing of taste qualities and the other to the reward system. The taste recognition system includes the nucleus of the solitary tract, parabrachial nucleus, the ventralis posteromedial thalamic nucleus, and the insular cortex. The reward system would include mainly the ventral tegmental area and the accumbens. Prefrontal cortex and amygdala are the candidates to be the interface regions between these two systems [15–17]. Despite the evidence that flavor familiarity influences neural activity in conditioned taste aversion as assessed by consumption tests, it is unclear what brain regions are activated in response to hedonic shifts in this learning paradigm. In addition to the fact that latent inhibition is of interest in its own right, the fact that pre-training exposure to the taste stimulus retards conditioning affords a direct comparison of the brain effects of identical taste-nausea parings between animals that form strong aversions (i.e. the non-exposed group) with those that will not form a strong aversion (i.e. the exposed group).

The neuronal underpinnings of conditioned taste aversion have been widely examined using c-fos immunocytochemistry [18–20]. However, the special functional properties of taste aversion learning may require alternative methods to fully explore the physiological bases of this learning. In contrast to other forms of learning, taste aversions are well known to form in a single trial and can be formed despite long-delays between the flavor and the illness [21,22]. Therefore, a method that assess long term changes in the brain activity would be particularly valuable. Due to the close relationship between the electrical activity of neurons and oxidative energy metabolism, an alternative way to study brain activity is by using histochemical techniques such as the mitochondrial enzyme cytochrome c oxidase (CO) [23,24]. Cytochrome c oxidase is a mitochondrial enzyme implicated in the oxidative phosphorylation process in which adenosine triphosphate (ATP) is generated. This histochemical technique identifies structures involved in long-term metabolic changes such as increased levels of enzymes or neurotransmitters, membrane protein synthesis, morphological changes (high postsynaptic density) and neuronal electrical increased activity, reflecting other process that also require ATP in the cell. CO histochemistry has been used in studies of learning and memory in a variety of animal species [25–29]. The use of CO histochemistry provides a range of advantages over the use of early gene expression as c-Fos immunohistochemistry, in particular c-Fos methodology indicates acute (seconds to minutes) transient changes in neural activation evoked by a stimulus as a result of a particular intervention and thus might not fully capture the processes involved conditioned taste aversion where conditioning can occur with long delays between flavor exposure and the induction of illness. In addition, CO histochemistry allows the assessment of cumulative changes throughout the learning process, which is critical in understanding the processes underpinning latent inhibition, where non-reinforced initial exposure can occur a matter of days before conditioning and testing. CO activity provides an accurate assessment of the metabolic history of brain regions because sustained increased or decreased in neuronal activity lead to corresponding changes in CO activity [30–32]. As such, it reflects the temporal dynamics of brain metabolism over the acquisition and retrieval of learning, not only in specific brain regions but also in terms of their functional connectivity [33].

Therefore, the goal of the present study was to examine, using CO histochemistry, changes in brain metabolic activity following latent inhibition of conditioned disgust. In particular, because CO activity is sensitive to manipulations between the day of measurement and up to a week before [27], this will reflect the impact of the whole behavioral preexposure, conditioning, and test procedure performed here. More specifically, we attempt to determine the functional connectivity between different brain regions involved in the development of nausea-induced conditioned disgust.

2. Materials and methods

2.1. Subjects

The subjects were 31 male Wistar rats weighing 190–339 g (258 g mean weight) at the start of the experiment. Upon arrival, they were individually housed in opaque plastic cages in a room maintained at 21 °C with a 12/12 h light-dark cycle. All experimental manipulations were performed during the light portion of the cycle. Food and water were available in the home cages throughout the experimental procedures. All experimental procedures were in accordance with guidelines for the care and use of laboratory animal of the Spanish (RD 12301/2005) and European (86/609/EEC) regulations concerning animal experimentation.

2.2. Cannulation surgery

In order to implant intraoral cannulas, the rats were anesthetized with ketamine (50 mg/kg) combined with medetomidine hydrochloride (0,15 mg/kg). A 3-cm square of fur was shaved on the back of the neck and the area was then wiped with an antiseptic product (Betadine). A thin-walled 15-gauge stainless steel needle was inserted at the back of the neck, routed underneath the skin, and brought out inside the mouth behind the first molar. The cannula was inserted through the needle, which was then removed.