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Effects of perinatal odorization on the development of food preferences in mice: a preliminary study

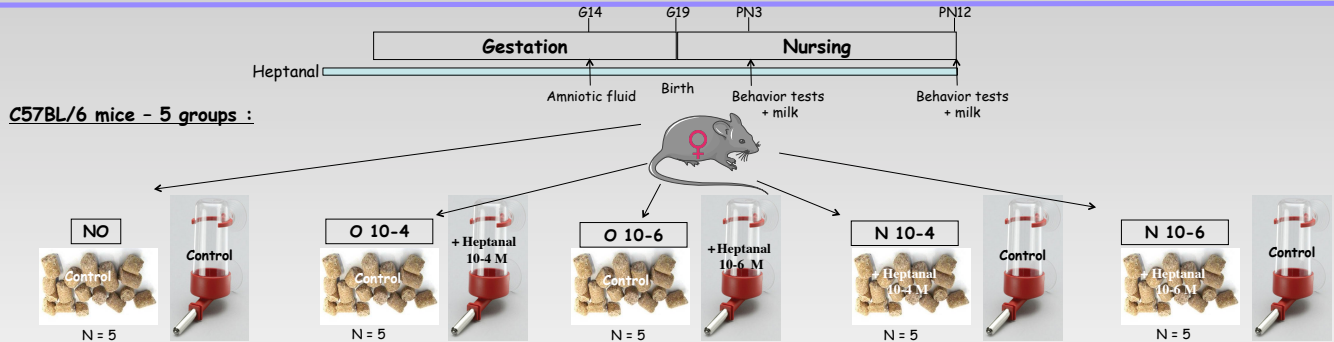
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AIM OF THE STUDY

The purpose of the thesis is to evaluate the consequences of the perinatal odorant environmental changes on the plasticity of the olfactory system and associated behaviors in mice strain (MI7-GFP) expressing an olfactory receptor coupled to a GFP reporter, whose preferential ligand is known (heptanal; Bozza et al., 2002). We wonder to examine the changes in the olfactory pathways induced by maternal odorization at two levels of the olfactory system, the mucosa and the bulb, using combined approaches from molecular (immunohistochemistry, electrophysiology, biochemistry, qPCR, DNA methylation) to behavioral ones (food choices, odorant detection thresholds).

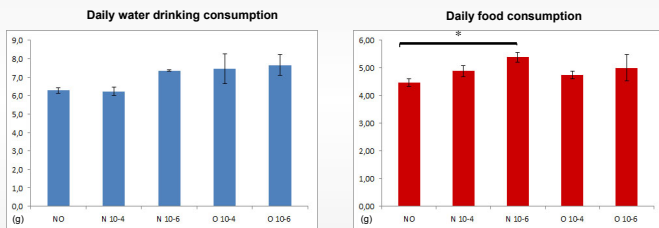
In a preliminary set of experiments, we tested different conditions of maternal exposition to heptanal (odorized water or food pellets at various concentrations) on C57/BL6 mice as a model. We measured the consequences of the odorization on food and water consumption, we checked the presence of the odorant in various biological fluids of mothers by GC/MS and we registered olfactory-driven behaviors related to olfactory maternal cues at postnatal day3 and day12 on odorized pups as compared to controls.



RESULTS

Metabolic parameters

Food and water consumption were registered three times a week until the day 14 of gestation in the 5 groups (n=5/group).

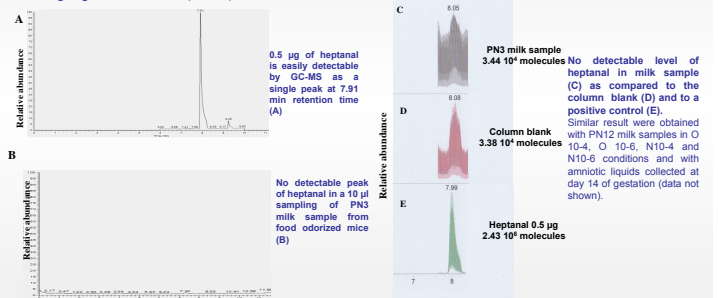


→ No significant difference in daily water consumption was observed between the five groups, whatever the odorant administration pathway.

