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Detection of novelty by perinatal rats

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Abstract

This study investigated the development of fetal/neonatal rats' ability to distinguish between a novel and familiar taste. Here, we report that neonatal rats alter their orofacial movements (e.g., mouth movements and licks) upon tasting saccharin (SAC) if it was experienced previously. We also sought to determine the origins and duration of this response. Fetuses of embryonic ages E17, E18, or E19 received an oral injection of 10 μL 0.3% SAC while in utero. These animals were then reexposed to SAC on postnatal day 3, (P3) and observations of orofacial motor responses were recorded. Only neonates that first experienced SAC on E19 exhibited a SAC-induced stimulation of mouthing and licking on P3. These data suggested that a taste-recognition memory (TRM) is maintained for up to 5 days (i.e., E19 to P3). However, in this paradigm, the youngest fetuses also have the longest retention interval. Could these data also reflect the limitations of the E17 and E18 fetuses in retaining the TRM? In a second study, we shortened the taste exposure–reexposure interval to 2 days in an attempt to detect the TRM in younger fetuses. As expected, E19 rats exhibited a TRM when tested on E21. However, neither the E17 nor E18 fetuses showed SAC-induced increases in mouthing and licking when tested 2 days after their initial exposure (E19 or E20). Finally, in order to determine whether a TRM could be detected in fetuses as well as neonates (see above), we conducted an additional study wherein E21 fetuses were tested before parturition. Like E21 neonates, E21 rat fetuses that had received SAC on E19 showed a differential response to SAC depending on whether it was novel or familiar. Thus, although E21 fetal orofacial movements were less frequent than those of the E21 neonate, the fetal-testing procedures were not sufficient to obscure the detection of a TRM. In summary, the data indicate that E19 rat fetuses can acquire a TRM and retain it for at least 2–5 days, whereas E17 and E18 fetuses cannot.

Keywords: Taste recognition memory; Fetus; Neonate; Rat; Novelty; Familiar; Memory; Saccharin; Motor responses; Ontogeny; Development

1. Introduction

As new information is sensed by an organism, it must be analyzed and sorted, and decisions must be made regarding its relevance in terms of possible benefit, danger, or insignificance. In particular, a decision about the information's novelty is one of the primary filters used as an organism determines whether or not the information should be attended to, and possibly encoded, for long-term storage [48]. Incorporating new information into long-term memory is presumably more conducive to survival than is duplicating information that may be already available in the memory store. Determining and seeking out novelty is also an adaptive means by which animals avoid stimulus re-exposure and therefore maximize the amount of new (i.e., nonredundant) information available [17]. This tendency to discriminate between novel and familiar objects has been exploited in early clinical assessments of intelligence in human infants [7].

Historically, the neonate (and certainly the fetus) was seen as unable to sense, respond or learn [14]. This view no longer reflects the data now at hand. Infants and, to a lesser extent, fetuses are now widely recognized as having a diverse behavioral repertoire and intellectual abilities for perceiving and interacting with their environment [36,37]. However, a major limiting factor in our knowledge of developing fetal and neonatal sensory/cognitive capacities is the difficulty one faces in assessing these abilities in organisms that have immature sensory and motor functioning. Because the gustatory and olfactory systems are fairly well developed late in gestation [46], our lab has been studying these systems as a means of assessing the ability of perinatal rats to detect and remember stimuli.

The data reported here, and elsewhere, suggest that young rats can discriminate between a novel taste and a familiar taste that was first experienced in utero. Smotherman [32] exposed E20 rat fetuses to either apple juice or saline via an amniotic fluid injection. When presented with a choice between apple juice and tap water, young-adult rats prenatally

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exposed to apple juice consumed more apple juice than did control rats lacking the prenatal experience with this taste. Similarly, Hepper [11] has demonstrated that if pregnant dams eat garlic, they produce offspring that exhibit an enhanced preference for the odor of garlic when tested 12 days after birth. Additional evidence suggests that the injection of citral (a lemon odor) into the amniotic fluid, in conjunction with immediate postnatal exposure to citral, induces a preference in pups to attach to nipples painted with citral [24].

The current study extends this literature. Here, fetuses were exposed to a novel taste (saccharin) or a control liquid (water). Days later, rats from each group tasted saccharin. We report that neonates exhibit patterns of orofacial motor-responding indicative of the novelty or familiarity of the taste. Further, we explored the ontogeny of these responses by testing fetal and neonatal rats at various ages and exposure–reexposure intervals.

