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Anisomycin infusions in the parabrachial nucleus and taste neophobia

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ABSTRACT

To investigate whether *de novo* protein synthesis in the parabrachial nucleus (PBN) is required for recovery from taste neophobia, anisomycin (a protein synthesis inhibitor) was infused immediately after consumption of a novel saccharin solution (Experiment 1). Unexpectedly, this PBN treatment caused a reduction in saccharin intake. In addition, we found that the anisomycin-induced suppression of tastant intake was attenuated by prior intra-PBN infusions of lidocaine (Experiment 2). This pattern of results raises concerns about using anisomycin to investigate memory consolidation processes in the PBN. Thus, a different manipulation may be needed to examine the nature of the neuroplastic changes that occur in the PBN during taste memory formation.

1. Introduction

The reluctance to consume a novel, and therefore potentially dangerous, food is termed taste neophobia (Barnett, 1956; Barnett, 1958; Best & Barker, 1977; Domjan, 1977; Lin, Amodeo, Arthurs, & Reilly, 2012). If no aversive post-ingestive consequences ensue, this neophobic reaction habituates as indicated by increased consumption until asymptote is achieved. On the other hand, if consumption of the new food is followed by gastrointestinal illness, the food is avoided on subsequent encounters. That is, a conditioned taste aversion (CTA) develops, in which the taste of the food (i.e., conditioned stimulus; CS) is associated with the aversive consequences (i.e., unconditioned stimulus; US; Garcia & Ervin, 1968; for recent reviews see Reilly & Schachtman, 2009). In each case, a memory of the taste (and its post-oral effect) is formed and this knowledge modulates subsequent intake of that food.

According to contemporary views of memory formation, the memory is stabilized over time through consolidation, a process that strengthens the initially labile trace via *de novo* protein synthesis (Davis & Squire, 1984; McGaugh, 2000; Nader, 2003; but see Gold, 2008). Taste memory is no exception and, regardless of whether it is safe (i.e., recovery from neophobia) or unsafe (i.e., CTA), requires consolidation in order to be permanently stored in long-term memory. For example, intracerebroventricular infusions of cycloheximide (a protein synthesis inhibitor) administered prior to lithium chloride (LiCl; a nausea-inducing agent) intoxication abolished CTA acquisition (Houpt & Berlin, 1999; Serova, Solov'eva, Lagutina, & Obukhova, 1995). Similarly, formation of a safe taste memory requires *de novo* protein synthesis. In this case, intracranial infusion of anisomycin into the nucleus accumbens (Pedroza-Llinás, Ramírez-Lugo, Guzmán-Ramos, Zavala-Vega, & Bermúdez-Rattoni, 2009) or gustatory cortex (Rodriguez-Ortiz, De la Cruz, Gutiérrez, & Bermudez-Rattoni, 2005) after exposure to a novel tastant prevented the habituation of taste neophobia. That is, the anisomycin-treated rats showed no recovery from neophobia when they were presented with the same taste again (i.e., the rats behaved as if the taste were novel even though they had prior experience with it). This pattern of results supports the hypothesis that *de novo* protein synthesis is required for a taste memory to be consolidated and stored in long-term memory.

The parabrachial nucleus (PBN), located in the dorsolateral pons, is thought to have a critical role in CTA acquisition (see Reilly, 1999, for a review). Rats with PBN lesions fail to show any signs of aversion to a taste after repeated taste-illness pairings (Di Lorenzo, 1988; Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Reilly, Shimura, & Norgren, 1998; Ivanova & Bures, 1990; Reilly, Grigson, & Norgren, 1993; Spector, Norgren, & Grill, 1992; Yamamoto & Fujimoto, 1991). Given that gustatory and visceral information converge in the PBN (Herbert, Moga, & Saper, 1990; Norgren & Leonard, 1971; Norgren & Leonard, 1973), the prevailing interpretation of the CTA acquisition deficit found in rats with PBN lesions involves a disruption of associative learning processes. That is, the PBN is thought to be the critical site for the plastic changes that underlie memory of the taste-illness association. An alternative

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interpretation, derived from the finding that decerebrate rats are incapable of CTA acquisition (Grill & Norgren, 1978), is that the PBN merely functions as a conduit through which information is relayed to forebrain structures (as yet unidentified) where the taste memory is stored (for further discussion see Reilly, 2009).

A straightforward method to unravel the nature of the involvement of the PBN (associative memory storage or information relay) is to examine the influence of intra-PBN infusions of a protein synthesis inhibitor (e.g., anisomycin) on CTA acquisition. However, proper interpretation of the results of such an experiment requires that it first be established that the PBN has no involvement in the processing of taste information. To the best of our knowledge no study has examined the effects of protein synthesis inhibition in the PBN on taste processing. This concern prompted the current study in which we sought to determine whether anisomycin infused into the PBN after exposure to a novel tastant (0.15% saccharin) influences subsequent performance directed at that tastant. If neurons in the PBN have a role in consolidating taste memory, anisomycin infusions might be expected to prevent the habituation of taste neophobia. On the other hand, if such processing occurs outside the PBN then we expect that anisomycin infusions have little influence on the recovery from taste neophobia.

