Physiology & Behavior 148 (2015) 51–57

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Change in the hedonic value of an aversive stimulus in the presence of a pre-exposed odor

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HIGHLIGHTS

- Newborns discriminate between different concentrations of saccharin and quinine.
- A familiar odor enhances intake of quinine, but not saccharine, in newborn rats.
- A familiar odor enhances grasping of an artificial nipple dispensing quinine.
- A familiar odor does not enhance grasping for saccharine.

ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form 10 December 2014
Accepted 13 December 2014
Available online 24 December 2014

Keywords:
Olfactory learning
Aversive solutions
Ontogeny
Newborn rats

ABSTRACT

Rats exhibit a sensitive period from the time of birth until postnatal day 10 during which they develop preferences for odors even if those odors are paired with a moderately aversive stimulus. It is still unknown whether pre-exposure to an odor produces alterations on intake responses of basic tastants, and on other patterns that indicate a change in the hedonic value of reward, such as nipple grasping behavior. The current study assessed the effect of pre-exposure to an odor immediately after birth on intake responses of appetitive and aversive tastants. The objectives were to assess if 3-hour-old rats adjust their behaviors to obtain different values of appetitive and aversive rewards in the presence of a familiar odor. Specifically we wanted to determine whether the intake of saccharin or quinine, administered through the artificial nipple, increases in the presence of the familiar odor. Results showed that 3-hour-old rats differentially respond to two different concentrations of saccharin and two concentrations of quinine. In the presence of the pre-exposed odor newborn rats increased intake and grasp responses to the artificial nipple containing quinine. This effect disappeared with a higher concentration of quinine. These results suggest that the pre-exposed odor generated a change in the hedonic value of the aversive reward.

Olfactory stimuli are critical during early stages of life in mammals. Altricial species are evolutionarily predisposed to learn behaviors that facilitate approach and attachment towards the caregiver, even in the context of parental abuse or neglect [14]. This age-specific predisposition declines as subjects undergo transition into adolescence and adulthood, yet the influence of early learning experiences during developmental sensitive periods can be long lasting.

Human fetuses can easily detect, via amniotic fluid, chemosensory stimuli derived from the maternal diet. Fetuses can inhale amniotic fluid from gestational week 24 onwards and by the end of gestation they are actively ingesting significant amounts of the fluid. These have been deemed the first taste and olfactory sensory experiences in humans [15] and can result in the encoding of associative and non-associative memories. For instance, it was found that infants whose mothers had ingested alcohol or anise during gestation exhibited greater orientation responses towards these odors [27,36].

Long-lasting memories, likely to affect intake and feeding behaviors at later stages of development, can also be formed after birth. Chemosensory cues present in breast milk can regulate sucking patterns [16,20]. The responsiveness to flavors seems to be precisely tuned during the early postnatal stage and drastic changes in sensitivity can occur in very short periods of time. For example, human infants readily accepted hydrolyzed protein formula (cow's milk formula modified to prevent allergies and protein intolerance in infants) at 3–4 months of age, despite the unpleasant taste of the food, yet they vigorously rejected the formula when tested at 5–6 months of age [16,18].

From postnatal day (PD) 0 to 10, the rat exhibits a sensitive period to develop odor preferences [28], although olfactory learning continues throughout the preweanling period, presumably allowing the infant to

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http://dx.doi.org/10.1016/j.physbeh.2014.12.041
0031-9384/

adjust to diet-related changes in the odor of the mother and her milk [14]. The reaction of the infant to the smell or taste of the substances available in the suckling context promotes further contact with their caregiver, and hence secures access to food, warmth and protection [30,38].

Neurobiological mechanisms that support odor learning operate in a complex manner in this specific stage of development. Odor presented during this sensitive period, even those without biological relevance, hyperstimulate the olfactory bulb, which in turn facilitate the acquisition of olfactory learning [14]. Unlike olfactory learning, the acquisition of conditioned fear is lessened during this developmental period, a phenomenon probably related to the hyporesponsivity of the HPA axis and the resulting immature processing of aversive stimuli in the amygdala. An increase in the levels of corticosterone (CORT) after PD 10 determines the end of the sensitive period in the rat [40], although basal and stress-induced CORT levels in the two- and three-week old preweanling rat are still much lower than in mature rats (see [31]). Amygdala activation and increased production of corticosterone in rats is associated with the beginning of locomotion, and therefore, it is necessary to develop protective mechanisms, such as aversion to predators' odors [28].