2. General methods

2.1. Subjects

The subjects were fetal and neonatal Sprague–Dawley rats (male and female) obtained from timed-pregnant dams supplied by Zivic-Miller Laboratories (Zelienople, PA). The date of conception (i.e., the first day that a vaginal plug was detected) was designated as "embryonic day 0" (E0). Pregnant animals (from which our subjects were derived) were individually housed in plastic "shoe box" cages (44.45 × 21.59 × 20.32 cm). Home cage temperature was maintained at 23–26°C under a 12/12-h light/dark cycle (lights on at 0600 h).

2.2. Fetal injections

Pregnant rat dams carrying E17 to E19 fetuses were briefly anesthetized with Isoflurane before they underwent a reversible spinal block procedure. A 30-gauge needle was used to inject lidocaine HCl 2% and epinephrine 1:100,000 (in a volume of 100 μL) between the first and second lumbar vertebrae. This procedure is effective in producing (a) a complete abdominal and hind-limb paralysis, (b) consistently long periods of spinal anesthesia (>45 min), and (c) complete recovery after the anesthesia. There is no indication that litters are adversely affected by this procedure [36,40].

The analgesic dam was restrained in a plastic holding apparatus, and her vision of the fetal injection procedure was restricted. Both uterine horns were exposed through a midline laparotomy, and the hind legs and lower abdomen were immersed in a warm bath (37.5°C ± 1°C) containing isotonic saline (Locke's solution) [8]. Both horns of the uterus were exteriorized through the abdominal incision, and the horns were allowed to float freely in the bath. E16+ rat fetuses can be seen through the walls of the uterus and positioned for accurate placement of injections [44]. We used a special submersible back light to facilitate these injections [26]. All fetuses in a particular litter received oral lavage with either saccharin (10 μL 0.30%; SAC) or H_2O (10 μL) via a blunted 30-gauge needle. Following the injections, the uterus was replaced, the abdominal wall and the skin of the pregnant rat sutured, and the wounds infused with a local anesthetic (Bupivacaine 0.25%) in order to produce postsurgical analgesia.

Even et al. [6] have reported that steroids present in one amniotic sac may diffuse across the fetal membranes to other fetuses in the uterus. Although we injected fluids into the mouth of the fetus, saccharin–water almost certainly also spilled into the amniotic fluid and may have moved into adjacent uterine compartments. If different pups in a litter had different oral injections, this could have confounded our conditioning procedure. For this reason, we did not mix different taste injections within litters. This procedure necessitated special data analysis techniques (see "Statistical analysis," below).

3. Experiment 1

3.1. Methods

3.1.1. Behavioral testing

On P3, rats that received oral lavage as fetuses were observed for their behavioral reactions after an oral injection of SAC. Rat pups were born via a normal vaginal delivery. Twenty minutes before the behavioral test, pups were separated from the dam and placed with littermates in a small plastic container sitting on a warm (38.5 ± 0.5°C) heating pad. This container was covered with gauze and maintained in a temperature-controlled incubator (ambient temperature = 28 ±1°C) until immediately before testing of the litter began. For the behavioral observations, neonates were placed in a warm (ambient temperature = 28 ± 1°C), high-humidity chamber on a glass plate warmed (via constantly circulating water) to 36 ± 1°C. Pups received oral lavage with 10 μL SAC through a blunt/smooth, 18-gauge, stainless-steel infusion needle. Subjects were then placed (ventral side down) on the glass plate. Using a mirror, behavior was videotaped from below the animal for 1 min before and after oral injection.

3.1.2. Dependent variables

Rat behaviors were recorded on videotape and later reviewed and scored with the help of the Observer computer program developed by Noldus Information Technology (Sterling, VA). Using a modification of the methods described by Smotherman et al. [33], we sorted observed behaviors into 12 exclusive and exhaustive categories of spontaneous fetal behavior. Because they seemed to be the most sensitive indicators of taste recognition, this paper focuses on orofacial movements: mouth movements and licks.

3.1.3. Treatment groups and age groups

Fetuses of embryonic ages E17, E18, or E19 received an oral injection of 10 μL, 0.3% SAC, or H_2O while in utero

(see above). In Experiment 1, these animals were subsequently exposed to SAC on postnatal day 3 (P3) before observations of motor responses were made. This study allowed us to determine the behavioral responses of P3 neonates to a novel or familiar taste experienced during different days of perinatal development. Throughout this paper, the groups of animals are designated by the subject's age during the first oral lavage and their age at time of the behavioral test. Therefore, the age groups in Experiment 1 were E17-P3, E18-P3, and E19-P3. The number of subjects/litters in each group were as follows: E17-P3, SAC preexposure: 16/4; E17-P3, water preexposure: 13/3; E18-P3, SAC preexposure: 14/4; E18-P3, water preexposure: 16/4; E19-P3 SAC preexposure: 10/2; E19-P3, water preexposure: 25/9.

