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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.

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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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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

base measuring 2.5 mm in diameter. The base of the surrogate nipple was attached to the end of an angled dental probe to facilitate presentation by the experimenter [33]. Polyethylene tubing (Clay Adams, Sparks, MD) extended throughout the length of the nipple and attached to a syringe containing the solutions. The syringe had a hole through the upper wall that generated a hydraulic system. Slight negative pressure, produced by the pups while attached to the nipple, was sufficient to extract voluntarily the fluids from the surrogate nipple. In turn, the artificial nipple had an alligator clip attached to it that allowed a cotton swab to be attached and present an odor at the time of nipple exposure. The individual subject was clamped in a semi-supine posture into a "vest" made from ultra-thin, elastic rubber. This light restraint prevented righting attempts but did not otherwise produce discomfort nor hinder the pups' movements.

1.4. Solutions

The saccharin solutions of 0.1% and 0.015% were prepared by diluting 100 mg or 15 mg, respectively, in 100 ml of distilled water. To prepare 0.1% and 0.2% quinine concentrations, 100 mg or 200 mg was diluted in 100 ml of distilled water, respectively (Sigma Aldrich, St. Louis, MO).

1.5. Procedure

Immediately after cesarean section pups were placed into heated chambers (based on the experimental condition) and exposed for 1 h to a cotton swab with the lemon odor or no odor, according to the group. Subsequently, they were placed for 3 (Experiment 1) or 2 h (Experiments 2 to 4) into an incubator based on odor exposure to avoid cross contamination of odors. The test began immediately afterwards. Pups were gently stimulated in the anal/genital region with cotton to induce urination and/or defecation. Subsequently, they were weighed to the nearest 0.01 g and placed individually into the test container for 6 min, on a mirror maintained at 35.5 °C ± .5 °C. The test involved the presentation of different solutions through the artificial nipple in the presence of lemon odor with a gentle application of the tip of the nipple to the perioral area of the subject for 6 min. Once the grasp response was made, the rats could obtain 0.1% or 0.015% of saccharin (Experiments 1 and 2) or 0.1% or 0.2% of quinine (Experiments 3 and 4) through the nipple. At the end of the session, pups were removed from the testing container, dried with a paper towel, weighed to the nearest 0.01 g and returned to the incubator. The oral grasp response involved an active movement of the head towards the surrogate nipple, which resulted in the tip of the nipple entering the oral cavity and the mouth closing around the nipple. From this response the following measures were obtained: a. Latency to grasp (time to first grasping response), b. total time of grasping (total time spent on the nipple or sum of the duration of all grasps), c. number of grasps (attachments initiated) and d. mean duration of an individual grasp response. Finally, body weight gain was calculated {100 × [(post-weight − pre-weight) / pre-weight]} as an estimate of fluid consumption. Order of testing for the different treatment groups was counterbalanced across the experiment.

Warmth and a conspecific were present during odor exposure and the 2–3 h interval to emulate the features of the nest environment. To eliminate the possible confounding of litter with treatment effects, only one subject of each sex from a given litter was assigned to a treatment condition [41]. Each condition included an equal number of male and female subjects.

All experiments were recorded in a continuous fashion and subsequently analyzed by two observers blinded to the conditions (reliability between experimenters included > 90% agreement).

1.6. Data analysis

In Experiments 1 and 4 analyses of variance were run for each dependent variable using Statistica version 8.0. The between-group ANOVAs used odor condition (prior odor exposure or no prior odor exposure) and concentration (high or low concentration) as independent factors. Analyses were supplemented by post-hoc comparisons [Tukey HSD test]. In Experiments 2 and 3 independent samples t Tests were run, where the grouping factor was odor condition (prior odor exposure or no prior odor exposure). Across experiments, sex of the animals did not affect any of the measures, thus the analysis was performed collapsing by sex. Significance was indicated when p values were less than or equal to .05.

Experiment 1. Responsiveness to a surrogate nipple providing a sweet taste (saccharin) in the presence of a pre-exposed odor.

Experiment 1 assessed the effects of stimulation with a familiar, pre-exposed odor on attachment to an artificial nipple delivering 0.1% or 0.015% saccharin. A 2 (odor pre-exposure) × 2 (saccharin concentration) factorial design was employed. Four groups were thus formed: odor-sac .1 (prior odor exposure — 0.1% of saccharin in the artificial nipple, $n = 14$), no odor-sac .1 (no prior odor exposure — 0.1% of saccharin in the artificial nipple, $n = 14$), odor-sac .015 (prior odor exposure — 0.015% of saccharin in the artificial nipple, $n = 13$), and no odor-sac .015 (no prior odor exposure — 0.015% of saccharin in the artificial nipple, $n = 14$).