During this sensitive period pups will display preference for odors, even after pairings of odor with an aversive stimulus of moderate magnitude. It has been suggested that this prevents the pups from acquiring aversion to the mother, who sometimes provides — during the course of maternal care behaviors — discomfort to the pup (e.g., bites, squashing). There have been attempts to translate these theories to explain cases of child abuse, who sometimes display attachment to caregivers that engage in maltreatment or negligence [29]. It should be noted that, despite this enhanced predisposition to acquire olfactory preferences, fetuses and neonates are able to acquire conditioned aversions. For instance, Gemberling & Domjan [8] found conditioned taste aversion in 1 day old rats; and Stickrod et al. [39] observed that fetuses exposed in utero to pairings of malaise and a taste/odor stimulus showed aversion to the taste/odor when tested at postnatal day 16.

The artificial nipple technique has significantly fueled the study of early neonatal learning. It employs a device fashioned out of latex, that newborn rats can grasp and attach to, and consume fluids dispensed through it. These behaviors, in turn, can be modulated by odor cues, normally provided by an odor-scented cotton swab attached to the nipple [33]. Previous studies showed that suckling and intake are reduced when the nipple provides quinine or a saline solution; whereas saccharin increases these behaviors. This result suggests that taste detection and discrimination influence suckling responsiveness very early in life [30]. Another study evaluated responses to a surrogate nipple containing nutritive or non-nutritive fluids, over repeated exposures. It was found that the first intake is regulated by chemosensory processes, while subsequent intake may be related to the nutritional value of the solutions [34].

A seminal study indicated that newborn rats can acquire robust olfactory conditioning 3 h after birth, when a lemon scent (conditioned stimulus, CS) was paired with intraorally delivered milk (unconditioned stimulus, US). Subsequent exposure to the CS triggered significant attachment to an empty, artificial nipple [6]. Later, studies that employed milk as the US in the nipple technique showed that newborn rats can acquire complex associative learning, such as second-order conditioning and sensory preconditioning [5].

Neonatal rats exhibit greater limb movement and grasping behavior towards an artificial nipple scented with a familiar, pre-exposed odor [22–25]. It is still unknown, however, if a familiar, pre-exposed odor can modulate intake of basic tastants such as quinine or sucrose. The hypotheses of the present study were that: a) the presence of a familiar odor would increase intake of saccharin and quinine, in newborn rats assessed via the artificial nipple, and that b) this modulation would be associated with behaviors indicative of a shift (from aversive to appetitive or, in the case of saccharin, an exacerbation of an inherent appetitive value) in the hedonic value of these tastants. These hypotheses were based on previous work suggesting that familiarity with odors or tastes early during sensitive periods of ontogeny can result in increased suckling, or increased acceptance of tastants or food later in ontogeny. For instance, Domínguez et al. [7]) observed that Wistar rats exposed to the chemosensory properties of ethanol during gestational days 17–20 exhibited greater consumption of a sucrose–quinine compound than controls exposed to vehicle in-utero. A number of studies [e.g., Kiefer et al. [12]] suggest that this tastant mimics the taste of ethanol. Pairings of a novel odor and ethanol administration in-utero seem to endow the odorant with reinforcing properties that enhance nipple attachment during the first suckling response [1]. Moreover, bitter and sour substances are innately rejected by humans, yet this pattern can be significantly altered by early exposure to the volatile component of flavors (for review and references, see [2]).

1. General methods

1.1. Subjects

Subjects were 165 male and female pups delivered via cesarean section from 29 Sprague–Dawley dams (Taconic, Germantown, NY) mated at the vivarium of the Department of Psychology at Binghamton University. Litter representation and number of subjects used in each experiment were as follows: Experiment 1 (55 animals, 7 litters), Experiment 2 (19 animals, 5 litters), Experiment 3 (35 animals, 9 litters), Experiment 4 (53 animals, 8 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) with birth occurring on E21 (P0). The vivarium had a 12 h/12 h light/dark cycle, with lights on at 7 am, and controlled temperature (22 °C) and humidity. All animals had ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Rats used in these experiments 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.

1.2. Cesarean delivery

Near term (E21) pups were delivered by cesarean section. Isoflurane (Baxter, Deerfield, IL; VetEquip, Pleasanton, CA) was used to anesthetize the dam during cesarean delivery. 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.

1.3. Apparatus

1.3.1. Heat chamber

Odor presentation was conducted in a controlled heat chamber (Microplate Incubator, Boekel Scientific, Feasterville, PA) maintained at 35 °C. Within the heating chamber, one male and one female littermates were placed into a plastic hexagonal cup (4 cm side, 2 cm high, 5 cm long). Animals pre-exposed to the odor were placed next to a cup with a cotton swab with .1 ml of a lemon scent (Lorann oils, Inc., Lansing, MI). Non pre-exposed animals remained in the incubator without any odor.

1.3.2. Surrogate nipple

The surrogate nipple was cast from rubber latex (AMACO rubber latex, Indianapolis, IN) and molded into a conical form to measure 12 mm long with a rounded tip measuring 1 mm in diameter and the