3.1.4. Statistical analyses

The data were analyzed via analyses of variance (ANOVAs) using a linear model (SAS, SAS Institute, Cary, NC) compensating for unequal *n* values. Because all the rats in a particular litter received the same conditioning treatment, we included litter as an independent, random, and nested factor (within the two preexposure treatments). This approach controls for litter effects and offers a direct statistical test of the significance of such effects [5,12]. In the analyses conducted here, effects attributable to litter were not statistically significant and therefore, subsequent analyses were run without this nested factor. Post-hoc analyses employed Duncan's multiple range test [16]. An $\alpha = 0.05$ was adopted throughout these tests.

3.2. Results

Perinatal rats discriminated between novel and familiar tastes. Pups that received oral lavage with saccharin on E19 exhibited significantly more mouth movements and licks (compared with control rats receiving oral lavage with water on E19) when reexposed to saccharin on P3 (see Fig. 1). However, this effect was not observed in rats preexposed to saccharin on either E17 or E18 and tested on P3. A two-way ANOVA (Treatment [SAC pretreatment or H_2O control] $\times$ subject age at time of initial and subsequent exposure [E17-P3, E18-P3, or E19-P3]) compared the mouth and lick movements of the rats after SAC lavage on P3. This analysis revealed significant treatment [$F(1, 88) = 5.21$, $p = 0.02$] and age-group $\times$ treatment interactions [$F(2, 88) = 3.07$, $p = 0.05$]. Post hoc analyses indicated that only the E19-P3 subjects exhibited a different orofacial response depending on the familiarity or novelty of the SAC administered on the test day.

4. Experiment 2

The data from Experiment 1 suggested that a taste recognition memory (TRM) is maintained for up to 5 days (i.e., E19 to P3). However, in this paradigm, the youngest fetuses also had the longest retention interval. Did younger fetuses

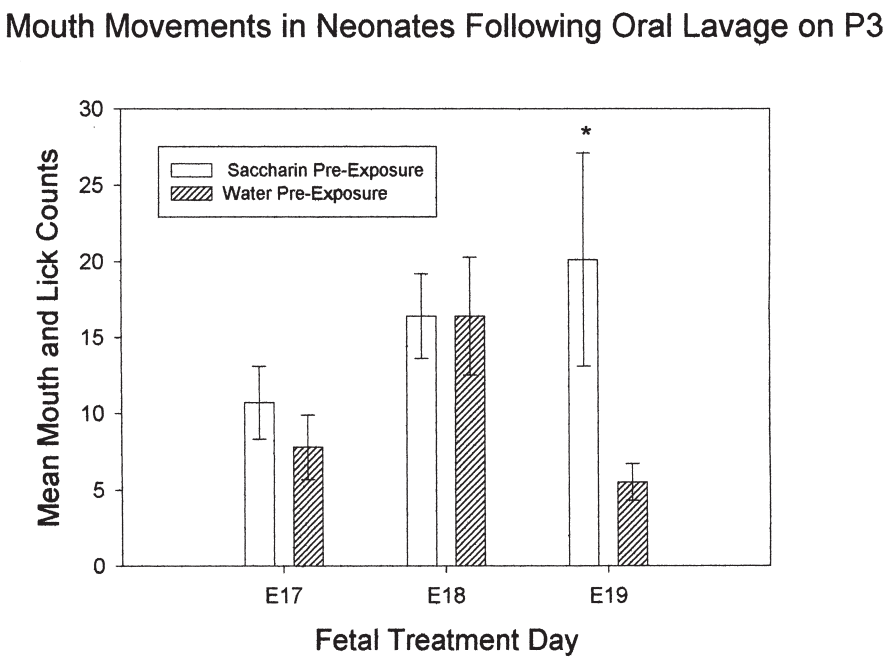

Fig. 1. Data from Experiment 1. Neonatal rats that tasted saccharin on E19 exhibited significantly (*, $p < 0.05$) more mouth movements and licks (compared with control rats that received oral lavage of water on E19) when reexposed to saccharin on P3. This differential behavioral response to a novel versus familiar taste was not observed in rats preexposed to saccharin on either E17 or E18. Variance indicators are the standard error of the mean (SEM).

forget the TRM? Could these data instead reflect the limitations of the E17 and E18 fetuses to sense the SAC and/or acquire the memory trace? In a second study, we shortened the taste exposure–reexposure interval to 2 days in an attempt to detect the TRM in younger fetuses.