1.7. Results

The ANOVA for mean duration of nipple attachment indicated a significant main effect of saccharin concentration. Animals exhibited a higher mean duration of grasps with the 0.1% saccharin solution, regardless of the odor condition at initial exposure, $F(1, 51) = 5{,}96$, $p < .02$ (Fig. 1). Neither pre-exposure to the odor nor the interaction between pre-exposure and saccharin concentration achieved significance ($p > .05$). The analysis for duration of grasps yielded a marginally significant effect of saccharin concentration, $F(1, 51) = 3{,}59$, $p < .06$. No differences for the two odor conditions were observed in terms of body weight gain, number of grasps and latency to grasp ($p > .05$).

These results indicate that newborn rats can adjust their nipple attachment behavior as a function of the magnitude of the appetitive reinforcer received. Our hypothesis of greater nipple approach in animals stimulated with the pre-exposed odor, however, was not confirmed.

Experiment 2. Responsiveness to a surrogate nipple providing a sweet taste in the presence of a pre-exposed odor, using a short interval between pre-exposure and test.

Experiment 2 replicated the procedures of Experiment 1, but used a shorter interval (i.e., 2 instead of 3 h) between pre-exposure and test, and pups were exposed to only 0.1% saccharin at test. This concentration was chosen because it had yielded the most robust response in the previous experiment. Two groups were formed: odor (prior odor exposure — 0.1% of saccharin in the artificial nipple, $n = 10$) and no odor (no prior odor exposure — 0.1% of saccharin in the artificial nipple, $n = 9$).

1.8. Results

Pups pre-exposed to lemon odor were not statistically different from nonexposed pups in terms of body weight gain, duration of grasps, mean duration of grasps, number of grasps and latency to grasp ($p > .05$; data not shown). This experiment confirms that exposure to a familiar odor does not alter grasps responses or intake to an artificial nipple providing a sweet (appetitive) taste.

Experiment 3. Responsiveness to a surrogate nipple providing a bitter taste in the presence of a pre-exposed odor.

