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The consolidation of inhibitory avoidance memory in mice depends on the intensity of the aversive stimulus: The involvement of the amygdala, dorsal hippocampus and medial prefrontal cortex

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ABSTRACT

Several studies using inhibitory avoidance models have demonstrated the importance of limbic structures, such as the amygdala, dorsal hippocampus and medial prefrontal cortex, in the consolidation of emotional memory. However, we aimed to investigate the role of the amygdala (AMG), dorsal hippocampus (DH) and medial prefrontal cortex (mPFC) of mice in the consolidation of step-down inhibitory avoidance and whether this avoidance would be conditioned relative to the intensity of the aversive stimulus. To test this, we bilaterally infused anisomycin (ANI-40 μg/μl, a protein synthesis inhibitor) into one of these three brain areas in mice. These mice were then exposed to one of two different intensities (moderate: 0.5 mA or intense: 1.5 mA) in a step-down inhibitory avoidance task. We found that consolidation of both of the aversive experiences was mPFC dependent, while the AMG and DH were only required for the consolidation of the intense experience. We suggest that in moderately aversive situations, which do not represent a severe physical risk to the individual, the consolidation of aversive experiences does not depend on protein synthesis in the AMG or the DH, but only the mPFC. However, for intense aversive stimuli all three of these limbic structures are essential for the consolidation of the experience.

1. Introduction

Memory can be inferred through changes in an animal's behavior sometime after a learning task and involves different phases including acquisition, consolidation and retrieval (Abel & Lattal, 2001). Among the different types of memory, such as working and spatial, emotional memory is related to situations with a significant affective character and in which fear/anxiety is felt. The process of memory formation is accompanied by protein synthesis. It has already been demonstrated in various different studies that the formation of long-term memories involving fear can be impaired by inhibiting protein synthesis around the time of learning or shortly afterwards (Bourtchouladze et al., 1998; Johansen, Cain, Ostroff, & LeDoux, 2011; McGaugh, 2000; Rosenblum, Meiri, & Dudai, 1993). Thus, during memory consolidation protein synthesis is required to transform newly learned information, acquired during the acquisition phase, into stable behavioral modifications (Nakayama, Yamasaki, Matsuki, & Nomura, 2013).

Several studies have made use of amnesic tools, such as sodium channel voltage dependent blockers (lidocaine and tetrodotoxin) (Choudhary, Yotsu-Yamashita, Shang, Yasumoto, & Dudley, 2003; Noack, Murau, & Engelmann, 2015; Quiroz et al., 2003; Tehovnik & Sommer, 1997) and protein synthesis inhibitors such as cycloheximide (Diaz-Trujillo et al., 2009) and anisomycin (ANI), in rodents subjected to various behavioral paradigms (Bekinschtein et al., 2007; Bourtchouladze et al., 1998; Cai, Pearce, Chen, & Glanzman, 2012; Canal, Chang, & Gold, 2007; Lattal & Abel, 2004; Nader, Schafe, & Le Doux, 2000; Naghdi, Majlessi, & Bozorgmehr, 2003; Nakayama et al., 2013; Rosenblum et al., 1993). Anisomycin is isolated from the bacterium *Streptomyces griseolus* and binds to the large subunit of eukaryotic ribosomes (Barbacid & Vazquez, 1974), thereby blocking peptidyl transferase activity and acting as a protein synthesis inhibitor. As a result, ANI

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can induce amnesia, as protein synthesis is necessary for long-term memory consolidation (Wanisch & Wotjak, 2008).

ANI has been widely used in different fear-motivated learning tasks, such as one-trial inhibitory avoidance tasks (Canal & Gold, 2007; Canal et al., 2007; Monleon Verdu, Arenas Fenollar, Vinader Caerols, Ferrer Ano, & Parra Guerrero, 2008; Qi & Gold, 2009) and one-trial step-down inhibitory avoidance tasks (Cammarota et al., 2004; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001). In one-trial step-down inhibitory avoidance tasks, rodents associate an elevated platform in a given context with a shock to the foot (US) when they step down from that platform (Gold, 1986). The conditioned response (CR) is to refrain from stepping down. If animals are tested without a shock, that response is then extinguished (Vianna et al., 2001). Paradigms similar to this have been used to investigate the interaction between protein synthesis inhibitors and the intensity of the US on memory consolidation. Some authors found that consolidation impairment induced by protein synthesis inhibitors only occurs with moderate, not high, foot-shocks (Diaz-Trujillo et al., 2009; Gonzalez-Salinas et al., 2015), while Gold and Wrenn (2012) reported an amnestic effect only in the case of high foot-shock intensity. In addition, there is no data in the literature about which brain regions are involved in the modulation of these different responses.

The involvement of limbic structures in the different phases of emotional memory has been reported in several studies (Canal & Gold, 2007; Fanselow, 1994; LeDoux, 2000; McGaugh, 2004; Taubenfeld, Wiig, Bear, & Alberini, 1999; Zhang, Fukushima, & Kida, 2011). Decades of research has shown that the rodent amygdala is an important site for the acquisition and storage of aversive memories (Ehrlich et al., 2009; Fanselow, 1994; LeDoux, 2000, 2012; McGaugh, 2004; Wilensky, Schafe, Kristensen, & LeDoux, 2006). Furthermore, the amygdala is able to modulate memories of emotional experiences in other brain areas, such as the cortex and the hippocampus (Kim, Lee, Han, & Packard, 2001; McGaugh, 2004; Pare, 2003; Roozendaal & McGaugh, 1997; Roozendaal, Nguyen, Power, & McGaugh, 1999).