As revealed in Experiment 1, intra-PBN infusions of anisomycin did not attenuate recovery from neophobia. Rather, and completely unexpectedly, these infusions significantly reduced saccharin consumption. That is, anisomycin infused into the PBN appeared to induce a CTA to the saccharin tastant. There is, to our knowledge, no evidence suggesting that protein synthesis inhibition produces CTA-like effects. Therefore, we hypothesized that this taste suppression might be a consequence of an effect of anisomycin on synaptic transmission (i.e., a transient, extraordinary large spike in neurotransmitter release at the infusion site; Canal, Chang, & Gold, 2007; Qi & Gold, 2009). Given that lidocaine, a local anesthetic, can attenuate this anisomycin-induced increase in release of neurotransmitters without influencing the effects on protein synthesis inhibition (Sadowski, Canal, & Gold, 2011), in Experiment 2 we sought to determine whether lidocaine, infused into the PBN before intra-PBN anisomycin administration, would negate the anisomycin-induced taste suppression found in Experiment 1. Thus, we examined whether lidocaine influences anisomycin-induced taste suppression and this is best achieved by using a tastant that supports high intake on Trial 1. Therefore 0.1 M NaCl, a stimulus supporting little taste neophobia, was used so that any attenuating effect of lidocaine would not be compromised by a floor effect on intake of anisomycin-infused animals. The concentration of anisomycin (62.5 μg/0.5 μl) was the same as that employed by Sadowski et al. (2011) whereas lidocaine (5 μg/0.5 μl) was used at half the effective concentration from that study.

2. Material and methods

2.1. Animals

Twenty-nine naïve Sprague–Dawley rats (Charles River Laboratory, Wilmington, MA) served as the subjects. After arrival in the laboratory, the rats were housed individually in plastic cages (26.5 × 50 × 20 cm) in a temperature controlled (21 °C) room with 12–12 light–dark cycle (lights on at 7:30 am) and given *ad libitum* food and water until the experiment started, at which time water access was restricted to 30 min/day (see below for details). The University of Illinois at Chicago Animal Care Committee approved all procedures employed in the present research. At all times, rats were treated according to guidelines recommended by the American Psychological Association (1996) and the National and the Institutes of Health (1996).

2.2. Surgery

A total of 20 rats received bilateral cannula implantations prior to behavioral testing. The rats were first anesthetized with intraperitoneal sodium pentobarbital (~65 mg/kg) and then fixed in a stereotaxic instrument (David Kopf Instruments; Tujunga, CA) with blunt earbars. A midline incision was made to expose the skull and a trephine hole (diameter ~3 mm) was drilled above the PBN in each hemisphere. A stainless steel guide cannula (33 gauge; Plastics One, Roanoke, VA) was inserted with the tip positioned 2 mm above the center of each PBN at the following coordinates: AP: −10.9 mm, ML: ±2 mm, DV: −4.6 mm. The cannulas were implanted at a 20-degree angle to avoid damaging the transverse sinus. Throughout the surgical procedure, body temperature was monitored with a rectal thermometer and maintained at ~37 °C with a heating pad (Harvard Apparatus, Holliston, MA). The rats in the non-surgical control group (Group CON; $n = 9$) were given pentobarbital injections but no surgery. The rats were returned to their home cages after recovery from anesthesia.

2.3. Apparatus

All behavioral testing occurred in the home cages. Fluids were presented in calibrated bottles each fitted with a silicone stopper and a steel sipper tube. Fluid intake was monitored to the nearest 0.5 ml.

2.4. Experiment 1: Intra-PBN anisomycin and taste neophobia

After recovery from surgery, rats were placed on a deprivation schedule of 15-min water access in the morning followed 4 h later by a second 15-min period of water access. When water intake stabilized, the rats with cannulas were divided into two groups based on the infusions to be given. Group SAL ($n = 9$) served as a second control group and received intracranial infusions of physiological saline whereas Group ANI ($n = 11$) received intracranial infusions of anisomycin (62.5 μg/0.5 μl; Sigma–Aldrich Co., St. Louis, MO). The neophobia trials (given in lieu of morning water access) were conducted in a 3-day cycle with a taste trial day followed by two water days. Each taste trial involved 15-min access to 0.15% saccharin followed by bilateral intra-PBN infusions of saline vehicle (Group SAL), or anisomycin (Group ANI), or no infusions for Group CON ($n = 9$). All infusions were 0.5 μl/side administered at a rate of 0.3 μl/min.

2.5. Experiment 2: Effect of lidocaine on anisomycin-induced tastant suppression

After completion of Experiment 1, the rats continued on the same fluid deprivation schedule of 15-min water access each morning and afternoon. The procedure of Experiment 2 was identical to that of Experiment 1, except 0.1 M NaCl was used as the tastant and two intra-PBN infusions were administered, the first immediately following removal of the stimulus bottle, the second 10 min later. In this experiment, rats were divided into four groups based on the drugs to be infused. Although the composition of the non-surgical control group (Group CON; $n = 9$) remained as in Experiment 1, the animals with implanted cannulas (one rat was dropped due to a clogged cannula) were counterbalanced for prior experimental conditions and divided into three groups: Group LIDO/SAL ($n = 6$) received intracranial infusions of lidocaine HCl (5 μg/0.5 μl; APP Pharmaceuticals, Schaumburg, IL) followed by physiological saline; Group SAL/ANI ($n = 5$) received saline then anisomycin (62.5 μg/0.5 μl); and Group LIDO/ANI ($n = 8$) was infused with lidocaine and anisomycin. As in Experiment 1, all infusions were 0.5 μl/side at infusing rate of 0.3 μl/min.