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Research article

Influence of prenatal pre-exposure to an odor on intake behavior of an aversive solution in newborn rats

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ABSTRACT

Early pre- or postnatal sensory experiences significantly influence flavor preference and food intake, and can induce liking for innately unpalatable flavors. Previous work found that newborn rats stimulated with an odor experienced shortly after birth exhibited heightened intake and seeking towards an artificial nipple containing quinine. This result suggests that odors made familiar trough early postnatal pre-exposure can shift the motivational value of unconditional stimuli. The objective of the current study was to assess the effect of an odor (lemon) experienced in-utero on the first intake responses towards an artificial nipple supplying quinine. The hypothesis, which was corroborated, was that stimulation with the olfactory stimulus experienced in-utero would increase the newborn's intake and grasp responses to the artificial nipple containing quinine. Exposure to the odor that had been pre-exposed in utero increased quinine intake and seeking (i.e., latency to grasp and total time in contact with the nipple, as well as number of and mean duration of nipple grasps) in 3-h-old pups. These results replicate those previously found with postnatal odor pre-exposure, and extend the phase for pre-exposure to the prenatal stage.

1. Introduction

Associative and non-associative odor learning in altricial species, such as the rat, facilitates the dam-pup dyad interaction during the first postnatal weeks. The odor of the dam is affected by the diet, and pups are born without fully developed visual and auditory systems. Odor learning, and smell in a broader sense, is thus key to find the nest and maternal nipple [8]. Tastes are associated with odors, and both stimuli constitute flavors [7].

The gustatory and olfactory systems are not yet mature during the latest stages of fetal life; yet this does not prevent detection and differentiation of, and learning from, the chemosensory cues present in the amniotic fluid [1]. These experiences affect postnatal behavioral responses and physiological reactions to odors and flavors [2]. It occurs inasmuch as flavors from the mother's diet are transmitted to the amniotic fluid and to her milk later on [3]. Prenatal learning of flavors may have been favored and selected through evolution because it favors the infant's acceptance of food and flavors ingested by his/her mother. Postnatal preference for flavors experienced during gestation has been observed in several mammalian species, such as humans [4], rats [2], cats [5] and piglets [6].

Mennella, Jagnow and Beauchamp [9] assessed whether exposure to a flavor during human gestation affected postnatal reactions to foods that had a similar flavor. Pregnant women consumed carrot juice during the last trimester of pregnancy. Their infants were assessed, when they were almost six months old, for responsiveness to cereals prepared with carrot juice. The authors reported significantly increased levels of enjoyment of that food in the children whose mothers had consumed carrot juice during pregnancy, compared to those of women who had not drunk carrot juice nor eaten carrots during that period.

Early pre- or postnatal sensory experiences not only influence preference for food or flavors that are part of the regular diet of the organism, but also induce liking for typically unpalatable flavors [4]. Kamenetzky, Suárez, Pautassi, Mustaca and Nizhnikov [10] pre-exposed rats, immediately after birth, to a lemon scent. Three hours later, the newborns were stimulated with a surrogate nipple odorized with

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lemon odor that provided either saccharin (0.1% or 0.2%) or an aversive quinine solution (0.1% or 0.2%). Greater intake and behavioral responsiveness, compared to a control, non-preexposed group, was found exclusively for the 0.1% quinine solution. It is conceivable that this might be due to the pre-exposed olfactory stimulus changing the hedonic value of this typically aversive substance [11]. This effect might be especially important when looking at preference of alcohol after exposure in the womb. Alcohol has a distinct bitter taste and it has been shown that gustatory stimulation with a quinine-sucrose compound evokes electrophysiological responses similar to those evoked by the drug [12].

The animal literature shows an extensive body of research indicating that prenatal exposure to alcohol produces long lasting memories in the organism that, along with early postnatal experiences with the drug, enhance its intake and sensory discrimination during infancy and adolescence [2,3,13–17]. For instance, Domínguez, López and Molina [18] found an increase in alcohol intake and lower duration of head and forelimb movements towards the odor of alcohol, in infant rats born from dams given 1 or 2 g/kg daily alcohol doses during gestational days (GD) 17–20, as compared to pups derived from vehicle-treated dams. This effect generalized to a sucrose-quinine compound, the psychophysical equivalent of ethanol for the rat [12]. These results suggest that fetuses can process the sensory attributes of alcohol and, as a result, encode specific memories that alter subsequent, postnatal responsiveness towards the drug.

