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Safe taste memory consolidation is disrupted by a protein synthesis inhibitor in the nucleus accumbens shell

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ABSTRACT

Consolidation is the process by which a new memory is stabilized over time, and is dependent on *de novo* protein synthesis. A useful model for studying memory formation is gustatory memory, a type of memory in which a novel taste may become either safe by not being followed by negative consequences (attenuation of neophobia, AN), or aversive by being followed by post-digestive malaise (conditioned taste aversion, CTA). Here we evaluated the effects of the administration of a protein synthesis inhibitor in the nucleus accumbens (NAc) shell for either safe or aversive taste memory trace consolidation. To test the effects on CTA and AN of protein synthesis inhibition, anisomycin (100 μg/μl) was bilaterally infused into the NAc shell of Wistar rats' brains. We found that post-trial protein synthesis blockade impaired the long-term safe taste memory. However, protein synthesis inhibition failed to disrupt the long-term memory of CTA. In addition, we infused anisomycin in the NAc shell after the pre-exposure to saccharin in a latent inhibition of aversive taste. We found that the protein synthesis inhibition impaired the consolidation of safe taste memory, allowing the aversive taste memory to form and consolidate. Our results suggest that protein synthesis is required in the NAc shell for consolidation of safe but not aversive taste memories, supporting the notion that consolidation of taste memory is processed in several brain regions in parallel, and implying that inhibitory interactions between both taste memory traces do occur.

1. Introduction

Memory consolidation is a process by which a new memory is stabilized over time (after an initial learning experience). This process leads to the formation of a long-term memory. For the memory to be stored in the long-term, changes in gene expression and synthesis of new proteins are required as underlying mechanisms for this memory consolidation, although this view has been challenged in recent years (see Gold, 2008). Consequently, memory consolidation can be disrupted by the administration of protein synthesis inhibitors, such as anisomycin. The effects of protein synthesis disruption have been observed in a wide variety of memory types, both in vertebrate and invertebrate species (Alberini, Milekic, & Tronel, 2006; Davis & Squire, 1984).

Gustatory memory is an extensively used memory model in the research of the different stages of memory formation (Welzl, D'Adamo, & Lipp, 2001). From an evolutionary perspective, the gustatory memory is greatly relevant since it increases the probability of an animal's survival, by allowing the recognition from a previous experience of what is safe to eat and what is not (Bermúdez-Rattoni, 2004; Welzl, D'Adamo, & Lipp, 2001). When a new taste is consumed, it generates a neophobic response, consisting in a reduced intake of the novel food. If no negative consequences follow ingestion, i.e. gastric malaise, the taste will be recognized as safe, leading to a gradual increase of its consumption in successive presentations (attenuation of neophobia, AN). However, if the ingestion of a novel food is followed by post-digestive malaise, the taste will later be recognized as aversive, leading to rejection of that taste in the future (conditioned taste aversion, CTA). Both safe and aversive taste memories depend on a neural representation of the taste that is probably stored in parallel in several brain regions; this neural representation has been called the taste memory trace (TMT) (Bermúdez-Rattoni, 2004).

One feature that makes gustatory memory a valuable memory model is the knowledge of the neural circuits involved in its formation (for review, see Bermúdez-Rattoni, 2004; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994). In this regard, previous studies have demonstrated that the gustatory insular cortex is a structure that plays an important role in taste memory formation (Yamamoto et al., 1994), mainly through glutamatergic (Ferreira, Gutiérrez, De La Cruz, & Bermúdez-Rattoni, 2002) and cholinergic (Gutiérrez, Rodríguez-Ortiz, De La Cruz, Núñez-Jaramillo, & Bermúdez-Rattoni, 2003) activity. Moreover, it has also been demonstrated that consolidation of both aversive (Rosenblum, Meiri, & Dudai, 1993) and safe (Rodríguez-Ortiz, De La Cruz, Gutiérrez, & Bermúdez-Rattoni, 2005) taste memory depends on *de novo* protein synthesis in the gustatory cortex.

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Similarly, the blockade of protein synthesis in the amygdala, another structure strongly implicated in taste memory formation (Bermúdez-Rattoni, 2004), disrupts aversive taste memory consolidation (De La Cruz, Rodríguez-Ortiz, Balderas, & Bermúdez-Rattoni, 2008). Together, these data suggest that consolidation of the TMT may depend on *de novo* protein synthesis in various structures in parallel.

One of the less investigated structures recently linked to gustatory memory is the nucleus accumbens (NAc). The NAc is a structure located in the basal forebrain, and is a major component of the ventral striatum (Zahm, 2000). The NAc is divided into two subregions: the motor NAc core and the limbic NAc shell (Zahm, 2000). The NAc has been implicated in reward and food ingestion (Kelley, 2004), locomotor activity (Swanson, Heath, Stratford, & Kelley, 1997; Zahm, 2000) and addictions (Di Chiara & Bassareo, 2007; Hyman, Malenka, & Nestler, 2006; Kelley, 2004; Wolf, 2002), and it is considered to be an interface between motivation and goal-directed behavior (Hyman et al., 2006; Wolf, 2002). There is evidence on the participation of both subregions of the NAc in learning and memory (Setlow, 1997), and specifically, its role in aversive learning such as cued (Haralambous & Westbrook, 1999; Levita, Dalley, & Robbins, 2002; Parkinson, Robbins, & Everitt, 1999; Schwienbacher, Fendt, Richardson, & Schnitzler, 2004) and contextual (Jongen-Rêlo, Kaufmann, & Feldon, 2003; Riedel, Harrington, Hall, & Macphail, 1997) fear conditioning has been documented. Importantly, there is growing evidence (Ramírez-Lugo, Núñez-Jaramillo, & Bermúdez-Rattoni, 2007) that the NAc is involved in aversive TMT formation, particularly the shell subregion through cholinergic, glutamatergic (Ramírez-Lugo, Zavala-Vega, & Bermúdez-Rattoni, 2006) and dopaminergic (Fenu, Bassareo, & Di Chiara, 2001) activity. Moreover, it has been demonstrated also that safe TMT formation depends on cholinergic activity in the NAc shell (Ramírez-Lugo et al., 2006). It has been shown previously that memory consolidation of an instrumental task (Hernandez, Sadeghian, & Kelley, 2002) and of a maternal memory (Li & Fleming, 2003) requires *de novo* protein synthesis in the NAc, but to date it is unknown whether protein synthesis in the NAc is necessary for TMT consolidation.