→ A significant difference was observed in daily food consumption for the N10-6 group compared to the NO group (20%), but no significant difference were found with the other groups.

GC-MS

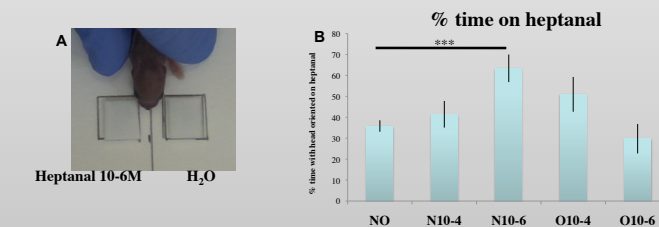
The presence of heptanal in the biological samples was checked by GC-MS on a SPME fiber (A and B) or following Argon fractionation (C to E).



→ Whatever the odorization conditions, no detectable levels of heptanal in biological fluids are observed by gas chromatography.

PN3 tests

PN3 pups were registered in a head-orientation assay adapted from *Al Ain et al., 2012*. A blotting paper was taped on a heating plate at 37°C. Two filter papers were stuck bilaterally at 3 mm of the midline on the blotting paper, one soaked with heptanal 10-6 M and the other one with water (figure A). Pups were held between the gloved thumb and index. The pups body was held aligned with the midline of the blotting paper. The duration of head orientation to each of the stimuli was measured for 2 min. Then, we calculated the percentage of time spent on sniffing heptanal for each pup (figure B).

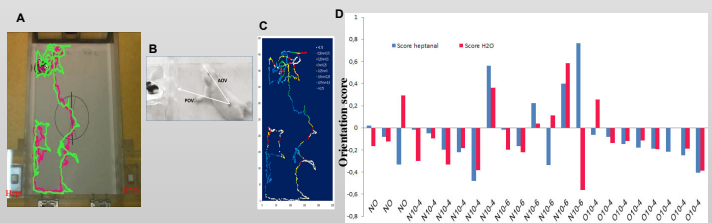


→ pups of the N 10-6 group spend more time with their head oriented on heptanal side as compared to the NO group

→ no significant difference was observed for other groups

PN12 tests

PN12 pups were exposed to an odorant choice test (water vs heptanal 10-6M) adapted from *Meunier and Nielsen, 2014*. They were placed in an open field "T" maze (A) and registered for 2 min by video. We obtained x,y coordinates of 2 markers (head and body center) using a video-tracking freeware. Two vectors were calculated: the animal orientation vector (AOV) and the perfect orientation vector (POV) (B). The angle between the 2 vectors in each frame was converted into a scalar product ranging from 1 (pup oriented directly towards the odor source) to -1 (facing the opposite direction) (C) and a mean scalar product was calculated for each odor source (D).



→ we observe a high variability in the orientation score obtained in all groups.

→ NO pups mostly display a negative score whatever the odor sources, indicating no clear odorant choice

→ same results are obtained with the O 10-4 and N 10-4 group.

→ N 10-6 pups scores are highly variable, with no heptanal preference.

(Animals of the O 10-6 group were not tested).

DISCUSSION

In this preliminary study, we developed an animal model of maternal exposition to heptanal in C57 BL/6 mice in order to test different conditions of perinatal odorization and we have developed functional behavioral tests.

Whatever the concentrations and the ways of odorant administration (food or drinking water), heptanal was not detected by GC/MS in 2 different maternal biological fluids collected at various ages. Furthermore, pups tested at PN3 and PN12 don't display any evidence for heptanal preferences, except for the N 10-6 group at PN3.

This result can be related to the mother higher daily food consumption in this group.

We hypothesize that the odorization levels are not sufficient to induce a measurable behavioral effect.

A new study using food odorized with higher heptanal concentration is underway.

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