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NMDA receptors in the basolateral amygdala and gustatory neophobia

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Abstract

The attenuation of gustatory neophobia occurs during repeated exposures to an initially novel taste solution that is increasingly perceived as safe and familiar. The present study examined whether NMDA receptors in the basolateral region of the amygdala (BLA) are involved in this important behavioral phenomenon. The results, which show that the attenuation, but not initial occurrence, of gustatory neophobia is dependent upon NMDA receptors in the BLA, are discussed with reference to a similar finding involving NMDA receptors in the insular cortex.

Keywords

Gustatory neophobia; Basolateral amygdala; MK-801; NMDA receptor; Rat

1. Introduction

Evolution has furnished animals with many kinds of adaptive behaviors that benefit survival. One of the most important of these behaviors involves an innate fear of new foods, a phenomenon called gustatory neophobia (Barnet, 1963; Domjan, 1976). Gustatory neophobia prevents the ingestion of large amounts of a new, potentially toxic food. In the absence of aversive post-ingestive consequences, the amount of the new food that is ingested increases over trials until asymptote intake for that particular food is achieved and the food is considered safe and familiar. The attenuation, or habituation, of neophobia demonstrates that animals are capable of discerning the difference between a novel solution and a familiar solution.

Experiments from our laboratory show that neurotoxic lesions of the insular cortex disrupt the acquisition of conditioned taste aversions (CTAs), particularly when the taste stimulus is novel (Roman, Nebieridze, Sastre, & Reilly, 2006; Roman & Reilly, 2007). These results suggest that the insular cortex has a role in gustatory neophobia. Indeed, we recently discovered that acute microinfusions of MK-801 (a non-competitive NMDA receptor antagonist) into the insular cortex, while having no influence on the initial magnitude of the neophobic response, prevents the attenuation of gustatory neophobia (Figueroa-Guzmán, Kuo & Reilly, 2006). These latter results indicate that NMDA receptors in the insular cortex are critically involved in the mechanism that governs the attenuation of gustatory neophobia.

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Relevant to present purposes, St. Andre and Reilly (2007; see also Morris, Frey, Kasambira, & Petrides, 1999; Nachman & Ashe, 1974) found that lesions of basolateral region (BLA), but not the central nucleus, of the amygdala selectively disrupt CTAs when novel taste stimuli are employed (for a review of this literature see Reilly & Bornovalova, 2005). Additionally, we have recently shown that lesions of the BLA disrupt the attenuation of neophobia to a taste stimulus but have no influence on the expression of the neophobic reaction to either aqueous odor or trigeminal stimuli (Lin, Roman, St. Andre & Reilly, in preparation). As with the insular cortex data discussed above, these results demonstrate that the BLA has a role in neophobia. Given the high levels of NMDA receptors and binding sites in the BLA (e.g., Monaghan & Cotman, 1985), the present study was intended to determine whether NMDA receptors in the BLA, like those located in the insular cortex, are involved in gustatory neophobia. The design and procedure were identical to our insular cortex study (Figueroa-Guzmán et al., 2006), except that MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine-maleate] was infused into the BLA.

2. Results

2.1 Anatomical

One MK1 rat was found to have a misplaced cannula in the left hemisphere, reducing the size of this group ($n = 3$). Behavioral data from this rat were excluded from the statistical analyses. Figure 1 shows the correct position of the tip of the microinfusion needle in the BLA.

2.2 Behavioral

A preliminary analysis of variance (*Statistica*, StatSoft Inc, Tulsa, OK) found no significant intake differences across the 6 saccharin trials for Groups CON and VEH ($F < 1$). Therefore, both groups were combined and hereafter referred to as Group CONTROL. A Group (CONTROL, MK1, MK2) by Trial (Trial 1 - 6) repeated measure analysis of variance was performed on the behavioral data and revealed a significant Group X Trial interaction, $F(2,10) = 7.69, p < .001$ (see Fig. 2). A Fisher's Least Significant Difference test was used to determine the nature of the interaction.

For CONTROL subjects, saccharin intake on Trial 1 was significantly lower than that on Trial 2 ($p < .001$) and consumption on Trial 2 was significantly lower than that on Trial 3 ($p < .001$). However, further pairwise comparisons found no significant differences on Trials 3 - 6 ($ps > .50$). These statistics indicate that the CONTROL rats displayed a neophobic response to the novel saccharin on Trial 1, an effect that was completely attenuated by Trial 3 when intake achieved asymptote for the familiar and safe saccharin solution.

