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Inactivation of the basolateral amygdala impairs the retrieval of recent and remote taste-potentiated odor aversion memory

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ABSTRACT

Memory reorganization as a time-dependent process can be investigated using various learning tasks such as the taste-potentiated odor aversion (TPOA). In this paradigm rats acquire a strong aversion to an olfactory cue presented simultaneously with a gustatory cue. Together these cues are paired with a delayed visceral illness. The basolateral amygdaloid nucleus (BLA) plays a key role in TPOA acquisition but its involvement in retrieval remains unclear. We investigated the involvement of the BLA in either recent or remote retrieval of TPOA. In each case, the number of licks observed in response to the presentation of either the odor or the taste was used to assess retrieval. Before the retrieval test, rats received a bilateral infusion of lidocaine to inactivate the BLA. We observed that both recent and remote TPOA retrieval tests induced by the odor presentation were disrupted in the lidocaine-injected rats. By contrast, the BLA inactivation had no effect upon the aversion towards the taste cue regardless of the time of retrieval. The present study provides evidence that BLA functioning is necessary for retrieval of aversive odor memory, even with a long post-acquisition delay.

1. Introduction

A wide range of evidence indicates that new memories need to be stabilized for permanent storage (Dudai, 2004; Sara & Hars, 2006). Synaptic consolidation involves the stabilization of changes in neuronal connectivity within localized networks. By contrast, the system consolidation is a more prolonged process which involves a gradual reorganization of the brain regions supporting memory. Once information has been stored in memory, it must be retrieved in order to be used to guide adaptive behavior. It has been shown that retrieval of recent and remote memory could lead to different patterns of brain activation (Frankland & Bontempi, 2005). For example, using a spatial learning paradigm, Bontempi, Laurent-Demir, Destrade, and Jaffard (1999) observed high hippocampal activity in mice subjected to a retrieval task on the 5th day after task acquisition whereas retrieval occurring 25 days after acquisition mainly activated the frontal region. A dynamic reorganization in the cerebral location of memories according to their age (i.e., the delay between acquisition and retrieval) was also observed for contextual fear memory (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004) and social transmission of food preference (Ross & Eichenbaum, 2006). Ding, Teixeira, and Frankland (2008) investigated the brain circuitry underlying retrieval of a conditioned taste aversion (CTA) memory, a kind of learned food aversion which is independent of hippocampal function. By showing a differential cortical activation according to either recent or remote CTA retrieval, these authors suggest that the reorganization of the neuronal substrates over time might not be limited solely to hippocampus-dependent learning tasks. Hence, it may be a general process which occurs regardless of memory content.

We addressed this issue in the present study by using a taste-potentiated odor aversion (TPOA) paradigm in the rat. Olfactory and gustatory cues are well-known to play a major role in food selection through their involvement in the acquisition of learned aversions (Bernstein, 1991). In contrast to CTA, conditioned odor aversion (COA), the association of a distal odor cue with delayed-malaise is difficult to establish. However, when an odor is presented together with the saccharin, poisoned rats can acquire an aversion toward the odor which is as strong as that shown toward the taste (Rusiniak, Hankins, Garcia, & Brett, 1979). This kind of conditioning has been named taste-potentiated odor aversion (TPOA) (Palmerino, Rusiniak, & Garcia, 1980). Due to its robustness, TPOA is a valuable behavioral paradigm for studying the retrieval of memory at recent and remote time points after acquisition. Various experiments using lesions, pharmacological injections, or mapping of immediate early-genes have examined the basic brain circuit involved in TPOA (Dardou, Datiche, & Cattarelli, 2007; Ferry, Sandner, & DiScala, 1995; Kiefer, Rusiniak, & Garcia, 1982; Lasiter,

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Deems, & Garcia, 1985) implicating, among other areas, the basolateral nucleus of the amygdala (BLA). The amygdala is well-known for its essential role in the processing of emotionally relevant experiences (LeDoux, 2000; McGaugh, 2004; Shionoya et al., 2006). In aversion paradigms, the rejection of the conditioned cue is associated with a negative emotional state. Due to its olfactory, gustatory and visceral afferents (McDonald, 1998), the amygdala could participate in the association of the conditioned cues with delayed-illness. Electrolytic (Bermudez-Rattoni, Grijalva, Kiefer, & Garcia, 1986) or excitotoxic (Hatfield, Graham, & Gallagher, 1992) lesion of BLA or the microinjection of the GABA-A agonist muscimol (Ferry et al., 1995) into the BLA have been shown to impair TPOA. Thus, the BLA appears to be critically involved in TPOA acquisition. However, its role in TPOA retrieval has yet to be completely elucidated. BLA injections of muscimol performed before either odor or taste tests did not impair TPOA retrieval (Ferry et al., 1995). However, immunocytochemical detection of *c-fos*, an indicator of brain activation, suggests that the BLA might be involved in TPOA retrieval (Dardou et al., 2007).