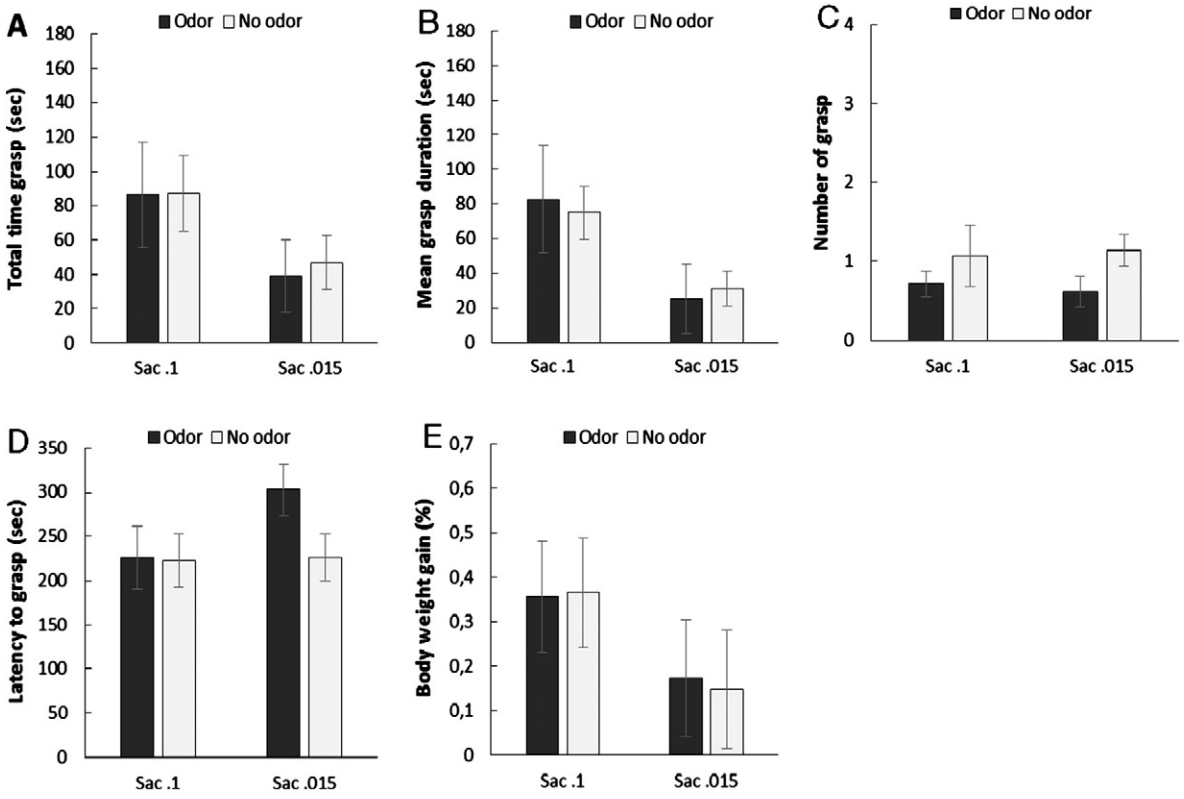

Fig. 1. Experiment 1. Mean percentage (±SE) of (A) total time grasp, (B) mean grasp duration, (C) number of grasp, (D) latency to grasp and (E) body weight gained, during the 6-min presentation of the scented artificial nipple containing 0.1% (left bars) or 0.015% of saccharin solution (right bars).

In Experiment 3, grasping and intake behaviors in the presence of a pre-exposed odor were evaluated with the nipple dispensing an aversive solution (i.e. quinine). Studies with newborn rats reported lower grasping and intake responses to the nipple when using quinine than when using saccharin solution or water [30], a result that suggests quinine induces an aversive hedonic state. Furthermore, tongue stimulation with quinine reliably elicits orofacial disgust reactions in different species, including the rat [4,9]. The aim of this experiment was to assess whether pre-exposure to an odor increases intake and grasp responses towards an artificial nipple containing quinine, in newborn rats. The interval between termination of odor pre-exposure and test was 2 h. Two groups were formed: odor (prior odor exposure — 0.1% of quinine in the artificial nipple, $n = 17$) and no odor (no prior odor exposure — 0.1% of quinine in the artificial nipple, $n = 18$).

1.9. Results

Fig. 2 shows latency to grasp the nipple, grasping frequency, total time attached to the nipple, average duration of each grasp, and body weight gain during the test. It seems that odor pre-exposure significantly facilitated approach and contact with the nipple, and had a facilitating effect on quinine intake. The statistical analysis supported these observations. Subjects pre-exposed to the olfactory stimulus showed, compared to non-pre-exposed animals, significantly greater percentage of body weight gain, $t(33) = 3{,}73$, $p < .0007$; total time grasps (attach duration), $t(33) = 6{,}12$, $p < .000001$; number of grasps, $t(33) = 3{,}70$, $p < .0008$; mean duration of grasps, $t(33) = 5{,}02$, $p < .00002$ and latency to grasp the nipple, $t(33) = -3{,}98$, $p < .0004$.

In summary, a pre-exposed odor enhanced seeking and intake of an otherwise aversive solution, such as 0.1% quinine. These results suggest that the pre-exposed odor may have produced a change in the hedonic value of the aversive reward.

Experiment 4. Responsiveness to a surrogate nipple providing a bitter taste solution in the presence of a pre-exposed odor: exploring the boundaries of the effect.

The previous experiment indicated that a lemon odorant, made familiar through pre-exposure, increased the seeking and acceptance of quinine, presumably by altering the hedonic value of this bitter taste. Experiment 4 tested the boundaries of this effect by increasing the concentration of quinine, and hence its innate aversiveness. Thereby the test was performed using 0.1% or 0.2% quinine, in four independent groups: odor-qui .1 (prior odor exposure — 0.1% of quinine in the artificial nipple, $n = 14$); no odor-qui .1 (no prior odor exposure — 0.1% of quinine in the artificial nipple, $n = 14$), odor-qui .2 (prior odor exposure — 0.2% of quinine in the artificial nipple, $n = 11$) and no odor-qui .2 (no prior odor exposure — 0.2% of quinine in the artificial nipple, $n = 14$).

1.10. Results

As illustrated in Fig. 3, pre-exposure to the lemon odor increased quinine seeking behaviors, in most of the measurements, yet only in those that received the lower concentration of quinine. For total time of grasping, the ANOVA (between factors: odor exposure and quinine concentration) as independent factors, revealed significant main effects of odor exposure, $F(1, 49) = 11.97$, $p < .001$, and concentration of quinine, $F(1, 49) = 19.32$, $p < .00006$. The interaction between these factors also achieved significance, $F(1, 49) = 11.65$, $p < .001$. Post-hoc tests revealed that odor-qui .1 grasped significantly more than odor-qui .2 ($p < .0002$), no odor-qui .1 ($p < .0002$) and no odor-qui .2 ($p < .0002$). Although the statistical analysis for frequency measurement yielded a main effect of concentration of quinine, $F(1, 49) = 4.66$, $p < .03$, pre-exposure to the odor was effective only for animals receiving 0.1% quinine, $F(1, 27) = 4.19$, $p < .05$, as planned comparisons revealed. Similarly, the analysis for latency to grasp the nipple revealed a main