4.1. Methods

The design of Experiment 1 did not allow for a standard interval between the first and second taste experiences. Therefore, in Experiment 2, different sets of E17, E18, or E19 fetuses received an oral injection of 10 μL 0.3% SAC or of H_2O while in utero and then received an oral injection of 10 μL of 0.3% SAC 2 days later. Thus, this second experiment maintained a standard exposure–reexposure interval of 2 days. A 2-day interval was selected because it has been shown that taste memories may require a period of 1–2 days for consolidation [23]. The age groups in Experiment 2 were E17-19, E18-20, or E19-21. The number of subjects/litters in each group were as follows: E17-19, SAC preexposure: 40/9; E17-19, water preexposure: 34/7; E18-20, SAC preexposure: 29/6; E18-20, water preexposure: 30/6; E19-21, SAC preexposure: 36/9; E19-21, water preexposure: 38/7. The E17-19 and E18-20 rats were tested via the fetal procedures described below. E21 rat pups were tested via the neonatal procedures. All other aspects of this experiment are as described previously. Twelve of the 16 litters tested on E21 were born via Cesarean section.

4.1.1. Fetal behavioral testing

If fetuses were tested, the pregnant dams were provided analgesia using an irreversible spinal block (0.1 mL 100% ethanol) via the general method described above (see Experiment 1). Both horns of the uterus were exteriorized through the abdominal incision, created 2 days before (during the injection procedure), and the horns were allowed to float freely in the Locke's solution bath. At least 15 min were allowed to elapse before onset of behavioral observations in order to allow the pregnant female and fetuses to fully recover from the Isoflurane anesthesia used during the spinal-block procedure. While still attached to the dam via the umbilical cord, fetuses were, one-by-one, removed from the uterus and floated in a 37.5°C ± 1°C Locke's solution bath. A blunt, 30-gauge stainless-steel injection tube was placed in each rat's mouth, and 10 μL SAC was injected into the oral cavity. Behavior was videotaped for 1 min immediately before (baseline) and after oral SAC injection.

4.1.2. Neonatal behavioral testing

If rats had not been born 4 h before the scheduled behavioral test on E21, they were removed by Cesarean section. Cesarean section was accomplished while the dam was provided analgesia using an irreversible spinal block (0.1 mL 100% ethanol) using the injection procedure described above. If rat pups had been born via a normal vaginal delivery, they were separated from the dam 20 min before the behavioral test. While awaiting testing, pups were placed, with littermates, in a small plastic container sitting on a warm (38.5 ± 0.5°C) heating pad. This container was covered with gauze and maintained in a temperature-controlled incubator (ambient temperature = 28 ± 1°C) until immediately before testing of the litter began. For the behavioral observations, neonates were placed in a warm (ambient temperature = 28 ± 1°C), high-humidity, chamber on a glass plate warmed (via constantly circulating water) to 36 ± 1°C. Pups received oral lavage with 10 μL SAC through a blunt/smooth, 18-gauge, stainless-steel infusion needle. Subjects were then placed (ventral side down) on the glass plate. Using a mirror, behavior was videotaped from below the animal for 1 min before (baseline) and after oral SAC injection.

4.1.3. Dependent variables and data analysis

As in Experiment 1, this study focused on orofacial movements (i.e., mouth movements and licks) recorded for 1 min immediately after SAC infusion. Neonates born via Cesarean section exhibited mouthing and licking responses that were statistically indistinguishable from those in pups that underwent a normal vaginal delivery. Therefore, the data from pups delivered vaginally and surgically were combined in the statistical analyses reported here. Our method of data analysis in Experiment 2 attempted to take into account some of the differences between the motor capabilities of fetal and neonatal rats. When we compared the motor responses of different aged animals, we used an ANCOVA. This technique employed, as a covariate, each animal's total activity (a total of head, mouth, lick, gape, curl, stretch, twist, roll, hindlimb, forelimb, face wipe, and twitch movements) during the 1-min baseline period immediately before oral lavage with SAC on the test day. Thus, the differing ability/motivation of different aged rats to move spontaneously was factored into our treatment of the data. Other features of the statistical analysis (e.g., use of a nested design) are similar to those described for Experiment 1.

4.2. Results

Responses to novel versus familiar SAC were not different for the E17-19 or E18-20 fetuses. However, as expected, E21 neonates exhibited differential responses to SAC depending on their history with this sweet taste from 2 days before (see Fig. 2). A two-way ANCOVA (treatment [SAC pretreatment or H_2O control] × subject age at time of initial and subsequent exposure [E17-19, E18-20, or E19-21]) compared the mouth and lick movements of the rats following SAC lavage 2 days after initial exposure to oral SAC or control H_2O. In order to account for the expected increase in general activity in developing rats, the total motor activity during the baseline 1 min before SAC lavage was used as a covariate. This analysis revealed significant treatment [$F(1, 200) = 5.44$, $p = 0.02$], age-group [$F(2, 200) = 16.93$, $p = 0.0001$], and age-group × treatment interactions [$F(1, 200) = 5.87$, $p = 0.003$]. There was also a significant covariate (baseline activity) effect [$F(1, 200) = 13.35$, $p = 0.0003$].

