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Endocrine and Immunological Alterations under Early Nasal Obstruction in Rats Wistar

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Abstract

For most animals smell is the primal sense. One they rely on to identify food, predators and mates. Indeed, for many organisms, odours arc their most efficient means of communicating with others and interpreting their surroundings. Innate behaviour in response to smell is essential to these organisms' survival and most likely result s from nonconscious perception of odours. This article is part of a research program dealing with the consequences of bilateral nasal obstruction (NO) during the postnatal development of mammals. Its aim was to test if the absence of nasal respiration and the related transition to chronic oral breathing could perturb the development of the individual. Therefore, a NO was induced in 8-day old rats (D8) and its effects were investigated 24 h after the treatment (D9), at the end of the obstruction period (D15) and six days after the reopening of the nostrils (D21). The results showed that NO affect some hormonal functions, these modifications were pronounced at D9, D15 and D21. Lastly, NO was associated with an atrophy of the brain at D15, it was maintained until D21. In rats, nasal obstruction can thus be considered like a multifactorial stressful situation. Its effects lasted until adulthood.

Keywords: Nasal obstruction; Olfactory deprivation; Lymphocytes; ACTH; Rat wistar

Introduction

For most mammalian species investigated so far, the female-offspring relationship has been shown to rely on multimodal systems of cues and signals, among which olfaction plays a predominant, sometimes unique, role in the orchestration of mutually adaptive responses. The scientific coverage of mammalian taxa on this point is obviously partial with some (e.g. rats, mice, rabbits, pigs, sheep, squirrel monkeys and humans) having received considerably more attention than others. The best understood system of female offspring communication is unquestionably that of the rat in which various functional aspects of behavioural and physiological fit are governed by olfaction. For example, newborns are guided to the nipples by amniotic/salivary cues spread by the female [1].

Newborn altricial mammals of many species learn the odor of their mother and use it in identifying and orienting to the mother. As the mother is the sole source of food, warm and protection, learning this odour is critical for the survival of the newborn [2]. In the rat neonate, olfactory cues from the mother and siblings are crucial in the establishment of early behaviours such as home orientation [3], huddling [4] and nursing [5]. A chronic olfactory deficit is therefore able to involve many consequences on the homeostasis of the young individual.

There are some methods of olfactory deprivation closer to the pathological conditions met in nature, where the lack of olfaction is generally associated with a nasal obstruction [6]. For example, the main technique employed to produce transient olfactory deprivation in neonatal animals consists in the obstruction of the nasal cavities, usually using a brief cauterization reducing airflow into the nasal cavity and thus odorant exposure [7].

Here, we present and discuss the consequences of early nasal obstruction on physiological, structural and behavioural variables during and after obstructive procedure, bilateral nostril cauterization was induced an olfactory deprivation on 8-day-old rats of both sexes.

Materials and Methods

Biological material and animal care

In the present study, we used wistar rats (Rattus rattus). Ten mixed-sex litters were born in the laboratory of the university. All animals were housed in standard cages on a constant 12-h light/12-h dark cycle with controlled temperature and humidity and were given access to food and water ad libitum.

Nasal obstruction procedure and experimental groups

At the age of 8 days, the litters were divided into three experimental groups. Untreated group (UT) was defined by the complete absence of manipulation (n=16). Sham group (SH) (n=16) and animals with nasal obstruction (NO) (n=16) were first anesthetized for a short period. They were placed for few seconds in a glass bell containing a cotton wisp impregnated with ether. Once the litters were anesthetized, a bilateral nasal obstruction was performed on NO animals as previously described by Meisami [7] and Waguespack et al. [8]. The selected method consisted in the cauterization of the external nostrils, which is the most common and simple procedure allowing reversible nasal obstruction in neonates. The tissue surrounding the external nostrils was burned by placing a surgical cauterizing instrument on the nostrils, consequently occluding the orifice of the nostrils. This procedure induced a complete nasal obstruction between PND 8 and

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PND 14 with 100% of the nostrils reopened at PND 15. In sham group, the nostrils were not sealed but the tissue above them was burned by placing the cauterizing instrument about 1–2 mm above each nostril. After the cauterization, the burn was washed with chlortetracycline (Aureomycine Evans 3%) to prevent a possible infection. SH and NO pups were kept warm (371°C) for 1 h and they were then returned to the mothers.

