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Aversion in the elevated plus-maze: Role of visual and tactile cues

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ABSTRACT

Thigmotaxis, a tendency to be close to vertical surfaces, leads rats to avoid open arms in the elevated plus-maze. Evidences support a role in thigmotaxis for the vibrissal sense as well as for vision. In this study, sensory inputs for both senses were manipulated in order to identify which of them mainly contributes to thigmotaxis. This was achieved by manipulating the length of rats' mystacial vibrissae, the presence of walls in the "open" arms and their transparency. As expected, rats avoided arms which lacked walls. On the other hand, rats did not avoid "open" arms surrounded by transparent walls as one could expect if they were using mainly vision while exploring the maze. Furthermore, these "open" arms were explored similarly to arms surrounded by opaque walls. Acute vibrissotomy resulted in minor effects in rats tested in a conventional elevated plus-maze. These findings suggest that vibrissotomized rats seemed to be able to compensate the absence of mystacial vibrissae by means of other sensory pathways (tactile or non-tactile) and by adjusting some exploratory aspects. Thus, the current results indicate that rats rely more on other sensory cues than on vision in avoiding open arms in the elevated plus-maze.

1. Introduction

The elevated plus-maze (EPM) is currently one of the most widely used animal models of anxiety. While being tested in an EPM, rodents show marked open arms avoidance (Handley and Mithani, 1984; Pellow et al., 1985). Anxiolytic or anxiogenic drugs increase or decrease, respectively, open arms exploration (Pellow et al., 1985). Aside from the conventional spatiotemporal measures (i.e., entries into and time spent in the arms), ethological measures have been indicated as reliable anxiety indexes (Cruz et al., 1994; Rodgers and Johnson, 1995). For instance, measures of risk assessment (primarily, stretched-attend postures – SAP) have proved to be highly valuable in identifying anti- and proanxiety drugs in the EPM (Rodgers and Cole, 1994).

It has been well established that anxiety-like behaviors observed in the EPM are triggered mainly by aversion of rodents to open spaces. A crucial motive for open arm avoidance has been suggested to be the impossibility of thigmotaxis––a natural defensive response in which rats remain close to vertical surfaces––rather than, for example, fear of heights or novelty (Treit et al., 1993). According to Grossen and Kelly (1972), rats naturally do prefer to be close to vertical surfaces in order to avoid avian predation. Whereas thigmotaxis is an important determinant of rat behavior in the EPM, it is still not well established the sensorial modality by which rodents perceive the openness of an environment. Most of the senses can possibly contribute to openness perception (for example, the rat can use odors, air flow or sound reflection cues for navigation – see, for example Wallace et al., 2002). However, studies about sensorial modulation of open space perception in rodents are concentrated in two types of inputs, those from the vibrissal sense and from vision.

There are suggestions that the vibrissae play a key role in thigmotaxis and other defensive behaviors. For example, besides openness, rats also avoid cliffs and, to evaluate depth, they seem to rely more on the vibrissal sense than on vision (Shiffman et al., 1970). Furthermore, there is evidence that animals submitted to unilateral vibrissotomy exhibit directional thigmotaxis asymmetry toward the intact vibrissae side while navigating in aquatic or terrestrial environments (Meyer and Meyer, 1992). In the EPM test, Belzung (1999) reports no open arm avoidance by mice without vibrissae.

On the other hand, evidences from studies which manipulated the amounts of light during the test session and the presence of transparent vertical surfaces in the open arms of the EPM suggest that vision is important for rodent perception of open spaces (Cardenas et al., 2001; Martinez et al., 2002). For example, rats explore more the open arms under complete darkness or dim light (01 lux) than under higher illuminations (Garcia et al., 2005, 2011).

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In addition, a study shows that the larger the gradient of luminosity between open and closed arms, the lower is open arm exploration (Pereira et al., 2005). When the open arms are provided with transparent walls, maze exploration is reported to be similar to the observed in a regular EPM (Martinez et al., 2002), suggesting that visual cues prevail over tactile ones.

Many previous studies provided evidences about the contributions from the vibrissae and from vision as mediators of thigmotaxis in the EPM. Indeed, no available evidence allows the exclusion of one of these two possible mediators. In this sense, a study in which both senses are evaluated in the same test can possibly be heuristic. Therefore, the current investigation was designed to test these two senses by manipulating vibrissae length (intact or cut), the presence of vertical surfaces (present or absent) and their transparency (opaque or transparent). Thus, if vision is the main sensory modality for thigmotaxis, similar exploration would occur in conventional open arms or in "open" arms surrounded by transparent walls – vibrissotomy would, at best, strengthen that similarity. On the other hand, if rats rely more on vibrissal sense, they would explore transparent walled "open" arms as if they were in regular enclosed arms – vibrissotomy would disturb exploration depending on the degree to which the sensory deficit could be compensated by other sensory inputs.