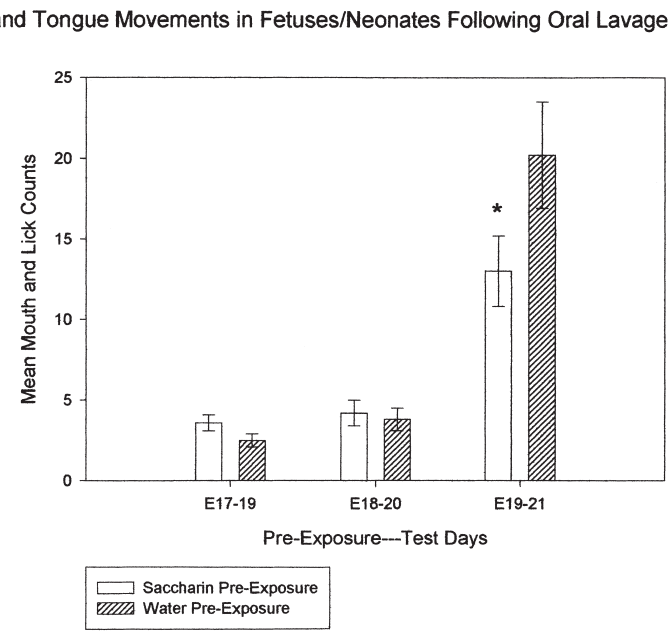

Fig. 2. Data from Experiment 2. Mouth and lick response of rats that received oral lavage of either saccharin or water on E17, E18, or E19 and then exposed to saccharin 2 days later (E19, E20, or E21, respectively). E21 neonates, for whom saccharin was a novel taste, exhibited significantly less mouthing and licking than did rats that were familiar with saccharin. Neither E19 nor E20 fetuses showed this differential response to a novel versus a familiar liquid. Variance indicators are the standard error of the mean (SEM).

Post hoc analyses indicated that the behavioral pattern associated with a TRM was not observed in rats preexposed to saccharin on E17 or E18. However, as in Experiment 1, animals that first tasted SAC or H_2O on E19 exhibited a differential response to SAC 2 days later. However, in this case, saccharin preexposure caused a relative decrease in mouth and lick movements. This effect could not be attributed to high levels of motor activity in the neonates because the orofacial motor effects were still discernible within an ANCOVA in which total baseline activity was accounted for as the covariate.

In contrast with our findings, Smotherman and Robinson [35] reported a differential response of E19 rat fetuses to an oral infusion of novel or familiar mint solutions. In this previous study, the mint was first presented to the animals in the *familiar* group on E17, while the animals in the *novel* group received an oral infusion of saline on that day. As compared with baseline activity, novel mint produced a suppression of total activity on E19, whereas familiar mint elicited no significant change from baseline movement. The differential response to the novel/familiar mint was seen only during the 5-s oral infusion of the mint.

Unlike Smotherman and Robinson's methods [35], our infusion procedures did not allow for recording of motor responses during the injection, and we typically analyzed the orofacial movements for a full minute immediately after delivery of our tastant (SAC). In an attempt to make our procedures as similar as possible to these previous studies, we limited an additional behavioral analysis to the first 5 s after the infusion. A one-way ANOVA compared the baseline activity of E17-E19 or E18-E20 fetuses that received either novel or familiar SAC. The baseline activity score represented the mean total activity of the fetus for a 5-s period during the 1 min before the infusion of SAC (i.e., baseline activity/5 sec = total frequency of fetal movements during the 1 min before infusion, divided by 60 [to get movements per second] and multiplied by 5 [to get average movements in 5 s]). This comparison revealed no significant difference between the baseline activity of the animals that were about to receive either the novel or familiar SAC. Other one-way ANOVAs compared (a) the mouth and lick responses of E17-E19 or E18-E20 fetuses after novel or familiar SAC and (b) orofacial changes from baseline (i.e., mouth and lick frequency from baseline activity) of E17-E19 or E18-E20 fetuses after novel or familiar SAC. Neither of these analyses revealed a significant difference in orofacial movements between the fetuses exposed to novel versus familiar SAC.

5. Experiment 3

The data from Experiments 1 and 2 suggested that E19 fetuses can acquire and retain a TRM for 2–5 days, whereas E17 and E18 fetuses cannot. However, the interpretation of these data is obscured by the fact that only neonates displayed the differential responses to novel and familiar SAC. Unlike fetuses that are always surrounded by fluids, neona-

tal rats are air breathing, and when a liquid is introduced into their mouth they have a good reason to clear the airway. This may help explain the relatively frequent mouthing seen in neonates as compared with fetuses (see Fig. 2).

