The basolateral amygdala has a critical role in food-matched visual-cue memory and post-ingestion food preferences in rats

Mahnaz Zamyad, Mehdi Abbasnejad, Saeed Esmaeili-Mahani, Vahid Sheibani, Maryam Raoof

The Basolateral Amygdala (BLA) has been shown to have an important role in food-related learning behaviors. Using a novel approach, we have evaluated the role of BLA in food preference and Food memory related to visual cues in rats. Thirty-two adult male Wistar rats, weighing 200–250 g, were used for the experiments. Electric lesion of BLA was produced by passing 1.5 mA of current for 7 s. Food-related behaviors and preferences were evaluated by using an automated apparatus. Geometric visual cues were also constructed. Food-deprived rats were presented with different diets in 6 consecutive trial performances. The number of visits, time consumed on each food zone and port, distance traveled in each visit, and the total amount of food eaten was evaluated. The changes in hippocampal c-Fos expression were determined by immunoblotting. The control sham group showed a high and low preference for biscuit and white flour, respectively. BLA lesion rats exhibited a shifted preference curve. In the sham group, a more significant amount of food consumption was associated with an increased number of references to each zone and port, along with more time spent there. Furthermore, a decrease in hippocampal c-Fos expression was observed in the BLA lesion animals. Taken together, the basolateral amygdala has a significant role in rats’ food-matched visual-cue memory and high-calorie/sweetness preferences.

**Keywords**

Food preferences, Learning and memory, Visual-cue, Basolateral amygdala, Lesion, c-Fos, Rats

**Abbreviations**

BLA: Basolateral amygdala
OFC: Orbitofrontal cortex
Introduction

Animal’s approach-behaviors to stimuli are related to the previously paired rewarding, escape, or avoidance experiences [1]. Post-ingestion consequences result in automatic selection or rejection of food, and through such experiences, the animals learn about the different characteristics of food [2].

The amygdala, especially its basolateral [3] and central [4] parts are involved in controlling feeding behaviors such as food preferences. Gustatory-related projections from parabrachial nuclei, insular cortex, and olfactory piriform cortex project to the basolateral amygdala (BLA) [5]. The orbitofrontal cortex (OFC) projections are required to the BLA for encoding the value of a reward [6]. BLA play important role in taste preference and olfactory classical conditioning. Lesions of the basolateral amygdala have been shown to be associated with taste-related disorder behaviors [7]. Furthermore, the BLA has a crucial role in experiencing the hedonic impact of the food outcomes [8, 9]. The BLA is also involved in the processing of sensory features of food, including smell, taste, buccal quick sense, and post-ingestion effects [10].

It has been demonstrated that the rats prefer to drink sweetened solutions over tap water, while radiation exposure caused a decrease in the consumption of sweetened solutions (taste aversion) [11, 12]. It has been demonstrated that animals can learn and memorize preferences regarding the characteristics of food. For instance, food-deprived rats prefer the flavor of a nutritious diet a few days after deprivation [1]. Furthermore, preferences for odors can be easily created or changed when the odor is matched with positive or negative reinforcemmen [13].

Studies have shown that both pure taste sensory information from the oral cavity, and post-ingestion factors are involved in c-Fos induction [14]. There is a positive relationship between the amount of food consumed and the expression of the c-fos protein. Several studies have shown that some nutrients, especially lipids and carbohydrates, induce c-Fos protein expression [15]. Also, it has been shown that the c-Fos protein expression is dramatically increased in the hippocampus of food preference trained rats [16].

It seems that when animals are given more opportunities for food selection, they prefer the most healthy (or rather, least unhealthy) option [17]. The calorific value and the nutritional composition of food are also the determinant factors influencing the food preference of animals [18]. It seems that animals can remember information about their food location. Moreover, place conditioning is a common, and potentially useful, procedure to assess the positive or negative motivational effects of exposure to various food stimuli [19, 20]. Nevertheless, it is unclear whether the BLA influences the food-matched visual-cue memory and post-ingestion food preference. Therefore, to elucidate the above, the present research has been designed using a new examination protocol.

Results

The amount of food consumed

Sham rats showed significantly increased preferences for biscuit (p < 0.001) and wholemeal + sugar (p < 0.05) than animals with a BLA lesion. However, there were no statistically significant differences in the preferences for white flour and wholemeal within the sham group. The preference order of BLA lesion groups was significantly (p < 0.001) different from that of the sham (p < 0.001).