The realization that the hippocampus is an important brain structure in processing memory is not a recent one (Turner, 1969). There is ample evidence that the hippocampus participates in processing memories, evaluated using behavioral tests containing spatial components, such as the Morris water maze (Dunbar, Rylett, Schmidt, Sinclair, & Williams, 1993) and the radial maze (Nelson, Bawa, & Finger, 1992), object recognition tasks (Soule et al., 2008), and also contextual fear conditioning, such as inhibitory avoidance (Kim, Rison, & Fanselow, 1993; Logue, Paylor, & Wehner, 1997; Matus-Amat, Higgins, Barrientos, & Rudy, 2004; Phillips & LeDoux, 1992; Taubenfeld et al., 1999). Although the hippocampus is related to the acquisition and retrieval of memories (Abel & Lattal, 2001; Fanselow & Dong, 2010), special attention has been given to the dorsal hippocampus when it comes to the consolidation stage of memory. Studies have shown that blocking protein synthesis in this brain area during inhibitory avoidance training can impair memory consolidation (Canal & Gold, 2007; Taubenfeld et al., 1999).

Another brain area that has been demonstrated to have an important role in the regulation or modulation of aversive memories is the medial prefrontal cortex (mPFC) (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Quirk, Garcia, & Gonzalez-Lima, 2006; Santini, Ge, Ren, Pena de Ortiz, & Quirk, 2004; Zhang et al., 2011; Zhao et al., 2005). For example, Corcoran and Quirk (2007) demonstrated that inactivation of the mPFC impairs the recall of fear memories learned the previous day. Further, the formation of inhibitory avoidance memories requires the expression of a gene in the mPFC, suggesting the mPFC is essential in the formation of this type of aversive memory (Zhang et al., 2011). Other studies have emphasized the role of mPFC in the consolidation of memories using diverse tasks, for example the odor–reward association (Tronel & Sara, 2003). In summary, there is evidence that the mPFC plays a critical role in various stages of memory formation over a broad range of tasks (review Euston, Gruber, & McNaughton, 2012).

Given that the synthesis of new proteins is necessary for the consolidation of memories and that the combination of protein synthesis inhibitors and different intensities of unconditioned stimulus have generated different behavioral responses regarding the consolidation of emotional memories, we hypothesis that the protein synthesis-dependent plasticity mechanisms aiding the consolidation of memories will vary with brain region and intensity of US. To address this, we investigated the role of the amygdala, dorsal hippocampus and medial prefrontal cortex of mice in the consolidation of step-down inhibitory avoidance under different intensity of US. This was done by bilaterally infusing anisomycin into these brain areas prior to training the mice in the one-trial step-down inhibitory avoidance task.

2. Material and methods

2.1. Animals

Male Swiss mice (Federal University of São Carlos, UFSCar, SP, Brazil), each weighing 27–37 g and aged 7–9 weeks at the time of testing, were housed in polypropylene cages (31 × 20 × 13 cm) in groups of five and maintained under a 12 h light cycle (lights on at 7:00 a.m.) in a controlled environment at 23 ± 1 °C. Food and drinking water were provided ad libitum, except during the brief test periods. All mice were experimentally naïve and the experimental sessions were conducted during the light period of the light cycle (11:00–15:00 h).

2.2. Drug

Anisomycin (Sigma–Aldrich, St. Louis, MO, USA) was diluted in a sterile 0.9% saline solution and then dissolved in 1 M HCl. The pH was adjusted back to 7 using 1 M NaOH (ANI, 40 μg/μl). The vehicle solution consisted of equal amounts of HCl and NaOH as the anisomycin solution and was used as the experimental control. The substances were coded and the coding was unknown to the experimenter at the time of testing and behavioral analysis. The dose of anisomycin used was based on the work of Wanisch and Wotjak (2008), who demonstrated that this dose of anisomycin was appropriate for influencing the contribution of protein synthesis to the temporal and regional aspects of memory consolidation. The authors also demonstrated that the effects of the ANI reversed 9 h after the injection.

2.3. Experimental procedure

2.3.1. Stereotaxic surgery and microinjections

Mice were anesthetized using an i.p. injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and treated, intramuscularly, with flunixin meglumine (Banamine: 2.5 mg/kg) before being placed in a Stoelting stereotaxic instrument (Stoelting Co, Illinois, USA). The stereotaxic coordinates for injection into the amygdala (AMG, −1.4 mm anteroposterior to the bregma (AP), ±2.7 mm lateral to the midline (L), −2.0 mm ventral to skull surface (V)), dorsal hippocampus (DH, AP −1.8, L ±1.6, V −1.5) and medial prefrontal cortex (mPFC, AP +1.7, L ±0.3, V −2.0) were taken from Paxinos and Franklin (Paxinos & Franklin, 2004). The stainless steel guide cannula (25-gauge) was implanted bilaterally in the right and left sides of the limbic structures, 1 mm above the injection site. It was then fixed to the skull with acrylic dental cement.

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