Experiment 3 was designed to determine if it was possible to detect a TRM by using the fetal testing methods reported in Experiment 2. Here, like in Experiment 2, we exposed E19 rat fetuses to SAC or H_2O. However, 2 days later (E21), fetuses were tested before parturition. We reasoned that if a TRM could be detected in both E21 fetuses and neonates, this might illustrate the capacity of the fetal testing method to reveal a TRM, if it exists.

5.1. Methods

The procedures were essentially the same as those used in Experiment 2. E19 fetuses received an oral injection of 10 μL 0.3% SAC or H_2O while in utero, and then, before behavioral testing, they received an oral injection of 10 μL of 0.3% SAC 2 days later (E21). Unlike Experiment 2, however, these subjects were tested as fetuses before parturition. This was accomplished by selecting litters that had not yet delivered early in the day on E21. The number of subjects/litters were as follows: E19-21, SAC preexposure: 19/5; E19-21, H_2O preexposure: 12/5. Oral injections and behavioral testing of fetuses were conducted as described previously.

5.2. Results

E21 rats, tested as fetuses, exhibited differential responses to SAC depending on whether it was novel or familiar. Like in Experiment 2, E21 rat fetuses that received SAC on E19 showed fewer mouthing and licking responses to SAC on E21 (mean ± SEM = 2.21 ± 0.88) than did rats exposed to H_2O on E19 (mean ± SEM = 5.08 ± 2.02). This difference was statistically significant [one-way ANCOVA: $F(1, 28) = 7.75$, $p = 0.01$]. There was also a significant covariate (baseline activity) effect [$F(1, 28) = 14.83$, $p = 0.001$] with fetuses preexposed to SAC on E19 exhibiting more mouthing and licking before the administration of SAC on E21. These data suggest that a TRM may be demonstrated in animals undergoing our fetal testing procedure.

6. Discussion

The data presented here indicate that perinatal rat pups exhibit differential orofacial reactions to a novel versus a familiar sweet taste and that this TRM is not readily acquired before E19. However, once acquired, the TRM may be retained for at least 5 days (E19 to P3). These findings are consistent with data from other labs indicating that preexposure to odor/taste stimuli in utero can influence postnatal reactions to that taste [11,24,37]. Moreover, our analysis of the origins and time course of a TRM are partial corroboration of Smotherman and Robinson's initial report [34] that preexposure to a taste (i.e., mint) on E17 has no residual effect on the motor behavior fetuses on E19.

However, it should be noted that Smotherman and Robinson later [35] took a more-detailed look at this phenomenon by analyzing motor activity during and immediately after the oral infusion of mint in E19 fetuses that were first exposed to mint (or control saline) on E17. These data were compared with a preinfusion baseline. This investigation revealed that rat fetuses exhibited immediate (i.e., during the infusion) differential responses to intraoral novel or familiar mint. The TRM effect was no longer present 5 s after infusion.

Our methods were somewhat different from those used by Smotherman and Robinson [35], and these differences may help explain the dissimilar findings. Our infusion method did not allow recording of mouth and lick movements during oral lavage. Instead, our fetal behavioral tests/analyses represented orofacial movements in the 1-min time period after the infusion. Further, we have typically analyzed the raw number of counts during this period rather than a change from baseline.

We did not have access to the mouth and licking movements exhibited during the infusion of SAC on the test day. However, in an attempt to perform a data analysis as similar as possible to that used by Smotherman and Robinson [35], we counted the orofacial movements in the 5-s period immediately after the SAC injection. Further, we calculated a change-from-baseline statistic for each animal. Like our other treatments of the data, these analyses did not reveal a TRM motor response in E17-E19 and E18-E20 fetuses. These findings, from 2 different laboratories, point us towards the conclusion that TRMs in E17-E19 and E18-E20 rats are very short lived (detectable during tastant administration only) and/or are significantly potentiated by combined gustatory/olfactory stimuli (e.g., mint).

Our methods of data collection and analysis were sensitive enough to reveal a TRM in E19-E21 and E19-P3 pups, but we did not record a TRM response in animals with first exposures to SAC/H_2O on E17 or E18. These data may be interpreted in several ways. Did the animals fail to sense the SAC? Did they sense the SAC but fail to retain the memory? We attempted to analyze retention factors that might influence TRM by using two different exposure–reexposure intervals: 6–7 days (Experiment 1) and 2 days (Experiment 2). This analysis showed that E17 and E18 subjects with the shorter retention interval had no more of an inclination towards demonstrating a TRM than did the subjects with the longer retention interval.