There were significant differences in the amount of wholemeal (p < 0.01), wholemeal + sugar (p < 0.001), and white flour (p < 0.001) consumed between the groups. The order of consumed food (in grams) in the sham group was a biscuit, wholemeal + sugar, wholemeal and white flour. The BLA lesion group has shown this order of preferences: wholemeal+ sugar, white flour, wholemeal and, biscuit, respectively (Figure 1A).

Traveled distance

The analysis of the distance traveled in wholemeal, wholemeal + sugar, white flour and biscuit meal-related zones revealed significant differences among the various food zones in the BLA lesion and sham groups (p < 0.001) (Figure 1B).

Number of visits

There was a significant difference between the sham and the BLA lesion group regarding the number of visits to the food zones and ports (Figure 2, A and B).

Time spent

As shown in Figures 3 A and B, the time spent in the biscuit port and zone was significantly higher than that of the other foods in the sham group (p < 0.01, p < 0.001). However, the rats with a BLA lesion spent approximately equal time in white flour, biscuit, and wholemeal + sugar ports. Compared to the sham groups, the BLA lesion group significantly more time spent in white flour ports (p < 0.001) and zones (p < 0.01). Nevertheless, they spent significantly less time in biscuit ports (p < 0.05) and zones (p < 0.001) in comparison with the sham rats (Figures 3A, and 3B).

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The relationships between the amount of food eaten with the visits to, as well as the time spent, in the related zones

There was a positive correlation between the amount of food eaten with the visits to (Figure 4A), as well as the time spent (Figure 4B), in the related zones in sham rats. Figures 4C and D indicate the relationship between the food intake of a subject about the number of visits made \((r = 0.45)\) and the spent time \((r = 0.37)\) in the BLA lesion group. A positive correlation was found in the sham group regarding the amount of food eaten in conjunction with the frequency and length of visits, but not in the BLA lesion group \((r = 0.75; r = 0.79, p < 0.001)\) \((n = 16)\).

Food memory related to visual cues Table 1 shows behavioral data in various zones with both empty and filled containers. Using the visual cues, the sham rats spent significantly more time in biscuit zones \((p < 0.001)\) than in any other. They also made more visits to the biscuit zones compared to the others \((p < 0.001)\).

The number of references, and the time spent in each zone, there were no significant differences between the empty and filled containers. The order of
Table 1. 
Acquisition of the spatial memory (comparing the data from 5th and 6th trials).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Zone</th>
<th>Whole meal(g)</th>
<th>Whole meal+sugar(g)</th>
<th>White flour(g)</th>
<th>Biscuit meal(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Number of port visits</td>
<td>Filled container</td>
<td>9 ± 3</td>
<td>12 ± 1</td>
<td>4 ± 0</td>
<td>15 ± 3***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Empty container</td>
<td>8 ± 0</td>
<td>14 ± 5</td>
<td>3 ± 1</td>
<td>16 ± 2***</td>
</tr>
<tr>
<td></td>
<td>Spent time in the zone (s)</td>
<td>Filled container</td>
<td>116 ± 4</td>
<td>230 ± 2</td>
<td>17 ± 1</td>
<td>237 ± 2***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Empty container</td>
<td>125 ± 2</td>
<td>213 ± 4</td>
<td>14 ± 1</td>
<td>230 ± 1***</td>
</tr>
<tr>
<td>BLA lesion</td>
<td>Number of port visits</td>
<td>Filled container</td>
<td>71 ± 1</td>
<td>117 ± 1</td>
<td>89 ± 1</td>
<td>72 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Empty container</td>
<td>52 ± 2</td>
<td>51 ± 1***</td>
<td>40 ± 2***</td>
<td>38 ± 1*</td>
</tr>
<tr>
<td></td>
<td>Spent time in the zone (s)</td>
<td>Filled container</td>
<td>1288 ± 4</td>
<td>1424 ± 3</td>
<td>1302 ± 2</td>
<td>2030 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Empty container</td>
<td>754 ± 2</td>
<td>563 ± 2</td>
<td>537 ± 1#</td>
<td>372 ± 1***</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. ***p < 0.001 indicates significant difference compared with the other meals in the control group; #p < 0.05 and ###p < 0.001 indicate significant differences compared with the data from the same zone with different container conditions (n=16).

Figure 3. 
The differences between the sham and BLA lesion groups regarding A) the time spent in different food ports and B) zones. *p < 0.05, **p < 0.01 and ***p < 0.001 indicate significant differences compared with the same meal in the sham group, and #p < 0.05, ###p < 0.001 indicate significant differences compared with the wholemeal and white flour in the sham group (n=16).

food preferences in the fifth trial was biscuit, wholemeal + sugar, wholemeal and white flour, respectively. Comparing the ordering results of the 5th and 6th trials indicated that the animals had the ability of spatial memory.

