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Research report

Expression of a conditioned place preference or spatial navigation task following muscimol-induced inactivations of the amygdala or dorsal hippocampus: A double dissociation in the retrograde direction

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ABSTRACT

Previous work indicates an essential role of the basolateral amygdala in stimulus-reward learning and the dorsal hippocampus in spatial learning and memory. The goal of the present, experiments was to examine the involvement of the amygdala and hippocampus in performance of tasks requiring stimulus-reward and spatial/relational learning and memory processes in the retrograde direction. Accordingly, this series of experiments tested the effects of temporary, inactivations directed at the basolateral nucleus of the amygdala or dorsal hippocampus on the, expression of a conditioned place preference (CPP) task or a spatial navigation water task. The results, of Experiments 1a and b showed that inactivations of the amygdala impaired the expression of a, previously acquired CPP but did not impair the expression of a learned spatial response required for, accurate performance of a spatial navigation task. The results of Experiments 2a and b showed that, inactivations of the dorsal hippocampus impaired expression of a learned response required for the, accurate performance of a spatial navigation task but did not impair the learned response required for, the expression of a CPP. Taken together, the results showed a functional dissociation between the, effects of amygdala or hippocampal dysfunction on the expression of these different classes of tasks.

1. Introduction

Accumulating research suggests that the amygdala is an essential component of a neural circuit mediating emotional learning and memory processes in the mammal [1,3,4,6,8,16,13,14,32,33]. One dominant idea is that the amygdala is essential for a particular type of classical conditioning in which, through experience, an animal links positive or negative events and the concomitant unconditioned responses, with neutral stimuli that predict them. The amygdala is thought to enable the relevant Pavlovian associative processes via anatomical and functional relationships with cortical and subcortical sites that provide online access to sensory information defining the external environment, and with hypothalamic, brainstem, and ventral striatal sites [19,34]. These latter brain sites can impact heart rate, respiration, hormone release, and neurotransmitter release like dopamine that occur during negative and positive experiences. These features of amygdala anatomy provide this neural system with a convergence of information which could support associations between sensory information in the external environment and negative and positive events.

One interesting issue that has emerged in the literature concerning the functional role of the amygdala in emotional learning and memory processes is the possibility that the amygdala is necessary for the acquisition of certain forms of classical conditioning but not the expression of these associations [15,26]. The results suggest that the amygdala is only involved in acquisition/encoding processes for these types of associations but it does not store these associations nor is it later needed for the expression of these memories in behaviour. These demonstrations have important implications for our understanding of the function of this brain structure.

The current experiments assess the generality of the claim that the amygdala is not necessary for the expression of appetitive classical conditioning by testing rats with amygdala inactivations during expression of the conditioned place preference (CPP) task. The CPP task was selected because of previous demonstrations

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that rats with basolateral amygdala lesions are impaired at the acquisition of this task. This deficit is thought to occur because normal performance requires stimulus-reward associations [16,2]. The effect of amygdala inactivations was also assessed on the spatial version of the water task, a task not dependent on the amygdala for acquisition [27]. We also assessed the effects of temporary inactivations of the dorsal hippocampus on the expression of the CPP and the spatial version of the water task. Previous work has shown that rats with damage to the dorsal hippocampus are impaired on the acquisition of various types of spatial memory tasks but not on tasks requiring stimulus-reward associations for normal acquisition [21,27]. Interestingly, recent results suggest that post-learning hippocampal damage may disrupt the expression of memories in tasks that are unaffected by damage made prior to learning [7].

2. Materials and methods

2.1. Animals

2.1.1. Subjects and handling

61 Long–Evans male rats were used for the experiments. Animals were paired-housed in clear Plexiglas hanging cages with beta-cob bedding. The animal room had an ambient temperature of 21 °C, 35% relative humidity, and was maintained on a 12:12 light–dark cycle. The subjects weighed between 375 g and 450 g, and were handled for 5 min each for 4 consecutive days prior to behavioural testing.

All rats used in these experiments participated in another experiment in which they received injections of amphetamine, or muscimol. One group (amphetamine) were trained on a novelty detection task and given post-training amphetamine injections. The other group was trained on a 1-chamber fear conditioning to context experiment and injected with muscimol. These subjects were randomly assigned to all of the different groups and experiments.

The main reason for using these subjects was to reduce the amount of animals used in our research as recommended by the Canadian Council on Animal Care. The rats trained on the conditioned place preference task were food-deprived to 90% of their free-feeding body weight 1 week prior to training.