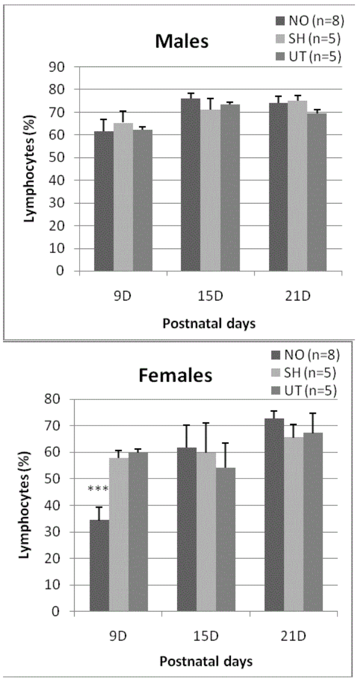

Figure 1: effect of early nasal obstruction on lymphocytes (%) in animals with nasal obstruction (NO) n=8 per sex versus untreated group (UT) n=5 per sex and sham group (SH) n=5 per sex, at 9, 15 and 21 postnatal days. P: significance level; NS: not significant (P >0.05), * significant (P<0.05), ** very significant (P<0.01), *** more significant (P<0.001).

Biological samples

At PND 9, PND 15 and PND 21, immediately following sacrifice; blood was collected in tubes that are treated with an anticoagulant. Plasma was immediately separated from cells by centrifugation (15 min at 650 g) then the extracts were stored at -181°C until the time of the assay. In order to assess the adrenal response after the induction of early nasal obstruction, the concentration of adrenocorticotropic hormone (ACTH) was measured. It is also used to measure the concentration of lymphocytes. After blood sampling, the brains were dissected and weighed.

Statistical analysis

Data in the text and figure legends are means ± standard errors for the indicated number (n) of animals. Statistical comparisons were made by analysis of variance (ANOVA) and differences were considered to be significant at P <0.05.

Results

Effect of early nasal obstruction on lymphocytes

As seen in figure 1, No difference was found in the rate of lymphocytes between the experimental groups of males, whatever their age (p>0.05). After induction nasal obstruction in females (9 PND), the lymphocytes level was significantly decreased p=0,009 in NO females (34.3 ± 5.01) compared to untreated (57.84 ± 2.7) and p=0,007 compared to sham females (59.85 ± 1.5).

Effect of early nasal obstruction on adrenocorticotropic hormone (ACTH): The plasma ACTH levels were significantly increased in NO animals after induction of bilateral nasal obstruction at 9 and 15 PND (Figure 2). This proliferative response of adrenocorticotropic hormone was persisted until 21 PND in females (p<0.001) and reached a high value (2000 ± 201.4) pg/ml compared to untreated (1300 ± 69.4) pg/ml and sham groups (1152.1 ± 98.5) pg/ml. There were not significant differences between the untreated and sham groups in males or in females (P>0.05).

Effect of early nasal obstruction on brain weight: Figure 4 shows a significant decrease of brain weight at the age from 9 to 15 days. The mass of brain was measured at 15 PND. The means were significantly different in males (P<0,001) between NO (0.35 ± 0.11) (g), sham (1.14 ± 0.05) (g) and untreated group (1.01 ± 0.14) (g). Also in females, absolute values were significantly different at 15 PND (P<0,001) in nasal obstruction group (0.37 ± 0.16) (g), sham group (1.12 ± 0.09) (g) and untreated group (1.17 ± 0.14) (g). The mass of brain was increased progressively after reopening of the nostrils (15 PND). Indeed, at 21 PND the means of males were (0.89 ± 0.12) (g) in NO animals, (1.31 ± 0.14) (g) in sham animals and (1.26 ± 0.07) (g) in untreated animals. There was also a significant difference in the brain weight of females at age of 21 days (p=0,003), in NO group (0.78 ± 0.13) (g), sham group (1.28 ± 0.08) (g) and untreated group (1.33 ± 0.09) (g).

Discussion

Our results revealed that the early nasal obstruction was associates with an increase plasma adrenocorticotropic (ACTH) level, it was more marked in females and lasted until 21 PND.

Proliferative activity of lymphocytes was diminished after induction of nasal obstruction in females but not in males. This is consistent with a previous report that considered that novelty-seeking behavior was capable to reflect the activation of HPA axis [9]. Early exposure to an episode of nasal obstruction impacts on pup viability, homeostasis is further imbalanced because of food-mediated maintenance of neonatal hormonal state. Thus, a few hours deprivation of mother's milk correlates with a significant reduction in thyroxin and an increase of plasma corticosterone levels [10-14].