Did the E17 and E18 fetuses never sense the SAC taste? The perinatal period is apparently a time when both qualitative and quantitative changes in sensation are occurring [1,28,41]. Thus, sensory factors may play a part in determining whether or not a TRM may be observed at different times during this period of development. However, it should also be noted that data from other laboratories [34] clearly suggest that fetuses have the ability to acquire a conditioned taste aversion (using mint as the conditioned stimulus [CS])

on E17. Likewise, we have reported similar data using SAC as a CS in E18 fetuses [18]. Other data [35] suggest that fetuses reduced their motor responding upon repeated oral lavage with lemon. Thus, it would appear that E17 and E18 fetuses can habituate to a smell/taste or form associations based on olfactory/gustatory stimuli.

If fetal gustatory systems are functional on E17/18, then the current data seem to be consistent with the interpretation that fetuses are less capable of acquiring or retaining a nonreinforced memory of the taste of SAC. It must be recognized that SAC has primarily gustatory characteristics, whereas mint and lemon have strong olfactory features as well. Smotherman and Robinson [34] have demonstrated a TRM in E17 fetuses exposed to mint. Thus, the data presented here suggest that the reduced salience of an exclusively gustatory stimulus may limit the ability of E19 or E20 fetuses to exhibit recognition of a gustatory stimulus originally applied on E17 or E18, respectively.

In Experiment 2, only rats tested via our neonatal testing methods demonstrated a TRM. Does this suggest that there are some aspects of this method that evoke the mouth/lick movements we recorded? Alternatively, did our fetal testing procedure inhibit our recording of a TRM? Behavioral ontogeny is determined not only by the maturational process but also by the pressures of the changing environment. For example, Robinson and Smotherman [27] have reported quite different levels of synchronous movements in rats of the same age but tested either in utero or ex utero.

There are several lines of evidence that suggest that the testing method (i.e., fetal tests in Locke's solution or neonatal tests in a relatively dry environment) may not be a critical factor in the recording of a TRM. First, in Experiment 1, all the rats were tested postnatally, but only the E19-P3 rats exhibited differential responses to novel vs familiar SAC. Second, TRMs (as defined as a different response to a familiar vs. a novel taste) may be demonstrated by either relative increases (e.g., in E19-P3) or decreases (e.g., in E19-E21 rats) in mouthing depending on the age of the neonate at time of test. This suggests that the neonatal procedure is not constraining and can accommodate different response topographies. Third, Experiments 2 and 3 demonstrated directly that the TRM could be recorded in E21 rats tested as either fetuses or neonates.

The orofacial motor analysis we report here is identical to the well-established procedure used by Smotherman and Robinson as an indication of conditioned taste aversions ([34]; for reviews, see [37–39]). Our measures of orofacial movements are also similar to the well-known taste reactivity test (TRT) developed by Grill and Norgren [10]. The TRT is useful in dissociating consummatory and appetitive taste-elicited behaviors. Like the TRT, we injected SAC directly into the oral cavity, and therefore appetitive measures were absent while direct measurement of both ingestion and aversion were available [31]. The licking and mouth movements we observed have been closely associated with the ingestion of sweet stimuli like sucrose and SAC [9]. Further, the rhythmic oral responses following infusion of sweet liquids are likened to consummatory taste preference measures like spout licking in several respects. Both are emitted in the same frequency range, organized in burst/pause patterns, and serve the function of oral transport of fluid into position for swallowing [15]. Other investigators have shown that licking frequency increases in direct proportion to the concentration of sucrose [13]. Thus, the motor responses measured in the current study are, in many ways, similar to other measures of taste preference or intake.

The establishment of a TRM may be influenced by the length of time that the fetuses experienced the taste in utero. In Experiment 1, E17 fetuses have the longest taste exposure-re-exposure interval. Are they experiencing the SAC for this whole period? Is the TRM of rats first exposed to SAC on E19, and tested with the taste 2 days later (see Experiments 2 and 3), underscored by remnants of the SAC present near birth? How long does the taste of SAC stay available in the amniotic fluid? A full answer to these questions would include information about the various means by which SAC may be absorbed/ingested by the fetus, rates of fetal metabolism/elimination of SAC, rates of maternal drug clearance, circulation of amniotic fluid, habituation rates of the fetal gustatory apparatus, etc. Unfortunately, only a limited subset of this information is currently available. In our experiments, we injected SAC into the mouth of the fetus. Thus, our animals presumably received an initial strong, gustatory sensation. After this exposure, it is likely that some of the SAC spilled into the amniotic fluid. We are not aware of experiments that have documented clearance rates of saccharin from amniotic fluid. However, there are data on other substances (e.g., alcohol) suggesting that the elimination may be quite rapid. Choroto et al. [4], for example, reported that the concentration of alcohol, injected in the amniotic fluid, was reduced by half in less than 30 min. It should be noted that, like saccharin, alcohol is poorly metabolized by the fetus (because of undeveloped alcohol dehydrogenase) [2], and therefore alcohol clearance is arguably a good model of SAC clearance. Apparently most clearance could be expected to occur via diffusion of these substances across membranes into maternal circulation [45].