2.1.2. Surgery

All surgical procedures were performed in accordance with the rules and guidelines set by the Canadian Council on Animal Welfare and the Institutional animal welfare committees. Thirty-two animals underwent bilateral basolateral amygdala (BLA) implants, and 32 animals were given bilateral dorso-hippocampus (DHPC) implants. Surgery was conducted while rats were anesthetized with isofluorane anesthesia (4% with 2 L/min of oxygen for induction and 2% after surgical plane was established) in a standard stereotaxic apparatus. An incision was made in the scalp and periosteum along the midline. The skin was retracted with 4 mosquito forceps to expose the skull surface, and trephining holes were drilled into the skull using a 2 mm drill bit and high speed drill. Rats were bilaterally implanted with 26 gauge guide cannulae into the BLA or the DHPC. The tip coordinates for the BLA cannulae were AP: −2.7; ML: ±5.0; DV: −6.4, and the tip coordinates for the DHPC cannulae were AP: −3.5, ML ±2, DV −3; all coordinates are in millimeters relative to the skull surface and to bregma. Two jewelers' screws were secured into the skull and dental acrylic was applied to hold the cannulae in place. Obdurators (made from 32 gauge wires) flushed with the tip of the cannulae were inserted to block foreign materials from entering the brain. Following surgery animals were given (0.1 ml) buprenorphine (Temgesic) as an analgesic. After surgery animals were monitored until they became active. Rats were single housed for 3 days to allow for individual recovery and were pair-housed and handled daily thereafter.

2.1.3. Micro-infusions

After behavioural training was completed, the rats were given an intracranial infusion 20 min before testing. For the infusions, each rat was wrapped in a small towel to keep it immobilized, and the obdurators were removed from the guide cannulae. To introduce saline or muscimol into the brain parenchyma, a 32 gauge injector tip attached to a Harvard mini-pump via polyethylene tubing (PE20), was passed through the cannulae to extend 1.0 mm beyond the cannulae tips. Rats were bilaterally infused with 0.9% saline or muscimol (1 μg/μl) at a rate of 0.33 μl/min for 3 min for the BLA infusions and 0.25 μl/min for 2 min for the DHPC infusions. The injector needles were left in place for an additional 2 min to allow for drug diffusion. Once the infusion procedure was completed the obdurators were placed into the guide cannulae and the rat was returned to its home cage before testing.

2.1.4. Histology

Upon completion of the experiment, animals were sacrificed with an overdose of euthansol (0.6 ml per animal administered *i.p.*). Animals were transcardially perfused with 60 ml of saline in 0.1 M phosphate buffer solution (PBS) with pH 7.4, followed by 60 ml of 4% formalin in 0.1 M PBS. Brains were removed and post-fixed in perfusate for 24 h and then transferred to a 30% sucrose and 0.02% sodium azide solution. Brains were frozen on a cryostat at −21 °C, and coronal sections were cut at 40 μm thickness. Sections near the target coordinates were mounted and stained in Cresyl violet (0.1%).

2.2. Apparatus and procedure

2.2.1. Conditioned place preference (CPP) task

2.2.1.1. Chambers. Two context chambers were used that differed on three dimensions: colour, shape and odour. One context was a black triangle-shaped chamber measuring 61 cm × 61 cm on its base and a depth of 30 cm; the other context was a white square-shaped chamber measuring 41 cm × 41 cm on its base and a height of 20 cm. The flooring of both chambers consisted of stainless steel bars spaced 1.5 cm apart. Due to this feature, clear Plexiglas panels were fitted on top of these bars throughout training. This allowed the experimenter to place food reward in the chambers for the paired condition. Both boxes contained a small plastic cylinder (pill bottle) that was mounted on one of the walls of the chamber. On each training day, a drop of each odorant, serving as the olfactory cue, was placed on a cotton ball and inserted within the bottle. Iso-amyl acetate served as the olfactory cue with the black triangle chamber, and eucalyptus served as the olfactory cue with the white square chamber. The two chambers were connected during pre-exposure by an alley (16.5 cm long × 11 cm wide × 11 cm high). The entire structure was placed on a Plexiglas table that was 100 cm above the floor. A mirror (91 cm long × 61 cm wide), inclined by 45 °C, was placed on the floor of the testing room providing the experimenter with a non-intrusive view of the chambers. A video camera was placed 60 cm in front of the mirror to record the pre-exposure and preference phases of the experiment.

2.2.1.2. Pre-exposure. Each rat was placed in the middle alley and allowed to freely explore the two chambers for 10 min. Dwell time was accumulated when both forepaws were past the threshold of the doorway into one of the chambers and ended when both forepaws were past the threshold of the doorway into the alley. After each rat was tested the entire apparatus was cleaned with a