



Research report

Vibrissal paralysis produces increased corticosterone levels and impairment of spatial memory retrieval



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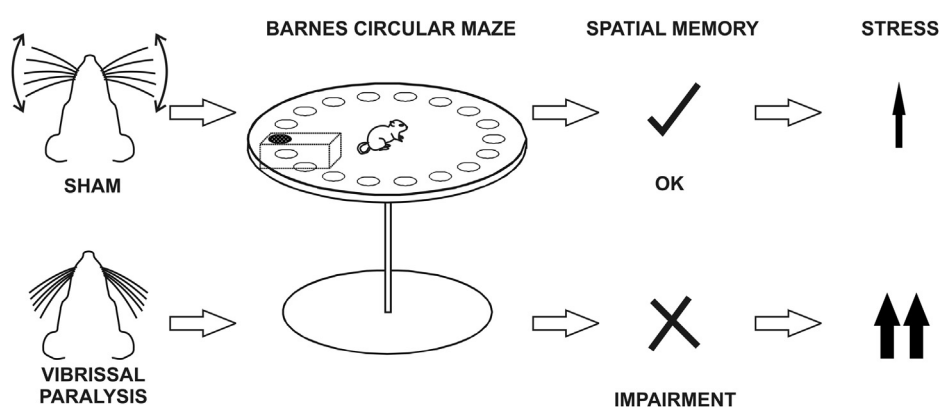
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HIGHLIGHTS

- Vibrissal paralysis was induced by bilateral facial nerve lesion in rats.
- Injured and control rats were trained and tested in a spatial memory task.
- Vibrissal paralysis induced retrieval but not acquisition impairment.
- Corticosterone response to training or testing was higher in injured rats.
- Paralysis-induced stress response potentiation may cause retrieval impairment.

GRAPHICAL ABSTRACT



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ABSTRACT

This research was aimed at establishing how the absence of active whisking in rats affects acquisition and recovery of spatial memory. The mystacial vibrissae were irreversibly paralyzed by cutting the facial nerve's mandibular and buccal branches bilaterally in the facial nerve lesion group (N = 14); control animals were submitted to sham-surgery (N = 15). Sham-operated (N = 11) and facial nerve-lesioned (N = 10) animals were trained (one session, eight acquisition trials) and tested 24 h later in a circular Barnes maze. It was found that facial nerve lesioned-animals adequately acquired the spatial task, but had impaired recovery of it when tested 24 h after training as compared to control ones. Plasma corticosterone levels were measured after memory testing in four randomly chosen animals of each trained group and after a single training trial in the maze in additional facial nerve-lesioned (N = 4) and sham-operated animals (N = 4). Significant differences respecting the elevation of corticosterone concentration after either a single training trial or memory testing indicated that stress response was enhanced in facial nerve-lesioned animals as compared to control ones. Increased corticosterone levels during training and testing might have elicited the observed whisker paralysis-induced spatial memory retrieval impairment.

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Abbreviations: AHEF, all holes exploration frequency; BCM, Barnes circular maze; CORT, corticosterone; HAB, habituation; HPA, hypothalamus-pituitary-adrenal; ROS, reactive oxygen species; SD, standard deviation; SEM, standard error of the mean; MWM, Morris water maze.

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1. Introduction

Rhythmic mystacial vibrissae movements (active whisking) are considered critical behaviour for mice, rats, and other rodents, enabling them to obtain relevant information from their close environment [1–4]. The whisking behaviour of such animals is involved in aspects such as orientation, locomotion, object localisation and texture recognition, swimming, food acquisition and three-dimensional perception [4–9]. This active movement allows them to decode spatial variations in their environment [10], discriminate surface orientation [11], gauge distances [12] and distinguish between different sound frequencies [13]. Vibrissae movements are crucial regarding aggressive, predatory and copulatory behaviour [14,15]; they are produced by alternating intrinsic and extrinsic muscle contractions in the whisker pad [3,16,17]. These muscles are innervated by the facial nerve's buccal and mandibular branches, thereby enabling the whiskers to protract and retract along the anteroposterior axis [3,18,19].

According to the pertinent literature, cutting whiskers or removing follicles (eliminating sensory input) have been the methods most used for studying the relevance of the vibrissae regarding task execution. So far, only our group has evaluated the relevance of active whisking in object recognition tasks leaving intact the input from passive sensory information through the vibrissae. Both the facial nerve's mandibular and buccal branches were bilaterally injured in such research, producing bilateral vibrissal paralysis which left passive sensory input information thorough the intact follicles unaffected. After injury, the rats were engaged in object recognition (position and texture) tasks. It was found that active whisking was necessary in these animals for textural but not for positional novelty detection [20].