It is known that fetuses are capable of swallowing [19,25], and this activity would also eliminate some portion of the SAC as it moves into the fetal, and then the maternal, blood supply. In fact, experiments by Nanbo [20,21] indicate that transplacental clearance from fetus to mother is more significant than fetal tissue clearance and that clearance rates change throughout the perinatal period. The clearance (of *p*-phenylbenzoic acid) from amniotic fluid to the rat fetus decreases from 7.3 mL/h on E16 to 3.3 mL/h on E21 [22]. However, this is more than compensated for by an increase in clearance by nonplacental elimination by the mother (increasing from 45.3 mL/h on E16 to 94.0 mL/h on E21). Thus, with the limited information available, it seems that overall clearance rate increase from E16 to E21. These data suggest that a substance would remain longer in the

uterus during the earlier stages of development and that our E17 and E18 fetuses may have had a longer exposure to the SAC. Despite this presumably longer exposure to SAC, E17 and E18 fetuses failed to consolidate information about SAC to the degree that E19 fetuses did.

Of course, clearance rates do not speak directly to the issue of how long the fetus experiences, and reacts to, the taste of a substance injected into the amniotic fluid. Smotherman and Robinson [35] have collected data relevant to this issue. Oral infusion of a 20-μL lemon bolus (providing a gustatory and olfactory CS) produced an initial increase in fetal activity followed by a gradual decline. After 1 min, the motor activity was indistinguishable from that exhibited by saline-control animals. Other observations indicate that repeated (1/min) pulsate infusions of lemon also produce a significant habituation of responding. Our studies infused half the volume presented by Smotherman and Robinson [35] and employed a CS (SAC) with only gustatory cues. Although the methods are not directly comparable to our procedures, Smotherman and Robinson's experiments suggest that the taste/motor reaction to a tastant may be transient—perhaps lasting less than a minute. Thus, for the purposes of our study, SAC availability in the amniotic fluid may be less critical than the relatively short length of time that the fetus tastes and reacts to gustatory stimulus.

Our data indicate that a TRM may be represented by a variety of age-dependent behavioral responses. Relative to controls, P3 pups exhibited more mouthing and licking when experiencing a familiar taste. However, E21 rats showed a relative decrease in orofacial movements to the familiar saccharin taste. It should be noted that these data could represent a simple difference in the nursing history of these animals. P3 pups had nursed (and had therefore experienced a wider variety of tastes), whereas the E21 pups have not. Alternatively, the particular behavioral expression of the TRM priming effect may represent the animal's adaptation to environmental pressures within an ontogenetic niche [36].

Development is not always uniformly linear or progressive. In fact, there are data suggesting waxing and waning of the ability to learn, retain, and demonstrate knowledge of new information. The existence of "periods of learning readiness" has been well established over the last 40 years [3,42]. For example, although virtually all rat fetuses exhibit a facial wiping response on E21, the incidence of facial wiping is reduced by 50% in newborn rat pups tested only a few hours after birth. Within 24 h, the wiping response disappears almost completely and remains absent until the end of the second postnatal week, when it reappears [38]. Such information fosters a view of the developing organism as occupying a succession of ontogenetic niches [50]. Periods of adaptation to a particular niche are interrupted by transitions to subsequent niches. Development within an ontogenetic niche is characterized by increasing behavioral diversity and organization, whereas periods of transition between niches may result in temporary slowing or regression of measures of development [39].

TRM has some similarity to the "priming" phenomenon that has been well studied in human subjects. Human priming often involves an increased facility for detecting or identifying words or other stimuli as a result of their prior presentation [43,48]. According to Vriezen et al. [49], "the mere presentation and processing of an item is sufficient to leave a trace in the perceptual representation system. It is the reactivation of this trace on subsequent presentations that accounts for the repetition priming effect" (p. 944) [29,30,47]. This facilitative effect is presumably mediated by a memory system separate from that involved in performance on explicit or direct tests of recall and recognition [43].

The apparent ability of fetuses to exhibit differential behavioral responses to a taste, dependent on its novel, or familiar, characteristics reinforces the current concept of the fetus and neonate as sophisticated sensors and responders to the uterine and extrauterine environment. Further, the TRM paradigm described here may be useful as a means to investigate "implicit" priming memory in perinatal rats.

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