The rats with a BLA lesion, however, showed significant differences with regards to the number of references and the time spent in each zone, according to whether it was filled or empty (p < 0.05, p < 0.001).

The effects of basolateral amygdala lesions on hippocampal c-Fos expression

The data showed significant differences in hippocampal c-Fos protein levels in different experimental groups by Immunoblot analysis. As shown in Figure 5, c-Fos expression was significantly increased in the sham group due to the food preference training (p < 0.01), which was reversed by BLA lesion. However, in BLA lesion rats, the c-Fos level was less than those in control animals (p < 0.001).

Discussion

Here we used four validated meal options according to the study done by Barnett et al., 1953 [21]. For the present study, we have developed a new apparatus and protocol. Considering the amount of food con...
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**Figure 4.**
The linear regression analysis of the relationships between the amount of food eaten and visits to and time spent in the related zones in the sham (A, B) and BLA lesion groups (C, D). *Significant regression, p < 0.001 (n=16).

**Figure 5.**
A) Effects of BLA lesion and food preferences on c-Fos expression in the hippocampus. B) Statistical comparison of c-Fos expression in the hippocampus between groups. **p < 0.01 and ***p < 0.001 indicate significant differences compared with the control group (n = 16). ##p < 0.01 indicate significant differences compared with the sham group.
sumed, Barnett et al., showed the following food preference order found in rats: biscuit, wholemeal + sugar, wholemeal and white flour. Our study confirmed the validity and reliability of these food preferences and protocols in rats. Furthermore, in the present study, we provided enough time for the animals to distinguish the post-ingestion consequences of food. Interference effects of post-ingestion products on eating certain foods or medication on food preferences have been extensively demonstrated [22].

In this study, a variety of food consumption variables including the number of references to the zones (whole surface of the square just in front of the food container) and ports (the entrance of the food containers), time spent, and distance traveled in each zone have been assessed. We noticed an association between the order of time spent in different zones and ports and the order of food preferences in rats. Illustratively, the order of the amount of time the rat spent in different zones was as follows: biscuit zone and port, wholemeal + sugar, wholemeal and white flour; the same as the previously discovered ordering of food preferences.

It has already been reported that rats have a preference for sweet taste [23]. Rats, like humans, have a natural bias towards consuming high-calorie food [18]. Moreover, the phenomenon of animals preferring high-fat foods has been considered a natural behavior. The high palatability and hedonic preference for fatty and sweet foods have already been reported. [24]. There are multiple causative factors including, texture, flavor, taste, and post-ingestive effect for high palatability of fat [25]. According to table 1, the biscuit has the highest amount of fat, protein, and calorie. It is also a high-carbohydrate meal. These specific features may help explain why biscuit was the first preference made by the rats. An increased appetite for high-calorie foods happens due to information received by the brain from the gastrointestinal tract through sensory nerves and chemical receptors [26].

On the other hand, the animals presented long-term memory formation driven by the visual cues associated with specific meals and locations. It has already been demonstrated that rats could learn to associate a specific stimulus, with a matched visual cue [27].

However, the present study is unique in that a food preference memory by use of matched visual cues has been used. The similar results of the 5th and 6th trials describe a positive post-ingestion consequence of foods, while the different data out of the two trials indicate negative post-ingestion effects. Indeed, the animals can learn to determine the suitability and preference of food according to the post-ingestion experiences [2, 28], and several types of mechanisms are involved in this regard [29].

In the present study, the preferred meal of the sham rats was biscuits. However, wholemeal + sugar, rather than a biscuit, ranks as the first choice for rats with BLA lesions. It suggests that the food preference is influenced by the carbohydrate-mediated post-ingestive effect [30] and the BLA may have a role in this regard. The rodent’s appetite for fat seems to be stronger than that for carbohydrates.

BLA lesions have been reported to be associated with taste disorder-related behaviors and with changes in a variety of taste and odor-related learning paradigms, including conditioned taste preference, taste-potentiated odor aversion, and conditioned taste aversion [3]. Since the BLA has a very crucial role in the induction of food-related memory, post-ingestive consequences of eating [28], and food behavior control [31], here we considered and evaluated all the mentioned conditions in rats with BLA lesions. Almost all of the data out of food consumption, time spent in, and visits to the zones and the ports, revealed that the rats with a BLA lesion had different food preferences than the sham animals. Besides, an induced BLA lesion has led to a change in the order of food preference in the 5th and 6th trials. The results indicate that the BLA is a critical region involved in Food memory related to visual cues.

