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### Research report

# Sexual odor discrimination and physiological profiles in adult male rats after a neonatal, short term, reversible nasal obstruction



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#### ABSTRACT

The present study was designed to examine behavioral responses (interpreted as preferences) to olfactory cues (nest bedding odor and odors of estrous and anestrus females) in adult male rats after they had a short term reversible, bilateral, nasal obstruction (RbNO) as developing rat pups. These results were compared to behavior of control (untreated) and sham operated male littermates. Behavioral tests and physiological parameters were analyzed 90 days after recovery of nasal breathing. Experiments investigated the time spent in arms or the center of a maze of male rats in response to odors from the nest bedding or from adult females. There were no differences in responses between untreated, sham and RbNO adult male rats to fresh and nest bedding odors. RbNO males spent more time in the center of the maze when given a choice of estrus or anestrus female odors, or bedding odors from untreated or sham operated female rats. In contrast untreated and sham male rats preferred the odors of estrous females and of untreated or sham females. Plasma corticosterone levels in the males increased during the behavioral tests. Plasma testosterone levels were significantly lower in RbNO males compared to untreated males and did not increase during the behavioral tests compared to sham operated males. Males from all groups had similar preferences for the odor of bedding from adult RbNO females. Plasma levels of cholesterol and triglycerides were increased in RbNO adults. In conclusion, short term nasal obstruction in males while juvenile has long term consequences on hormones and behavioral preferences, thus potential partner selection when adult.

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#### 1. Introduction

Under normal conditions lack of olfaction is generally associated with the obstruction of the nasal cavities (Seiden and Duncan, 2001) which, if carried out early, could have negative effects on subsequent adult activities as growth of the neuronal population in the olfactory bulb extends up to post-natal day 20 (Bayer, 1983). Our previous study revealed indeed that reversible, bilateral, nasal obstruction (RbNO) induced in eight-day old rats led to an activation of the stress response with increased corticosterone as the final effecter (Gelhaye et al., 2006a,b). The literature shows that increased plasma levels of corticosterone are associated with the expression of anxiety-related behaviors such as hyperactivity

(Cao et al., 2007), with modifications of novelty-seeking behavior (Alemany, 2008; Gelhaye et al., 2006a,b, 2011), and social play in juvenile rats (Beatty and Costello, 1983; Risser and Slotnick, 1987).

Through olfactory deprivation and activation of the HPA axis, early nasal obstruction could disrupt, on the one hand establishment of exploratory behavior, a preliminary necessary for the emancipation and the dispersion of young mammals, and on the other hand the hormonal homeostasis of young individuals (Leon, 1992; Rossier and Schenk, 2003). Nasal obstruction generates numerous effects on the olfactory bulb, including reduction of its volume (Gelhaye et al., 2006a,b; Padzys et al., 2012) and a variety of neurochemical and functional changes (Brunjes, 1994). However, in spite of the blockage of the nasal cavities, odorants could, theoretically, reach the olfactory epithelium *via* the nasopharynx and this retronasal perception could mediate odor-guided behaviors in rodents (Coppola et al., 1994; Chapuis et al., 2007).

In many species, including humans, chemosensory stimuli function as social cues that impact reproductive hormones and behavior (Doty, 2001; Jacob et al., 2001; Wirsig-Wiechmann, 2001). In

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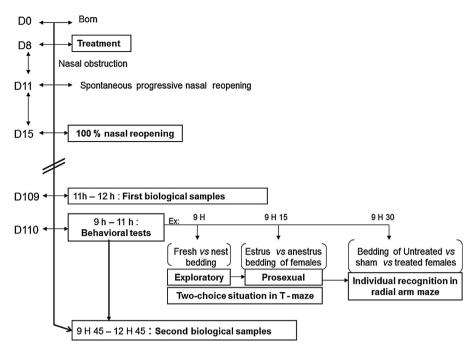


Fig. 1. Time line of experimental protocol.

rodents, female odors (or pheromones) activate neurons in limbic circuits mediating male reproductive behavior and elicit gonadal steroid release in sexually naïve males (Meredith, 1998). The expression of reproductive behavior in sexually naïve male Syrian hamsters is absolutely dependent on female pheromones present in vaginal secretions and their transduction by the vomeronasal system (Meredith, 1986; Meredith and Howard, 1992). In male hamsters and rats, exposure to female pheromones elicits a rise in testosterone within 60 min (Richardson et al., 2004; Wood et al., 2004). Furthermore, female chemosensory stimuli can be used to establish a classically conditioned endocrine response to a neutral stimulus, confirming their roles as unconditioned stimuli for evoking reproductive responses in males (Graham and Desjardins, 1980).

Olfactory recognition often appears to be important in establishing the bond between mammalian individuals. The young distinguish their own mother from other females by recognizing her distinctive odor (Hongo et al., 2000). An early imprinting with the correct odor may influence not only the young animal's future recognition of and relations with its mother, but also its selection of a mate having a similar odor when it becomes adult. Mammalian pheromones used for individual recognition may volatilize directly from the body of the animal, or it may be deposited onto a substratum as a scent mark. A scent mark has the advantage of allowing an animal to identify the previous presence of either a known or an unknown individual of the same species in a particular area (Calamandrei, 2004). These scent marks may act as loci for the general exchange of information such as the individual identity, as well as the age, sex, breeding condition, and social status of the marking animals (Müller-Schwarze and Müller-Schwarze, 1971; Hudson, 1993).

The aim, therefore, of the present study was to evaluate in 110 days old adult male rats the effect of neonatal RbNO on normal exploratory behavior and on sexual odor differentiation. For the latter we used uniquely odor cues as it is well known that rats make ultrasonic vocalization during mating (Barfield and Thomas, 1986; Matochik and Barfield, 1991). The effects of early olfactory deprivation on the stress response (corticosterone) on plasma levels of sexual hormones (progesterone, oestradiol and testosterone), and

biochemical states (glucose, proteins, lipids) were also studied in adult rats.

Our hypothesis was that early short-term olfactory deprivation (RbNO) would have a significant effect of some parameters of exploratory and/or prosexual behavior. Nest bedding odor recognition and choice of sexual partner odor (bedding from estrus and anestrus females) were therefore investigated in a two-choice situation. As vertebrates are frequently characterized as being able to recognize the physiological status of a sexual partner, we therefore tested also the choice of odors (estrus) from untreated, sham or early nasally obstructed female rats in a three choice situation.

#### 2. Methods

#### 2.1. Animal care

Male and female Wistar rats (origin IFFA-CREDO) were used in these experiments. These pups were born in the laboratory from 15 litters, culled to 10 pups per litter (5 males and 5 females) to ensure normal body growth. We used three male from each litter for each of the biological and behavioral tests. We used all females from each litter for behavioral tests. The animals were housed in standard cages under controlled temperature conditions  $(22\pm1\,^{\circ}\text{C}).$  Food (pellets of 12 mm, Harlan Interfauna Iberica SA) and water were available ad libitum throughout the experiment. From birth, the rats were kept on a reversed 12:12 light–dark cycle (dark period  $08:00-20:00\,\text{h}).$ 

Methods were schematically in Fig. 1.

#### 2.2. Nasal obstruction procedure

At the time of the experiments they conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (no. 85-23, revised 1996), the recommendations of the European Community Council for the Ethical Treatment of Animals (no. 86/609/EEC) and the regulations of the University of Lorraine. All efforts were made to minimize animal suffering.

At 8 days of age, the litters were first anesthetized by hypothermia (10 min at -18 °C), then weighed and then semi randomly

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