Thyroid, renal, adrenal, and gonadal hormones play a key role in early development. An early deficiency in thyroid hormones disturbs brain development (specifically the olfactory system) [15] and delays the maturation of muscles (especially orofacial muscles) [16,17]. Vasopressin and corticotrophin-releasing hormone (CRH) both play a synergistic role in stimulating the release of adrenocorticotropic hormone (ACTH) [17], so vasopressin could possibly enhance the CRH effect during the first days of nasal obstruction-induced oral breathing. This "stress" reactivity might mediate response to nose-blocking surgery and/or dehydration induced by oral breathing [18].

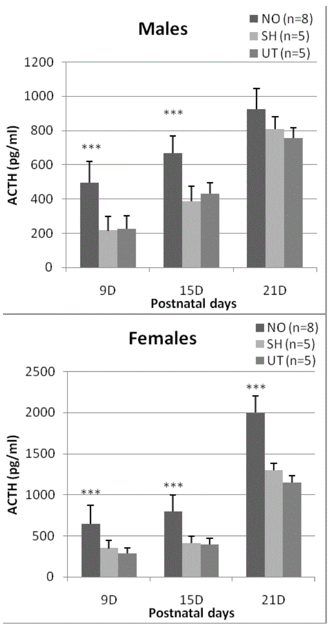

Figure 2: effect of early nasal obstruction on adrenocorticotropic hormone (ACTH) in experimental groups at 9, 15 and 21 days of age. P: significance level; NS: not significant (P >0.05), *significant (P<0.05), **very significant (P<0.01), ***more significant (P<0.001).

The stress response induced by narial obstruction in 8-day-old rat pups is also evidenced by the hypertrophy of adrenal glands 72 h after treatment [12]. Adrenal hypertrophy is more marked in females (+ 68% in CN females and +29% in CN males, compared to controls) on PND 21 [10]. These effects did not persist over the long term (PND 90). An increase in plasma testosterone was observed during the nasal obstruction episode and on PND 90 [19].

This suggests that nasal obstruction via the olfactory bulb influences gonadotropin secretion that might be mediated by altering gonadal steroid feedback. Nose blocking affects the immune system by suppressing the proliferation of B-lymphocyte precursors [10]. Thymus weight was reduced only in CN females. The thymus is particularly sensitive to stress-associated glucocorticoids, which induce thymocyte apoptosis. Although not documented by our own experiments, nasal obstruction has far reaching consequences on biological rhythms. It can impair nocturnal sleep and induce diurnal lethargy [20,21]. We cannot exclude that it also induced biorhythmic maladaptation in rat pups, in terms either of hyporeactivity when they had to suck the nursing dam or of hyperactivity due to high corticosterone levels. Finally, a brief period of nasal obstruction affects mother-offspring interactions and decreases offspring's food intake [11].

Lastly, in our experiment early nasal obstruction was associated with an atrophy of the brain in both sexes. As much as the brain is considered an "immune-privileged organ," the relationship between the nasal cavity and the brain is also a "privileged" and "intimate" one. First, the nasal cavity is the only place in which central nervous tissue (the olfactory neurons) is directly exposed to air carrying chemicals, pathogens, pollutants, and allergens. Second, the transfer of large molecules and pathogens into the brain via the intranasal pathway has been reported [22,23]. It is important to mention that the intranasal pathways may, via olfactory and trigeminal pathways, deliver large molecules into brain areas that play a key role in mood regulation and dysregulation more efficiently than intravascular administration [24]. In addition, it has been shown that intra-nasal instillation of recombinant cytokines, including IL-6 and IL-12, affects the course of experimentally induced neurologic disorders in rats [25,26].

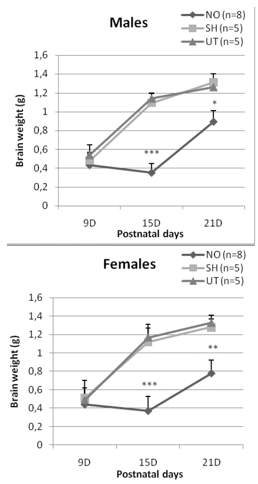

Figure 3: Effect of early nasal obstruction on brain weight in experimental groups at 9, 15 and 21 postnatal days. P: significance level; NS: not significant (P >0.05), *significant (P<0.05), **very significant (P<0.01), ***more significant (P<0.001).

In conclusion, we have shown that short term nasal obstruction in 8 days old rats can have profound effects of the capacity of these rats to develop. Olfactory deprivation can thus be considered like a multifactorial stressful situation. It is interesting that nasal obstruction was of only a short duration and very early in the life of the rat but the impacts lasted until adulthood.

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