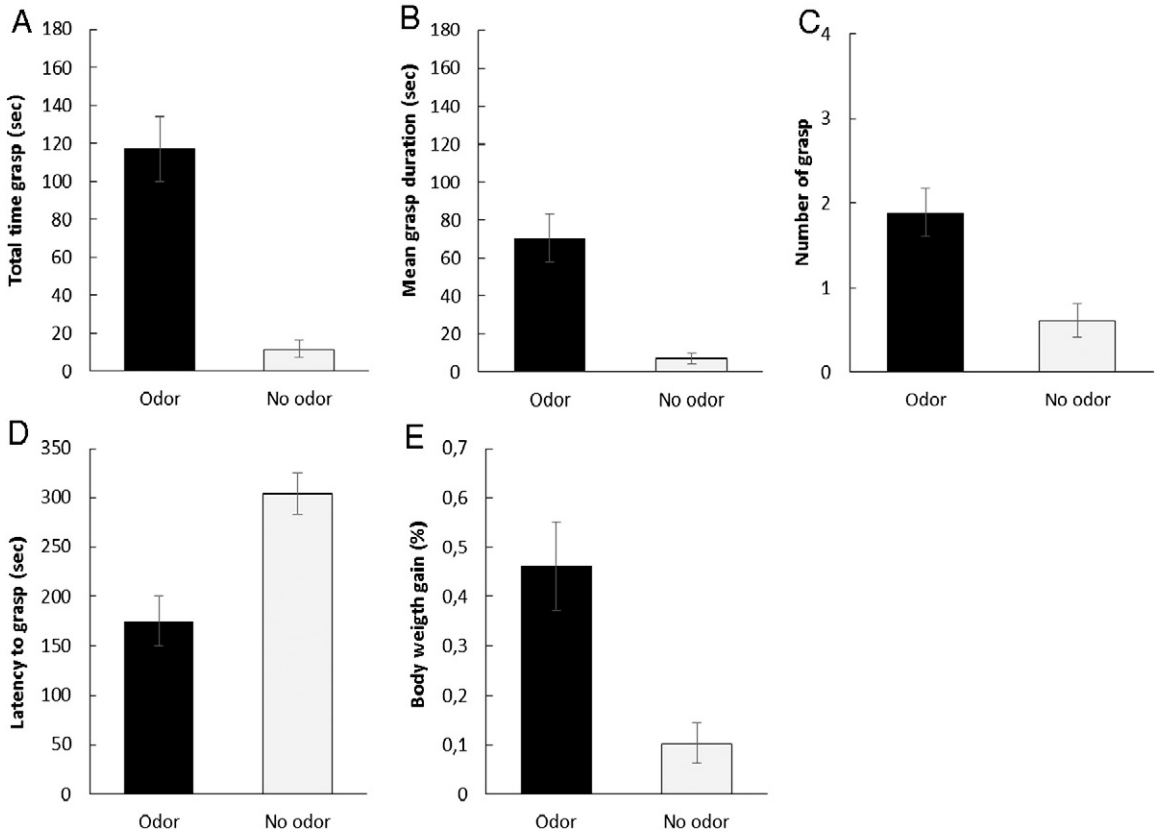

Fig. 2. Experiment 3. Mean percentage (±SE) of (A) total time grasp, (B) mean grasp duration, (C) number of grasp, (D) latency to grasp and (E) body weight gained, during the 6-min presentation of the scented artificial nipple containing 0.1% of quinine solution.