The aim of this study was to determine if *de novo* protein synthesis in the NAc shell is required for TMT consolidation. In order to evaluate if the protein synthesis blockade in the NAc shell could impair safe TMT consolidation, anisomycin infusions were performed in the AN paradigm. The possible necessity of protein synthesis in the NAc shell for aversive TMT consolidation was explored also by infusing anisomycin in the CTA paradigm; short-term memory (STM) and long-term memory (LTM) tests were conducted. It has been proposed previously (Gutiérrez et al., 2003) that the consolidation of safe TMT gradually inhibits the formation of aversive TMT. Thus, the likelihood of aversive TMT formation will be lesser as more time has passed between the first contact with that taste and the possible onset of gastric malaise (Gutiérrez et al., 2003). To assess if the blockade of safe TMT consolidation could allow the formation of aversive TMT in the NAc shell, a third experiment using a latent inhibition paradigm in the CTA was conducted. Latent inhibition (LI) is a behavioral phenomenon characterized by a retardation in conditioning to a stimulus as a result of a previous non-conditioned exposure to that stimulus, so that more associations between conditioned and unconditioned stimulus are required to achieve conditioning (Lubow & De La Casa, 2005). The LI has been reported also to occur for CTA (Revusky & Bedarf, 1967). In a third experiment anisomycin or vehicle was infused into the NAc shell immediately after the pre-exposure to saccharin, and the next day the animals underwent a CTA protocol, using the same gustatory stimulus. STM and LTM tests were conducted.

2. Materials and methods

2.1. Subjects

Male Wistar rats, weighing 270–310 g at the time of surgery, were used. They were housed in individual plastic cages and maintained on a 12/12 h light/dark cycle; all manipulations and behavioral procedures were done during the light phase. Food and water were provided *ad libitum* except when noted in behavioral procedures. Experiments were performed in accordance with the Rules in Health Matters (Ministry of Health, Mexico) and with approval of the local Animal Care Committee.

2.2. Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The animals were implanted bilaterally with 12 mm, 23-gauge stainless steel guide cannulae, aimed 2.5 mm above the NAc shell (AP + 2 mm, lateral ± 1 mm, ventral −5.3 mm, from Bregma; Paxinos & Watson, 1986). The cannulae were fixed to the skull with dental acrylic cement, anchored with two surgical screws, and blocked with stylets to prevent clogging. Following surgery, animals were allowed to recover for 4 days, with food and water *ad libitum*.

2.3. Drug administration

Artificial cerebrospinal fluid (ACSF, NaCl 125 mM, KCl 5 mM, $NaH_2PO_4H_2O$ 1.25 mM, $MgSO_47H_2O$ 1.5 mM, $NaHCO_3$ 26 mM, $CaCl_2$ 2.5 mM, glucose 10 mM; De La Cruz et al., 2008; Rodríguez-Ortiz et al., 2005; Rosenblum et al., 1993) was used as vehicle. Anisomycin (Sigma) was dissolved in equimolar concentration of HCl, and brought to a final concentration of 100 μg/μl in vehicle (Rodríguez-Ortiz et al., 2005; Rosenblum et al., 1993). It has been reported before that this dose of anisomycin causes >90% of protein synthesis inhibition in the insular cortex (Rosenblum et al., 1993).

Intra-accumbens microinjections were given to hand-restrained, conscious animals. Stylets were withdrawn from the guide cannulae, and 30-gauge injection needles were inserted, extending 2.5 mm from the tips of the guide cannulae. Injections needles were connected via polyethylene tubing to two 10 μl Hamilton syringes, driven by an automated microinfusion pump (Carnegie Medicine, Stockholm, Sweden). A total volume of 1 μl per hemisphere was delivered in the NAc shell, at a rate of 0.5 μl/min. After microinfusions were completed, the injection needles were left placed in the guide cannulae for an additional minute to allow diffusion of the solutions into the tissue. The total volume and infusion rate used have been reported before (Ramírez-Lugo et al., 2006; Sederholm, Johnson, Brodin, & Södersten, 2002).

2.4. Behavioral procedures

2.4.1. Experiment 1. Neophobia and attenuation of neophobia

After the 4-day recovery period from surgery, rats were deprived from water for 24 h. The following 4 days after water deprivation, rats had access each day to 40 ml of tap water for 15 min. This water intake was measured, and its mean was considered as baseline.

Experimental behavioral protocol is briefly schematized in Fig. 2A. On day 5 rats had access for 15 min to 40 ml of a novel taste (saccharin 0.5%); the saccharin intake was registered and considered as the neophobic response. The next day animals had access for 15 min to 40 ml of the same concentration of saccharin, and the volume ingested was measured in order to evaluate the atten-