Inspection of Fig. 2 suggests, and statistical analysis confirmed, that there were no saccharin intake differences between the CONTROL subjects and the rats in groups MK1 ($F < 1$) and MK2 ($F < 1$) on Trial 1. The rats in each experimental group did, however, drink significantly less saccharin than the CONTROL subjects on Trials 2 and 3 ($ps < .05$). No infusions of MK-801 were given on Trials 4 - 6 and, on these trials, there were no saccharin intake differences between Group CONTROL and either set of experimental rats ($ps > 0.15$). These statistics demonstrate that, irrespective of concentration, MK-801 had no influence on the magnitude of the initial neophobic reaction to the novel saccharin solution on Trial 1. MK-801 did, however, prevent the attenuation of neophobia as seen in the CONTROL subjects on Trials 2 and 3. In the absence of MK-801 infusions, fluid intake rapidly recovered in the experimental rats over the final 3 trials such that all groups achieved the same level of saccharin consumption at asymptote.

Of further interest, saccharin intakes on Trials 1 and 2 ($p > .40$) and Trials 2 and 3 ($p = .35$) were not significantly different in the MK2 rats. A statistically identical pattern of results was found in the MK1 rats ($p > .60$ and $p = .07$, respectively). Finally, the performance of the two experimental groups was not significantly different on each of the 6 saccharin trials ($ps > .40$). These latter statistics indicate that each concentration of MK-801 infused into the BLA was equally effective at completely preventing the attenuation of gustatory neophobia.

3. Discussion

As expected, the CONTROL subjects showed a pronounced neophobic response when they first encountered the novel 0.5% saccharin solution. Indicating partial habituation, these rats drank significantly more saccharin on the second trial than the first trial, a volume that was also significantly lower than that ingested on the third trial. Finally, by the third trial saccharin consumption achieved asymptote. Overall, this represents a normal acquisition curve for intake of a taste solution that is not followed by aversive post-ingestive consequences and indicates the rats were learning that the saccharin solution is familiar and safe. Intra-BLA infusions of MK-801, a non-competitive NMDA receptor antagonist, had no influence on the initial occurrence or magnitude of the avoidance response to the novel taste stimulus. It might be noted that AP5 (DL-2-amino-5-phosphonopentanoic acid), a selective and competitive NMDA receptor antagonist, has been reported similarly to have no influence on neophobia. Using a very different paradigm from that employed in the present study, ingestion of novel (cheese) and familiar (chow) foods in a novel environment, Burns, Everitt and Robbins (1994) found that intra-BLA infusions of AP5 did not affect any measure of neophobia including amount of food consumed, duration of eating and the latency to contact or eat food. Continuing with the present results, intra-BLA infusions of MK-801 prior to saccharin access fully prevented the attenuation of the neophobic response. Moreover, in the absence of MK-801 infusions, the persistent and undiminished neophobic response to saccharin showed an immediate and complete recovery. Thus, there were no differences between the CONTROL and MK-801 groups on Trials 4 - 6. Although there were no significant intake differences between the two groups of MK-801 subjects, the forgoing pattern of behaviors was most clearly evident in the rats given the highest dose of the NMDA antagonist.

The behavior of the rats infused with MK-801 over the first 3 saccharin trials seems most readily explained as the product of a disruption of a post-acquisition mechanism. Moreover, the finding of an immediate and complete recovery from neophobia once the MK-801 was no longer administered provides strong indication that the memorial information about prior experience with saccharin was available to the rats on the fourth saccharin trial and therefore that NMDA receptors in the BLA are involved in neither the acquisition nor consolidation mechanisms. Rather, the data suggest that functioning NMDA receptors are critical for the retrieval of a consolidated taste memory. This hypothesis is readily testable because it predicts that infusions of MK-801 into the BLA prior to access to a habituated taste stimulus (like, for instance, Trial 6 in the present design) would disable the retrieval mechanism and the rats would show a full neophobic response (i.e., saccharin intake would be comparable to the level seen on initial exposure to the novel taste).

Using the same experimental design and behavioral procedure, our previous work (Figueroa-Guzman et al., 2006) found that MK-801 infused into the insular cortex produced a similar, but not identical pattern of results on gustatory neophobia as those reported herein for the BLA. That is, as with BLA infusions of MK-801, rats with insular cortex infusions of MK-801 showed a persistent neophobic response that did not habituate until the infusions were terminated. Unlike BLA infusions, however, the termination of MK-801 infusions into the insular cortex did not result in an immediate and complete recovery from neophobia. Rather, the rats in the insular cortex experiment showed a partial habituation response that was fully

recovered only on the second saccharin trial after termination of MK-801 infusions (i.e., on trial 5). Accordingly, we are inclined to propose that MK-801 infused into the insular cortex disrupts proper consolidation of the taste memory