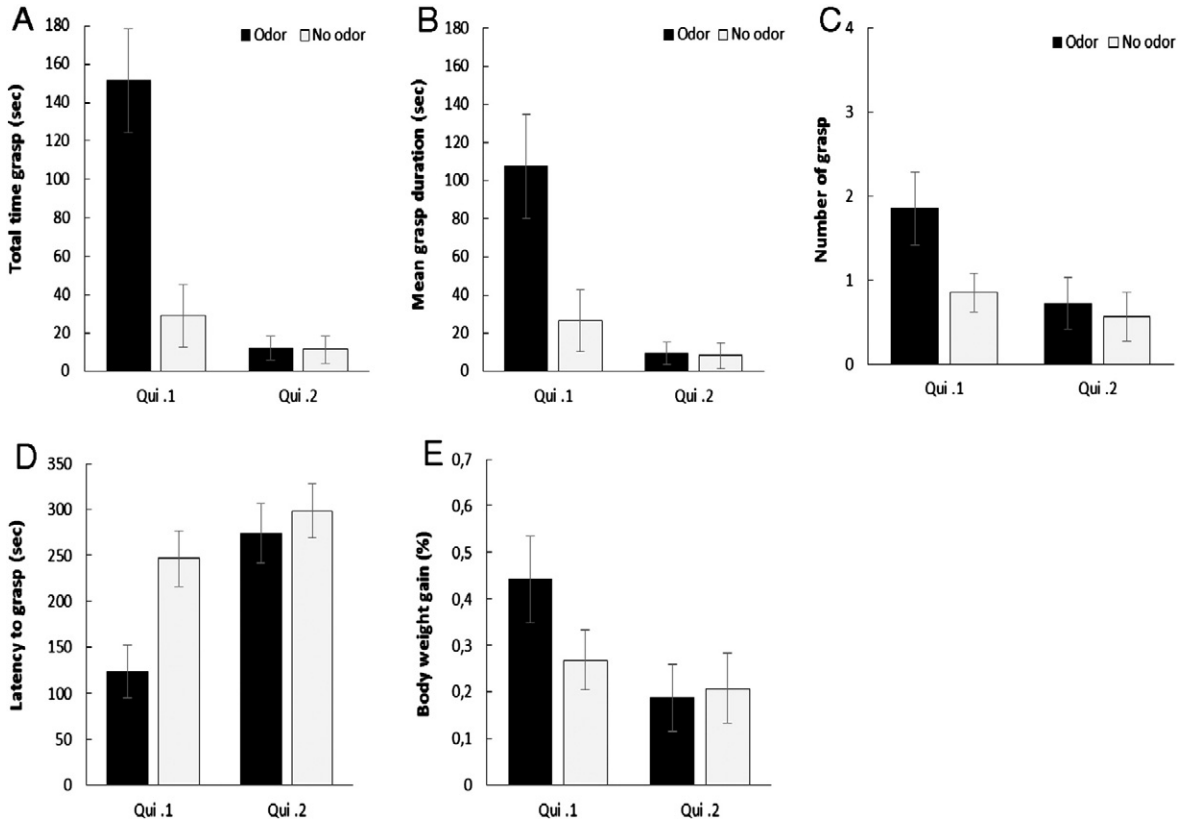

Fig. 3. Experiment 4. Mean percentage (±SE) of (A) total time grasp, (B) mean grasp duration, (C) number of grasp, (D) latency to grasp and (E) body weight gained, during the 6-min presentation of the scented artificial nipple containing 0.1% (left bars) or 0.2% of quinine solution (right bars).

effect of odor exposure, $F(1, 49) = 5.92$, $p < .02$ and concentration of quinine, $F(1, 49) = 11.09$, $p < .002$. Planned comparisons yielded significant differences between odor-qui .1 and no odor-qui .1, $F(1, 27) = 8.81$, $p < .006$. Analysis of mean duration of grasps revealed a significant main effect of odor exposure, $F(1, 49) = 5.56$, $p < .02$, concentration of quinine, $F(1, 49) = 11.05$, $p < .002$, and a significant odor exposure by concentration of quinine interaction, $F(1, 49) = 5.17$, $p < .03$. Post-hoc tests revealed that odor-qui .1 differs from odor-qui .2 ($p < .002$), no odor-qui .1 ($p < .007$) and no odor-qui .2 ($p < .0009$). The ANOVA for percentage of body weight gained yielded a main effect of concentration of quinine, $F(1, 49) = 4.04$, $p < .05$. Animals that received 0.1% of quinine solution showed significantly greater body weight gained than pups that received 0.2% quinine.

These results replicate those found in Experiment 3 and suggest that pre-exposure to the lemon odor is not effective to promote subsequent quinine seeking and intake when the intensity of the aversive solution is increased.

2. Discussion

Altricial species have mechanisms that facilitate the infant's attachment process through a great plasticity of maternal odor learning. Given the fact that maternal odor changes based on diet and pups are born without fully developed visual and auditory systems, such plasticity in odor learning allows the pups to find the nest and maternal nipple through smell [32]. During the sensitive period, that lasts the first 10 days of a rats life, facilitation in the learning of odors is produced to both maternal and neutral smells [14]. In this study, rats were exposed to a neutral odor (i.e., lemon odor) immediately after birth.