In the present study, the following food preferences training, the amount of hippocampal c-Fos protein was increased. It is in line with the previously reported study that showed that in the dorsal and ventral hippocampus, the expression of c-Fos is increased in rats trained on socially transmitted food preference [13]. The BLA is strongly correlated with food-related learning and memory [3]. It also has connections with the hippocampus [32]. These connections seem to be necessary for visual cue discrimination and spatial memory formation. A lesion in the BLA negatively affects both reward-related learning and conditional learning and memory [33]. Also, c-Fos protein is induced by a wide range of stimuli and is a reliable indicator for neural activity such as food-related learning behaviors [13] and increases after learning and memory induction. In the present study, c-Fos expression decreased after BLA lesion. The reduction in the expression of c-Fos may be due to the role of the basolateral amygdala in food-related learning and memory; however, this issue needs further investigation.

Changes in the food preferences of the BLA lesion-affected rats have already been reported by Edmund et al. in 1973 [34]. However, our study employed both an innovative apparatus and protocol that produced more data related to feeding behaviors. Moreover, in the present study, we provided enough time for the animals to experience post-ingestion con-

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sequences. We also assessed visual cue-conditioning with food-dependent memory. The amygdala, especially the basolateral nucleus (BLA), has been involved in visual processing [35]. This area has connections with visual association areas, including primary and secondary, and supplementary visual cortices.

The present study sought to fill the gap in the related literature by examining the relationships among BLA function, changes in rat’s food preference, and visual cue-dependent memory. We propose that besides the amount of food consumption, the number of visits to the food zones or ports, the time spent and traveled distance in the zones might also be valuable to assess food preference in rats. Moreover, we found that the results of food-matched visual-cue-dependent memory assessment are negatively affected by a lesion in the BLA of rats.

**Materials & Methods**

**Subjects**

In this study, a total of 32 Wistar male rats aged two months (adult young), weighing 200–250g, were used. The rats (n=16) [36] were randomly assigned into two groups and evaluated for their food behaviors: the sham-lesion control group was given no treatment, and the BLA lesion group received electrical stimulation and stereotaxic surgery. The rats were kept in a temperature-controlled room at 23±1°C, with a standard 12 hours light/dark cycle. The animals received food and water ad libitum. All experimental procedures were approved by the Animal Research Ethics Committee of Shahid Bahonar University, Kerman, Iran (IR.UK.VETMED.REC.1398.018).

**Food type**

Four different diets have been considered according to a previous study [21] as follows: wholemeal, wholemeal + sugar, white flour, and biscuit (hard-baked) for testing food-related behaviors. To prevent a familiar effect, the ordinary laboratory rat food was powdered or mixed (50 percent) with the ingredients mentioned above (Table 2). According to Kasper and Johnson [18], rats present approach bias towards high-calorie and high-fat foods. Each of the ingredients was chopped into pieces no larger than 0.5 cm.

**Surgery**

The rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) (Stoelting Co., USA), and mounted on a stereotaxic instrument. The skulls were exposed and two holes were drilled into the skull over the BLA at stereotaxic coordinates: AP = –2.28, ML = ±5, and DV = 8.6 mm from bregma. A bipolar electrode (Teflon-coated stainless steel, 0.125 mm diameter, Advent Co., UK) was positioned in the BLA, and an electrical lesion was produced by passing 1.5 mA of current for 7 s [37]. Animals were allowed one week to recover from surgery. At the end of the experiments, the correct location of the lesions was verified histologically.

**Apparatus**

An automatic device made of black Plexiglas (60 cm long × 60 cm wide × 30 cm high), was used. The floor was imaginarily divided into nine identical squares. As shown in Figure 6A, the device was equipped with water and food storage containers and an electronic sensor to provide information on the animal’s locations, through container weight changes (Figure 6A).

The data of the four middle areas that include the ports (zones 2, 4, 6, and 8) presented areas to the animal’s preference for the container. Four corner squares (1, 3, 7, and 9 squares) were provided for resting (rats like rest in corners). The central square (square 5) was used as an animal release site. The device was supported by special software that assessed the number of visits to each zone, port, the location of the rats, the time spent, and distance traveled in each zone and port, food consumption per visit, as well as the total food consumption.

**Experimental design**

One day before the test phase, following recovery from the surgery, rats were habituated to the test environment. Each rat was allowed to freely explore the chamber that was free of food, for 15 min. If an animal spent more time in a specific area or didn’t show exploratory behavior, it was retested.

Figure 6.

A) Different parts of the preference meter device. B) The region of damage in BLA-lesioned rats.
behavior, it would be excluded from the experiment.