The present study aims to investigate the contribution of the BLA to TPOA retrieval. Since brain networks sustaining memories might be reorganized as time elapses from acquisition, we examined the role of the BLA when rats were submitted to either a recent or a remote retrieval test. In both conditions, the number of licks observed in response to the presentation of either the odor or the taste was used to assess retrieval. Before the retrieval, the rats received bilateral infusions of lidocaine, a sodium channel blocker to transiently inactivate the BLA. Analysis of behavior in the lidocaine-injected rats compared to the vehicle-injected controls allowed us to determine the effect of BLA inactivation upon TPOA memory according to the time of retrieval (recent or remote) and to the kind of conditioned cue (odor or taste).

2. Materials and methods

2.1. Subjects

The subjects were 66 male Wistar rats (CERJ, France) weighing between 220 and 250 g at the beginning of the experiment. The animals were housed in individual cages and kept in a holding room at 21 °C under a 12 h light/dark schedule (6:00 a.m. lights on). Food and water were supplied *ad libitum* in the home-cage. Rats were given 7 days to acclimate to colony conditions before the beginning of the experimentation. During the acclimation phase, the rats were handled daily by the experimenter for 5 min. All animals and experiments were conducted in accordance with protocols approved by the European Communities council directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize the use of animals.

2.2. Surgery

Rats (310–370 g at the time of surgery) were anesthetized for surgery with an i.p. injection of Ketamine (Merial, 5 g/100 ml), 2.3 ml/kg and Xylazine (Bayer, 2 g/100 ml), 0.46 ml/kg. Rats were placed in a stereotaxic frame, in a flat skull position with the incisor bar set at −3.5 to −4.0 mm. The stereotaxic coordinates of the BLA were taken from the atlas of Paxinos and Watson (1986). Bilateral burr holes were drilled 2.8 mm posterior and 4.8 mm lateral to bregma for the BLA. Rats were implanted with two 11 mm 22 gauge stainless guide cannulae to a depth of 6.8 mm from the skull. The cannulae were fixed with dental acrylic cement and secured by three skull screws. Stylets were inserted into the guide cannulae to prevent clogging.

The rats designed for recent TPOA retrieval were submitted to surgery after the acclimation phase. They recovered for 4 days in their home-cages with food and water *ad libitum* before beginning the TPOA conditioning procedure.

The rats designed for remote TPOA retrieval underwent the surgery on the 8th day after TPOA acquisition in order to reduce the total duration of the experiment. Thus, the post-surgery recovery period of 4 days was included in the resting time between the TPOA acquisition and its remote testing. Preliminary experiments carried out in the laboratory (unpublished data) allowed us to check that performing surgery after TPOA acquisition had no effect on TPOA retention. Moreover, the vehicle-injected rats used in the present study allowed to ensure that the long-term retention of TPOA memory was not impaired.

2.3. Microinjection procedure

Lidocaine hydrochloride (4% in 0.1 M phosphate buffer saline, Sigma) was injected in the BLA 20 min before the retrieval test. It was infused over 2 min (flow rate of 0.35 μl/min). A total volume of 0.7 μl per hemisphere was delivered. After infusion, the needles were retained in the guide cannulae for an additional 2 min before withdrawal to allow diffusion of the solution into the tissue and to minimize dragging of the liquid along the injection track, and then withdrawn. As controls, some rats received bilateral vehicle injections in the BLA. Flow rate, volume, and time were similar to those employed for the lidocaine injections. During the microinjection time, rats were gently handled by the experimenter. However, some of the rats had unexpected movements and the solution was not correctly injected on both sides of the brain. In that case, we considered that the injection was not successful and these rats were excluded from further analysis.

2.4. Behavioral apparatus

TPOA conditioning and testing were performed in five automated lickometers designed in the laboratory. As previously described by Dardou et al. (2007), it consisted of plexiglas cylindrical chambers (diameter 24 cm, high 40 cm, made of clear plastic) allowing observation of the rat behavior during the session within a sound and smell resistant room. A drinking tube with a glass spout passing through an oval hole in the plexiglas wall provided access to water. The cylinder was mounted on a platform with a stainless steel grid floor (below which was placed a litter tray). A metal wire inside the tube and the steel floor were connected to an electronic device driven by a computer which recorded the number of licks via a Labview interface (International Instruments). On average, one lick delivered 3.1 μl of liquid to the rat. On each glass tube, an odorized filter paper was fixed at 1.5 cm from the aperture of the drinking tube. In view of analysis of the rat behavior, all the sessions in the lickometer chambers were recorded by a video-camera.

2.5. Behavioral procedures

2.5.1. TPOA conditioning

First, the rats underwent a 7-days habituation phase during which they had water for 10 min per day in the lickometer chambers. They also received some additional water from a bottle in the home-cage for 5 min between 11:30 am and 12:00 pm each day. Rats were daily weighed to verify their adaptation to the water-deprivation. On the 8th day, the rats were submitted to the TPOA conditioning which consisted of simultaneous presentation of an odorized filter paper with 2 μl of benzaldehyde (Sigma) and of 0.1% sodium saccharin (Aldrich) solution for 10 min in the chamber. Then, the rats were returned to their home-cages. Thirty min-