However, no studies have evaluated the importance of vibrissae active movement regarding spatial memory tasks to the best of our knowledge; just one study has reported the effect of whisker removal (unilateral or bilateral) in rats regarding tasks in the Morris water maze (MWM) [21]. The authors reported a significant increase in latency after vibrissae removal regarding the rats locating the exact position of the submerged platform; they pointed out that spatial task execution by rats in which the vibrissae had been removed had similar deficiencies to those in rats having hippocampal (CA1 pyramidal cells) loss due to cerebral ischemia [22]. They also proposed that CA1 cell loss caused by ischemia prevented the animal translating sensory information coming through the vibrissae system [21]. Such observations thereby pose the question of whether an animal requires active sensorial input through the whiskers to solve a spatial maze (i.e. will an animal be able to accurately solve a spatial task by receiving only passive sensory input through its vibrissae?). This led therefore to the main goal of this work which was to answer the aforementioned question by using animals having intact vibrissae in experiments but which were unable to actively move the vibrissae.

Furthermore, the relevance of active whisking in the aforementioned multiple functions suggests that an ability to execute voluntary movements also represents an inability to confront potentially stressful environmental challenges. This poses a further question: Does an inability to actively move the whiskers imply a stress response which is triggered during spatial task execution? This is especially interesting, given that spatial memory can be affected by many external and internal factors, including stress.

It has been shown that stress can enhance or impair memory associated with numerous tasks, like object recognition, classical conditioning and spatial navigation. This effect could deviate, depending on stress response intensity, the kind of stressor used, the kind of memory assessed and the phase during which stress is induced [23–30].

Many instruments have been developed for evaluating spatial memory ability, such as the MWM and the Barnes circular maze (BCM), both involving similar tasks for evaluating a rodent's ability to learn and remember how to locate a specific maze region when being guided by distal extra-labyrinth visual cues located around the instrument, implying hippocampal spatial memory [31]. Despite being similar, the MWM has an aversive and stressful component in itself [32], given that an animal must swim uninterruptedly to reach the goal. A single exposure to the MWM produces higher activation of the hypothalamus-pituitary-adrenal (HPA) axis, causing higher corticosterone release compared to BCM exposure [29].

Considering the above, the present study was aimed at establishing whether an inability to execute voluntary whisking affects spatial task learning and performance in the BCM and (if differences were found) whether such behavioural changes could be due to hormonal changes associated with stress response. The approach involved injuring the facial nerve's mandibular and buccal branches to paralyse the lower third of the face (bilaterally), thereby impairing active whisking but keeping passive sensory input intact (both vibrissae and the trigeminal nerve remained intact); it also involved evaluating learning and spatial task recovery, using the BCM in both control (sham-operated) and facial nerve-lesioned rats, for measuring plasma corticosterone levels as an indicator of hypothalamic-pituitary-adrenal (HPA) axis activation in control and facial-lesioned animals.

2. Materials and methods

2.1. Subjects

Twenty-nine adult male Wistar rats (*Rattus norvegicus*) weighing 300 ± 20 g (mean \pm SEM) were used as subjects; they were supplied by the National Institute of Health in Bogotá. Animals were housed in polycarbonate boxes ($38 \times 32 \times 18$ cm) in groups of four, under a 12-h light/dark cycle (lights on at 07:00 h). Subjects were kept at room temperature (21 ± 2 °C) and had *ad libitum* access to food and water.

2.2. Procedures

Rats were randomly assigned to one of the following experimental groups: Sham or Lesioned (D1, Fig. 1C).

False lesion surgery (Sham): Eleven randomly-chosen subjects were exposed to sham surgery in aseptic conditions and under general anesthesia (75 mg/kg ketamine and 9 mg/kg xylazine, administered intraperitoneally). Briefly, buccal and mandibular branches of the facial nerve (Fig. 1A) were dissected through a 0.5-cm horizontal incision above the mandibular angle, but left them intact; the skin wound was sutured with discontinuous 4-0 silk stitches. Previous studies have demonstrated that simple exposure of the facial nerve do not causes neither axonal degeneration nor motor neuron death [33].

Facial nerve lesion surgery (Lesioned): Ten randomly-chosen subjects were exposed to facial nerve transection surgery in aseptic conditions and under general anesthesia (75 mg/kg ketamine and 9 mg/kg xylazine, administered intraperitoneally). Briefly, the facial nerve's buccal and mandibular branches were dissected and cut through a 0.5-cm horizontal incision made above the mandibular angle a 2-mm segment of the proximal stump was removed in each sectioned nerve branch to avoid nerve repair and the skin wound was sutured with discontinuous 4-0 silk stitches (Fig. 1A). Nerve branch identity and lesion effectiveness were confirmed by electrical stimulation (for more details see [20]).

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