2. Materials and methods

2.1. Subjects

Subjects were 71 male Wistar rats weighing 250–300 g, obtained from Londrina State University/UEL (Paraná, Brazil) which were housed in groups of 5 per cage (40 cm × 34 cm × 17 cm). They were kept under regular 12:12 h light cycle (lights on at 7:00 a.m.) in a temperature (25 ± 1 °C) and humidity (55 ± 5%) controlled environment. Food and drinking water were freely available, except during the brief test periods. All rats were experimentally naïve and used only once.

2.2. Ethics

Experiments were carried out in accordance with the norms of Brazilian Neuroscience and Behavior Society, which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals. All experimental procedures had been previously approved by the Committee for Animal Research of Londrina State University (CEEA/UEL 11/09).

2.3. Apparatus

Three types of plus-mazes were used in the experiments (Fig. 1):

(A) *Conventional maze (CM):* a standard elevated plus-maze which consisted of two open arms (50 cm × 12 cm × 1 cm) at right angles with two closed arms (50 cm × 12 cm × 40 cm) connected to a central platform (12 cm × 12 cm). The apparatus was constructed from varnished wood (floor) and transparent Plexiglas (walls) and was raised to a height of 50 cm above floor level. To prevent the rats from falling, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. The walls in the closed arms were covered with brown paper in their outer surface.

The other two plus-mazes were similarly constructed, but they were different in the open arms construction. Actually, for these mazes, the word "open" was used only for labeling the position of the arms (in reference to the closed arms and to the room).

Table 1
Illumination (lux) in each part of each type of maze.

Location	Conventional	Transparent	Opaque
"Open" arms	66.7/66.7	71.0/73.2	42.0/42.0
Closed arms	44.1/44.1	43.0/43.0	42.0/44.1
Central square	58.1	58.1	45.2

(B) *Transparent walls maze (TW)*: the "open" arms were surrounded by 40-cm high transparent Plexiglas walls.

(C) *Opaque walls maze (OW)*: similar to the TW, however with the outer Plexiglas walls surface of the "open" arms covered with brown paper.

2.4. Procedure

All experimental procedures were conducted between 01 p.m. and 04 p.m. under the illumination of a 60-W bulb light fixed 2.4 m above the apparatus (Table 1 shows illumination levels in each maze).

Seventy-one rats were randomly allocated to three groups (n = 23–24); one group to be tested in the CM, another one in the TW maze and the third group in the OW maze (in each testing day, one maze was replaced by another in the same testing room). For all these groups, 5 min before testing, each animal was subjected to a vibrissae removal procedure. Cut group: whole extension of mystacial vibrissae was cut with a scissor (no more than 1 mm remained) (n = 11–12) and Intact group: mystacial vibrissae were sham cut (i.e., vibrissae were touched with the side of a scissor and then it was closed in order to produce the same sound) (n = 12). The vibrissae (or the sham) removal procedure duration was about 30 s. For testing, each rat was carefully placed in the center of the CM, TW or OW maze, facing one of the closed arms. All procedures were performed by the same experimenter. Test sessions lasted 5 min and the apparatus was cleaned with 5% ethanol and dried with paper towels in between trials. All sessions were video-recorded by a camera fixed above the apparatus.

2.5. Behavioral scoring

Behavioral scoring was performed by a trained observer (intra-observer concordance: >90%). The observer, while scoring, was unavoidably aware of the type of maze, but was not aware about the vibrissae condition (video quality did not allowed such identification). An ethological scoring software package developed by Dr. Morato's group at Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP, Brazil (retrieved from http://scotty.ffclrp.usp.br/page.php?6) was used. Behavioral parameters comprised conventional spatiotemporal and ethological measures which are regarded as important in the elevated plus-maze (see, for example, Cruz et al., 1994; Rodgers and Johnson, 1995) and their latencies. Entries into and time spent in the closed and "open" arms were recorded. Total distance traveled in each type of arm by the animals was estimated from the number of crossings (the maze arms were divided into 10-cm squares on a transparent plastic mask placed over the TV set screen). An entry into an arm (or into a square within an arm) was scored after all four paws entered it. The frequencies of the following behaviors were recorded: Rearing (animal rising on the hind limbs both touching and not touching a wall surface), Stretched Attend Posture (SAP) (when the animal stretches its body forward and then retracts to the original position with no locomotion) and Head Dipping (animal sticking the head outside the maze border and toward the floor). Head dippings and SAPs are often assigned (see, for example, Rodgers and Johnson, 1995), depending on the part of the maze where they occur, as unprotected (in the open arms) or protected (in the center or the