Pre-exposure studies that employ alcohol are subjected to an important caveat. This drug has a distinctive flavor yet exerts potent pharmacological effects that can, by themselves, facilitate subsequent alcohol acceptance [19]. Yet pre-exposure to other flavor or tastants, that lack pharmacological activity, yield similar results. Increased preference for garlic odor was found in pups whose mothers had consumed garlic during gestation, compared to offspring of mothers who had not ingested garlic [20]. Similarly, Nicholaides et al. [21] found greater salt appetite in adult offspring of dams that had consumed a diet rich in salt during gestation. In an intriguing study, Smotherman [22] assessed consumption of an apple juice solution in juvenile rats that had been (or not) exposed to the flavor on day 20 of gestation, via injection into the amniotic fluid. Animals pre-exposed to the solution exhibited significantly higher levels of intake than either untreated pups or pups pre-exposed to saline. Rat fetuses receiving pairings of apple juice solution and lithium chloride (a potent emetic agent) in-utero showed conditioned taste aversion to that solution when they were evaluated at postnatal day 10 [23]. Altogether, these studies indicate that in-utero exposure to flavors during the last days of gestation can yield memories that last up to adulthood and generate greater preferences for those flavors, or increased responsiveness to its sensory attributes [2].

The present study determined whether odor pre-exposure during late gestation, via contamination of the amniotic fluid, could yield the same effects on quinine intake as the odor pre-exposure shortly after birth. We assessed the effect of stimulation with an odor that had been experienced in-utero, on the first responses towards an artificial nipple supplying quinine.

2. Materials and methods

2.1. Subjects

Thirty-four pups, derived from 8 Sprague–Dawley dams (Taconic, Germantown, NY) mated at the vivarium of the Department of Psychology at Binghamton University (temperature: 22 °C; 12-h light-dark cycle with lights on at 0700 h), were employed. Specifically, 18 pups were stimulated with water prenatally, and these were representative of 4 litters; whereas 16 pups (representative of 4 litters) were exposed to lemon prenatally. We aimed at using only one male and one female from each dam, yet due to logistic problems in the supply of dams, we had to use more than one male and one female from the same dam in 4 of the litters.

Vaginal smears were collected each day during a 7-day breeding period to time each pregnancy. The first day of detectable sperm was designated as embryonic day 0 (E0). All animals had ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. The rats were maintained and treated in accordance with the guidelines for animal care and use established by the National Institutes of Health (1986), within an AAALAC-accredited facility.

2.2. Apparatus

The surrogate nipple was constructed with a hollow tip made of rubber and shaped conical, attached to a dental explorer and connected through a cannula to a syringe containing the solution. The syringe had a hole in the top wall, which generated a hydraulic flow that was activated and controlled by the animal when it voluntarily suctioned. The dental explorer had an alligator clip attached to the top, which had a cotton swab soaked in the lemon scent [24].

2.3. Procedure

2.3.1. Pre-exposure to the olfactory stimulus

During GD21, one hour before the C-section, the pregnant female rats were intragastrically administered with 0.015 ml/g of lemon solution (16, 8% v/v) via i.g., or with an equal volume of vehicle (distilled water). Lemon odor was chosen because it is widely used as a salient odor that is not harmful to the pups [25–27]. The pups were assigned to treatment groups in a random fashion and precautions were taken to conceal treatment assignment to the behavioral coders.

2.3.2. Caesarean section

One hour after, a C-section was performed under a continuous supply of isoflurane (Baxter, Deerfield, IL; VetEquip, Pleasanton, CA). A midline incision was made through the abdominal wall to expose the uterine horns. A small incision into each amniotic sac allowed externalization of the pups. The umbilical cord was pressed for a few seconds and then cut and the membranes were removed. Finally, each pup was placed into a plastic container (12 cm long × 12 cm wide × 6 cm high) lined with a moist, sterile gauze, on a heating pad. Once the cesarean section was completed, the anesthetized dam was sacrificed. Immediately after, pups were placed during 3 h in an odorless incubator (Microplate Incubator, Boekel Scientific, Feasterville, PA) kept at 35 °C ± 1; until the test began. The 1 h interval between gavage and C-section was meant to minimize the possibility of an association between the lemon odor and the manipulations inherent to the C-section. Our aim was to familiarize (pre-expose) pups with the lemon odor and not to condition this odor to the event of C-section.

2.3.3. Test

Before the commencement of the test, the pups were gently stimulated in the anogenital region with cotton to induce urination or defecation. Subsequently, they were weighed to the nearest 0.01 g and placed individually on a mirror maintained at 35.5 °C ± .5 °C. Then, the test began and lasted for 6 min, during which the offspring were stimulated with the artificial nipple on the perioral area, in the presence of a swab soaked in lemon essence. The experimenter held the swab at about 2 cm from the pup's snout via a dental explorer tool which, itself, was connected to the swab through a crocodile clip. Grasping the nipple allowed the pup to obtain a 0.1% quinine solution, which was selected based on our previous work [10]. Specifically, quinine acceptance is usually quite low, which made it a good fit for the present study, which assessed the possibility of observing heightened acceptance of a taste provided in contiguity with a pre-exposed odor. At the end of the session, pups were dried with a paper towel, weighed to the nearest 0.01 g and returned to incubator.

The oral grasp response involved an active movement of the head