There is a controversy in the literature regarding the ability of rats to recognize flavors at early stages of development. Although previous literature suggests that rats do not detect bitter tastes until PND 10–12, inasmuch as taste buds are not functionally mature until that age [10,11,37], vast evidence reflects that newborn rats detect and discriminate several flavors, which is evidenced in their ability to respond differentially to them [13,30]. They also react differently to solutions with diverse nutritional qualities [34], and acquire complex associations derived from first and second order conditioning, and sensory preconditioning preparations [5,6].

This work also shows that shortly after birth, rats discriminate between concentrations of saccharin and between concentrations of quinine. Given a high concentration of saccharin, there is an increase in the duration and mean duration of grasps to the nipple, compared to animals receiving a lower concentration, regardless of the pre-exposure to the odor condition. Overall, these antecedents show that the intake behavior of newborn rats involves sophisticated mechanisms of taste detection. Future research should elucidate the physiological mechanisms involved in detecting flavors and the interaction of taste and smell senses during early ontogeny of the rat.

The most surprising result of this investigation is that in the presence of a pre-exposed odor, newborn rats increase intake and grasp responses to the artificial nipple containing quinine, which is considered an aversive solution [4]. Odor pre-exposed animals exhibited greater percentage of body weight gain and grasping behaviors, and a decrease in latency to the first grasp towards the nipple, in comparison to the control, non-pre-exposed group.

An important caveat, however, was the lack of a group pre-exposed to lemon odor right after birth, but tested without the presence of the odor. This additional control could have helped discard alternative explanations related to non-specific changes in behavior due to pre-exposure alone. The addition of a group of animals pre-exposed to lemon but tested under a different yet similarly salient odor could also have helped understand the generalization of the phenomena under analysis. Another useful control would have been animals that received water in the presence of the pre-exposed odor. This condition could have helped discard the possibility that the increased appetitive responses were emitted due to mere exposure to the odor. This possibility, however, seems unlikely when considering that the pre-exposed odor did not affect saccharin seeking and intake, nor responsiveness towards the higher concentration of quinine.

Experiment 4 assessed the boundaries of the odor pre-exposure phenomenon. Facilitation of quinine seeking and intake occurred with a moderate (0.1%), but not with a more concentrated (0.2% quinine) solution. Previous work indicated that experiences with chemosensory stimuli produce a change in responses to stimuli of the same sensory modality [24,26]. New information provided by this study is that an olfactory stimulus, that acquired familiarity due to the occurrence of explicit pre-exposure, increased intake of an aversive tastant. The effect did not occur when the nipple delivered an appetitive stimulus (Experiments 1 and 2).

The results could not be explained by mere familiarity with the stimulus. The groups receiving saccharin and pre-exposed to the odor did not exhibit an increase in their responses, although the possibility of a functional ceiling effect cannot be discarded (although the response to quinine exceeded that of the highest concentration of saccharin in almost all measures, suggesting that a ceiling effect is not a highly plausible possibility). Furthermore, moderate and strong concentrations of quinine by themselves produced minimal intake and grasping responses. It seems that in order for this exacerbated increase in behavior to appear, two conditions must occur: odor pre-exposure, and subsequently the odor has to be presented in conjunction with an aversive taste, in this case, quinine. These results could indicate that pre-exposure to an odor produces an effect of facilitation of intake against aversive solutions, or it could reflect an associative process. This is the first evidence of such phenomena, and future research is necessary to reveal the mechanisms involved.

This phenomenon was observed within a sensitive period in which there is considerable odor learning plasticity. In this period, odor preferences occur, even when a new odor is paired with an aversive stimulus such as electric shock [35], tail pinch [41] or rough handling generated by maternal care [35]. Enhanced responsiveness in the present and in previous studies by Sullivan could be due to the fact that during the sensitive period aversive rewards — irrespective of whether they are flavors or painful tactile stimulation, rough handling or tail pinch — undergo an hedonic shift after being paired with odors. This could suggest the presence of a protective mechanism against aversive stimuli to facilitate attachment to the caregiver.

These results relate to studies conducted with human infants. These subjects seem to exhibit a sensitive period, before 4 months of age, during which solutions typically rejected are accepted. For example, hydrolyzed protein hydrolysate milk formulas (PHFs) are extremely unpalatable compared to cow milk-based formulas (CMFs) because of bitter and sour taste components. Infants showed greater acceptance of PHFs at ages < 3.5 months old [17,21]. From 4 months of age and until adulthood, PHF is rejected, unless the individual has been exposed to PHF during early life. Other related, yet important problems that could be addressed with the model proposed are the acceptance of unpalatable drugs or food, during early infancy. The unpleasant flavor of medicine may cause babies to reject its intake, and result in dose deficiencies, which in some cases can be life-threatening [19]. Similarly, healthy foods such as vegetables are usually rejected at least partly due to their bitter taste (e.g., broccoli). Food consumed during childhood is generally accepted in later stages of development and these habits generated at early stages, contribute to individual health. Several diseases are associated with an unhealthy diet, such as obesity and diabetes [3]. Research with animal models, which consider specific associations of tastes and odors, may help develop techniques to gain acceptance of unpalatable drugs in pediatric populations, as well as food with bitter taste.