The test stage includes 6 trials in total with 15 min inter-trial intervals. The trials were as follows: In the first trial, container A was provided with 10 g wholemeal. Rats were placed in the central zone with dark cylindrical-shaped Plexiglas (to prevent their inclination to stray sideways). They were allowed to have access to this food for a 12-h period. The feeding behavior-related data was assessed and analyzed with the help of the aforementioned software.

The second trial began after the whole meal was removed from container A and when the apparatus had been thoroughly cleaned. Then, wholemeal + sugar was put in container B, and as with the previous trial, animals were released in the central square, and the feeding behavior was evaluated for 12 hours. The third and fourth trials were then conducted in the same manner, with white flour (container C) and biscuit (container D).

In the fifth trial, each of the various diets found in containers A-D were made simultaneously accessible. Dedicated visual cues were also included. The visual cues helped the animal to remember the taste memory. This trial was designed the animal’s food preference is influenced by previous experience with food consumption. For this trial, latency to the first container is also important. During the final trial, the food containers were empty.

With the help of the software, the following criteria were evaluated for each of the trials: the number of visits to each food zone and port, the distance traveled and the time spent in each zone and port, the food consumption per visit, and total food consumption.

**Histology confirmation of lesion**

Histological examination of the lesion was performed, just after decapitation, the rats’ brain was removed and stored in 10% formalin for at least three days. Also, serial transverse sections (30 µm) were cut, and the lesion sites were determined according to a rat brain atlas (Figure 6B).

**Western blot analysis**

After preparing the RIPA buffer (10 mM Tris–HCl: pH 7.4, 1 Mm ethylenediaminetetraacetic acid, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1% NP-40 and protease inhibitors, 0.1% Na-deoxycholate, and 1 mM sodium orthovanadate) added to the hippocampal tissue of the brain of rats for Lysis. 40 µg of protein per sample were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinyl difluoride membrane. Then for two hours, blots were blocked with 3% nonfat milk in 0.1% tween-tris-buffered saline, followed by overnight (at 4 °C) incubation with c-Fos primary antibody. The primary antibody was detected with goat anti-rabbit IgG antibody peroxidase-conjugated secondary antibody. The antibody-antigen complexes were detected by the ECL system and exposed to Lumi-Film chemiluminescent detection film (Roche, Germany). To assess the intensity of the blotting bands, the Lab Works analyzing software was used. We used β-actin as the loading control. The expression values were presented as c-Fos / β-actin ratio for each sample.

**Statistical analysis**

Behavioral data are presented as mean ± SEM and were evaluated using two-way ANOVA with statistical significance set at p <0.05, followed by Tukey’s post-hoc correction for multiple comparisons, where applicable. Regression coefficient r was used for calculating the dependency between food consumption and the number of visits, as well as the time spent in each zone.

**Authors’ Contributions**

MA: designed the study protocol, MZ: collected the data, SEM: carried out the statistical analyses, MA, VS, and MR drafted the manuscript.

**Acknowledgements**

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**Conflict of interest**

The authors declare that there are no conflicts of interest.

### Table 2.

<table>
<thead>
<tr>
<th>Food stuff</th>
<th>Description</th>
<th>Cal/10g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholemeal</td>
<td>Wholemeal* + pellet</td>
<td>33.30</td>
</tr>
<tr>
<td>Wholemeal + sugar</td>
<td>Wholemeal+ sugar*+ pellet</td>
<td>33.76</td>
</tr>
<tr>
<td>White flour</td>
<td>White flour *+ pellet</td>
<td>30.10</td>
</tr>
<tr>
<td>Biscuit meal</td>
<td>Biscuit meal* + pellet</td>
<td>3830</td>
</tr>
</tbody>
</table>

*Results of analyses carried out by Cereals Research Station:

<table>
<thead>
<tr>
<th>Food stuff</th>
<th>Total fat/10g</th>
<th>Protein/10g</th>
<th>Carbohydrates/10g</th>
<th>Sugar/10g</th>
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</thead>
<tbody>
<tr>
<td>Wholemeal</td>
<td>0.34 g</td>
<td>1.3 g</td>
<td>4.1 g</td>
<td>0.6 g</td>
</tr>
<tr>
<td>Wholemeal + sugar</td>
<td>0.34 g</td>
<td>1.3 g</td>
<td>7.5 g</td>
<td>5.6 g</td>
</tr>
<tr>
<td>White flour</td>
<td>0.1 g</td>
<td>1 g</td>
<td>7.6 g</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Biscuit meal</td>
<td>0.85 g</td>
<td>3.2 g</td>
<td>6.8 g</td>
<td>2.24 g</td>
</tr>
</tbody>
</table>

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References


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