Finally, as alluded to earlier, neurotoxic lesions of the BLA or insular cortex impair the initial expression of gustatory neophobia in a behavioral procedure identical to that used in the present experiment (Lin et al., in preparation). These permanent lesion data, then, stand in mark contrast to the behavioral results obtained from NMDA receptor blockade of the BLA and insular cortex. Clearly, these two structures must have multiple roles in gustatory neophobia. Future research will not only need to determine the pharmacological substrate of the initial response to a novel taste stimulus (involving detection of taste stimulus as novel and the consequent expression of a fear response which is manifest as a reluctance to consume the novel stimulus) but will also seek to establish the complete neurocircuitry of these two complementary behavioral effects (i.e., initial expression of, and the recovery from, gustatory neophobia). This endeavor, it is hoped, will lead to the development of a new psychological theory of gustatory neophobia that will move beyond the two-process model proposed by Nachman and Ashe (1974) over 30 years ago on the basis of their BLA lesion results.

4. Experimental procedures

4.1 Subjects

A total of 21 male Sprague-Dawley rats weighing 320-350 g were used in this experiment. They were housed individually under a 12/12-h light dark cycle in a room maintained at 22°C with water and food ad libitum (except where indicated below for experimental purposes). All the experimental treatments and tests were conducted during the light phase of the cycle.

4.2 Surgery

Fourteen rats received bilateral implantation of 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) under pentobarbital anesthesia (50mg/kg) using aseptic stereotaxic procedures at the following coordinates: AP -2.8 mm, M/L ± 5.0 mm, DV -6.5 mm (Paxinos & Watson, 2005). The guide cannulas were secured in place with acrylic dental cement and screws. The tip of the guide cannula was located 3.0 mm above the BLA. Following surgery, all rats were given a ten-day recovery period. During this time, two rats lost their guide cannula, reducing the overall number of cannulated subjects to twelve. Following post-operative recovery, the rats were placed on a water deprivation schedule that allowed 15 min access to water every day. After intake stabilized (11 days), 0.5% saccharin solution was substituted for water every third day for a total of six saccharin trials.

The subjects were divided in two controls groups (CON, $n = 7$; VEH, $n = 4$) and two experimental groups (MK1, $n = 4$; MK2, $n = 4$) according to the treatment they received on saccharin Trials 1 - 3. The rats in Group CON, an intact control group, received no surgery and no intracranial infusions whereas those in Group VEH, an infusion control group, were given bilateral BLA infusions of phosphate buffer saline (PBS). Comparison of the performance of the rats in Group CON with those in Group VEH permits a determination of whether the inevitable neural damage consequent to cannula implantation influenced fluid intake. The MK1 group received bilateral BLA infusions of 1 μ g/1 μ l of MK-801 (Sigma, St. Louis, MO) dissolved in PBS whereas the MK2 rats were given bilateral BLA infusions of 2 μ g/1 μ l of MK-801. Given to free moving rats 20 min before saccharin access on Trials 1 - 3, the 1.0 μ l infusions per hemisphere occurred at a rate of 0.5 μ l per min delivered through a 28-gauge stainless steel injector attached via polyethylene tubing to a 10 μ l Hamilton syringe located in an automated microinfusion pump (Cole-Parmer, Vernon Hills, IL). No BLA infusions

occurred on saccharin Trials 4 - 6. Volume of fluid ingested during the 6 saccharin trials served as the dependent measure.

4.4 Histology

At the end of the behavioral experiment, histologically analysis was used to confirm the proper placement of the injector tips and to verify the absence of any tissue damage in the BLA. Briefly, the rats were perfused transcardially with 0.9% saline followed by 10% formalin. The brains were sectioned with a freezing microtome (40 μ m) and sections through the BLA were mounted on gelatin-coated slides and stained with cresyl violet. The position of the injector tips were analyzed using a Zeiss Axioskop 40 microscope equipped with a Q-Imaging Camera running Q-Capture software (Quantitative Imaging Corporation, Burnaby, BC, Canada).

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Fig. 1.

The left panel shows, in coronal section, a schematic representation of the location of the basolateral amygdala (shaded in black and modified with permission from Paxinos and Watson, 2005). The right panel indicates the position of a cannula track (arrow) in the basolateral amygdala of a rat from Group MK2.

Fig. 2. Mean ($\pm$ SE) group fluid intake (ml) for control subjects (CONTROL) and for rats infused with 1 $\mu\text{g}/\mu\text{l}$ (MK1) or 2 $\mu\text{g}/\mu\text{l}$ (MK2) into the basolateral amygdala 20 min prior to the first three of six saccharin trials.