The newborn rat represents a neurological model of the human in the third trimester of gestation, thus this preparation could be an animal model of intake behaviors and factors that modulate these behaviors in

preterm infants [34]. Further research is necessary to determine if this is a suitable animal model for studying such phenomena with human babies, but these early results provide fruitful avenues to address the ontogeny of intake responses to aversive taste stimuli.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2014.12.041.

References

[1] Abate, Varlinskaya, Cheslock, Spear, Molina, Neonatal activation of alcohol-related prenatal memories: impact on the first suckling response, Alcohol. Clin. Exp. Res. 26 (10) (2002) 1512–1522.
[2] G.K. Beauchamp, J.A. Mennella, Early flavor learning and its impact on later feeding behavior, J. Pediatr. Gastroenterol. Nutr. 48 (1) (2009) 25–30.
[3] G.K. Beauchamp, A. Mennella, Flavor perception in human infants: development and functional significance, Digestion 83 (2011) 1–6.
[4] K.C. Berridge, Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns, Neurosci. Biobehav. Rev. 24 (2000) 173–198.
[5] S.J. Cheslock, E.I. Varlinskaya, J.M. High, N.E. Spear, Higher order conditioning in the newborn rat: effects of temporal disparity imply infantile encoding of simultaneous events, Infancy 4 (2003) 157–176.
[6] S.J. Cheslock, E.I. Varlinskaya, E.S. Petrov, N.E. Spear, Rapid and robust olfactory conditioning with milk before suckling experience: promotion of nipple attachment in the newborn rat, Behav. Neurosci. 114 (2000) 484–495.
[7] H.D. Domínguez, M.F. López, J.C. Molina, Neonatal responsiveness to alcohol odor and infant alcohol intake as a function of alcohol experience during late gestation, Alcohol 16 (2) (1998) 109–117.
[8] G.A. Gemberling, M. Domjan, Selective associations in one-day-old rats: taste-toxicosis and texture-shock aversion learning, J. Comp. Physiol. Psychol. 96 (1) (1982) 105–113.
[9] H. Grill, R. Norgren, The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats, Brain Res. 143 (1978) 263–279.
[10] W.G. Hall, T.E. Bryan, IV. The ontogeny of feeding in rats: taste development as measured by intake and behavioral responses to oral infusions of sucrose and quinine, J. Comp. Physiol. Phsychol. 95 (1981) 240–251.
[11] D.L. Hill, R.M. Bradley, C.M. Mistretta, Development of taste responses in rat nucleus of solitary tract, J. Neurophysiol. 50 (1983) 879–894.
[12] S.W. Kiefer, K.G. Hill, D.L. Coonfield, F.A. Ferraro, Ethanol familiarity and naltrexone treatment affect ethanol responses in rats, Alcohol 37 (2005) 167–172.
[13] A.P. Kozlov, E.S. Petrov, E.I. Varlinskaya, N.E. Spear, Taste differentiation in the context of suckling and independent, adultlike ingestive behavior, Dev. Psychobiol. 48 (2006) 133–145.
[14] M.S. Landers, R.M. Sullivan, The development and neurobiology of infant attachment and fear, Dev. Neurosci. 34 (2012) 101–114.
[15] S.V. Lipchock, D.R. Reed, J.A. Menella, The gustatory and olfactory systems during infancy: implications of development of feeding behaviors in the high risk neonate, Clin. Perinatol. 38 (2011) 627–641.
[16] J.A. Mennella, G.K. Beauchamp, The human infant's response to vanilla flavors in mother's milk and formula, Infant Behav. Dev. 19 (1996) 13–19.
[17] J.A. Mennella, G.K. Beauchamps, Developmental change in the acceptance of protein hydrolyzate formula, J. Dev. Behav. Pediatr. 17 (1996) 386–391.
[18] J.A. Mennella, G.K. Beauchamp, Development and bad taste, Pediatr. Asthma Allergy Immunol. 12 (1998) 161–163.
[19] J.A. Mennella, G.K. Beauchamp, Optimizing oral medications for children, Clin. Ther. 30 (2008) 2120–2132.
[20] J.A. Mennella, C.P. Jagnow, G.K. Beauchamp, Prenatal and postnatal flavor learning by human infants, Pediatrics 107 (2001) 88–94.
[21] J.A. Mennella, L.D. Lukasewycz, S.M. Castor, G.K. Beauchamp, The timing and duration of a sensitive period in human flavor learning: a randomized trial, Am. J. Clin. Nutr. 93 (2011) 1019–1024.
[22] S.S. Miller, N.E. Spear, Olfactory learning in the rat neonate soon after birth, Dev. Psychobiol. 50 (2008) 554–565.
[23] S.S. Miller, N.E. Spear, Olfactory learning in the rat immediately after birth: unique salience of first odors, Dev. Psychobiol. 51 (2009) 488–504.
[24] S.S. Miller, N.E. Spear, Mere odor exposure learning in the rat neonate immediately after birth and 1 day later, Dev. Psychobiol. 52 (2010) 343–351.
[25] J.C. Molina, M.G. Chotro, Association between chemosensory stimuli and cesarean delivery in rat fetuses: neonatal presentation of similar stimuli increases motor activity, Behav. Neural Biol. 55 (1991) 42–60.
[26] J.C. Molina, N.E. Spear, Consequences of early exposure to alcohol: how animal studies reveal later patterns of use and abuse in humans, in: M.E. Carroll, J.B. Overmier (Eds.),Animals Research and Human Health, 2001, pp. 79–85.
[27] S. Moriceau, T.L. Roth, T. Okotoghaide, R.M. Sullivan, Corticosterone controls the developmental emergence of fear and amygdala function to predator odors in infant rat pups, Int. J. Dev. Neurosci. 22 (2004) 415–422.
[28] S. Moriceau, R.M. Sullivan, Neurobiology of infant attachment, Dev. Psychobiol. 47 (2005) 230–242.
[29] M.E. Nizhnikov, E.S. Petrov, E.I. Varlinskaya, N.E. Spear, Newborn rats' first suckling experience: taste differentiation and suckling plasticity, Physiol. Behav. 76 (2002) 181–198.
[30] R.M. Pautassi, M.E. Nizhnikov, N.E. Spear, Ethanol-mediated appetitive conditioning in infant rats, but not corticosterone release, is dependent on route of ethanol administration, Dev. Psychobiol. 54 (2012) 98–104.
[31] P.E. Pedersen, C.L. Williams, E.M. Blass, Activation and odor conditioning of suckling behavior in 3-day-old albino rats, J. Exp. Psychol. Anim. Behav. Process. 8 (1982) 329–341.
[32] E.S. Petrov, E.I. Varlinskaya, W.P. Smotherman, The newborn rat ingests fluids through a surrogate nipple: a new technique for the study of early suckling behavior, Physiol. Behav. 62 (1997) 1155–1158.
[33] E.S. Petrov, M.E. Nizhnikov, A.P. Kozlov, E.I. Varlinskaya, T.A. Kramskaya, N.E. Spear, Repetitive exposures to a surrogate nipple providing nutritive and non-nutritive fluids: effects on suckling behavior of the newborn rat, Appetite 43 (2004) 185–194.
[34] C. Raineki, S. Moriceau, R.M. Sullivan, Developing a neurobehavioral animal model of infant attachment to an abusive caregiver, Biol. Psychiatry 67 (2010) 1137–1145.
[35] B. Schaal, L. Marlier, R. Soussignan, Human fetuses learn odors from their pregnant mother's diet, Chem. Senses 25 (2000) 729–737.
[36] G.J. Schwartz, H.J. Grill, Comparing taste-elicited behaviors in adult and neonatal rats, Appetite 6 (1985) 373–386.
[37] G. Stickrod, D.P. Kimble, W.P. Smotherman, In utero taste/odor aversion conditioning in the rat, Physiol. Behav. 28 (1) (1982) 5–7.
[38] R.M. Sullivan, Developing a sense of safety, in: J. King, C. Ferris, I. Lederhendler (Eds.),Neurobiology of neonatal attachment, Roots of Mental Illness in Children, 2003, pp. 122–132.
[39] R.M. Sullivan, M.A. Hofer, S.C. Brake, Olfactory-guided orientation in neonatal rats is enhanced by a conditioned change in behavioral state, Dev. Psychobiol. 19 (1986) 615–623.
[40] K.J. Upton, R.M. Sullivan, Defining age limits of the sensitive period for attachment learning in rat pups, Dev. Psychobiol. 52 (2010) 453–464.
[41] R.R. Holson, B. Pearce, Principals and pitfalls in the analysis of prenatal treatment effects in multiparous species, Neurotoxicol. Teratol. 14 (1992) 221–228.