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Maternal Ingestion of Ortho-Aminoacetophenone During Gestation Affects Intake by Offspring

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NOLTE, D. L. AND J. R. MASON. *Maternal ingestion of ortho-aminoacetophenone during gestation affects intake by offspring.* PHYSIOL BEHAV **58**(5) 925–928, 1995.—Ingested flavor chemicals cross the placental barrier and occur in the fetal blood and amniotic fluid. This occurrence is detectable by the fetus, and can influence post parturition feeding. In the present experiment, pregnant mice were offered either 0.1% ortho-aminoacetophenone emulsions (OAP) or water throughout gestation. OAP is normally avoided by mice, apparently on the basis of chemosensory characteristics. Subsequently, offspring were offered 0.5%, 0.25%, or 0.1% OAP in one-bottle tests at 26 or 88 days of age. Offspring of mothers given OAP drank greater amounts of OAP than did offspring of mothers given water. Enhanced acceptance of OAP was not detected in mice exposed to 0.1% OAP as adults for a duration similar to that given during gestation. We conclude that fetal experiences with OAP lowered sensitivity and/or raised tolerance for the compound.

Chemosensory In utero Learning Mouse *Mus musculus* Ortho-aminoacetophenone

OLFACTORY and gustatory stimuli cross the placental barrier (15,31) and are present in the fetal blood and amniotic fluid (11,24). These substances can stimulate olfactory and taste receptor systems (4,17), stimulate ingestive behaviors, and possibly, influence receptor development (5).

There is also evidence that prenatal chemosensory experience can influence postnatal chemosensory responsiveness. For example, rats born to mothers given ethanol when pregnant tend to ingest more ethanol as pups than rats born to mothers without such experience (3,25,26). These effects are evident even when the exposure period is limited to intraperitoneal injections on day 8 of gestation (21). Fetal experiences with food flavors also affect subsequent preferences of rats. For example, offspring of dams that ingest garlic or apple juice during gestation exhibit preferences for these stimuli relative to the responsiveness of control animals (12,30). Rat fetuses also form conditioned aversions that are expressed postnatally (29,32).

In the present experiment, we investigated whether intake of ortho-aminoacetophenone (OAP) by gestating mice would affect the responsiveness of their offspring. OAP is normally aversive to mice (22,23), apparently on the basis of chemosensory characterisitcs. An avian trigeminal irritant (18), the specific aversive mammalian chemosensory properties of OAP have not yet been identified.

MATERIALS AND METHODS

Subjects

Forty-two pairs of experimentally naive 90–95 day old CF-1 mice (*Mus musculus*, 42 females, 42 males) were caged as pairs (27 × 21 × 14 cm) under a 12:12 light:dark cycle (light onset 0700 h) at 23°C. Wayne Rodent Blox and water were provided ad lib, except as described below.

Chemicals

Ortho-aminoacetophenone (OAP; CAS # 551-93-9) was obtained from Aldrich Chemical Company (Milwaukee, WI) and mixed with tap water to prepare a 0.1% (mass/mass) stock emulsion. This substance reduces fluid intake by mice and virtually eliminates ingestion at concentrations ≥ 1.0% (23).

Fetal Exposure

Pairs of mice were randomly assigned to 2 groups. One group was given tap water to drink while the other group was given

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0.1% OAP. Treatments were initiated when female and male mice were paired. Each day at 0800 h, all females were examined for the presence of a vaginal plug. The day a plug was detected was considered to be day 0 of gestation. All female mice were given plain water to drink after day 18 of gestation. Regardless of gestational day, males were separated from females after 18 days, caged individually and given plain water.

All pups were born on day 19 or 20 of gestation. The number of litters and pups per litter were similar between groups. Litters were not cross-fostered to naive females, so a possibility exists that pups may have been exposed to OAP while suckling. We think this is unlikely, however, because of evidence that many flavors are undetectable in milk 7 hours after flavor ingestion (1).

On postparturition day 11, all litters were reduced to 6 male pups, and 15 OAP and 15 control litters were selected for subsequent behavioral testing. Litters were weaned at post parturition day 21, but litter-mates continued to be housed together.

Behavioral Assays

For each of the treatment groups, two cohorts of offspring were tested. One cohort was tested to determine the response of offspring to OAP at weaning and the other cohort was tested to determine their response as subadults. Three pups from each litter were randomly assigned to the cohort to be tested at weaning while the remaining siblings were assigned to the cohort to be tested as subadults. Pups from each cohort were individually caged for testing on days 25 and 87, respectively. A third cohort, adult males exposed to OAP for 18 days while caged with the gestating females, was also tested to determine their subsequent acceptance of OAP.

Test procedures were identical for all cohorts. First, there were four days of adaptation to an 18 hour water deprivation schedule. Adaptation procedures for the first cohort were initiated when pups were 26 days old and at 88 days of age for the second cohort. Adaptation procedures for the adults began the day after they were separated from the females. On each of these days, animals were presented with tap water in a graduated 10 ml sipper tube at 0900 h. At 1500 h, the tubes were removed until the following morning. Adaptation was followed by four days of pretreatment. The only difference between adaptation and pretreatment was that drinking was measured to the nearest 0.2 ml between 0900 and 1200 h. Pretreatment was followed by 4 days of treatment. Treatment was similar to pretreatment, with the following exception. For treatment tests, each cohort was randomly divided into three subcohorts. One pup from each litter was randomly assigned to each of these subcohorts. Each subcohort ($n = 15$) was presented with a different OAP concentration (0.5%, 0.25%, 0.1%) during the three hour drinking measurement period. Adult males were also divided into three subcohorts ($n = 7$) and presented the same OAP concentrations during the treatment period. To prepare the OAP stimulus emulsions, a stock emulsion (0.5%) was sonicated for 60 minutes, stirred for 30 minutes, then aliquots were diluted with tap water.

Analysis

First, a one-way analysis of variance (ANOVA) was used to ensure that pretreatment intake by all subcohorts within an age cohort was similar. Difference scores were then calculated for each mouse by subtracting the mean treatment intake from the mean amount of water ingested during the pretreatment period (Fig. 1). Accordingly, high scores indicate a relative avoidance of the stimuli while scores that approach zero reflect an indifference. Difference scores for each cohort were assessed in separate 2-factor ANOVAs (34). In each case, mice were nested within treatments and the 2 factors were exposure (2 levels) and concentration (3 levels).

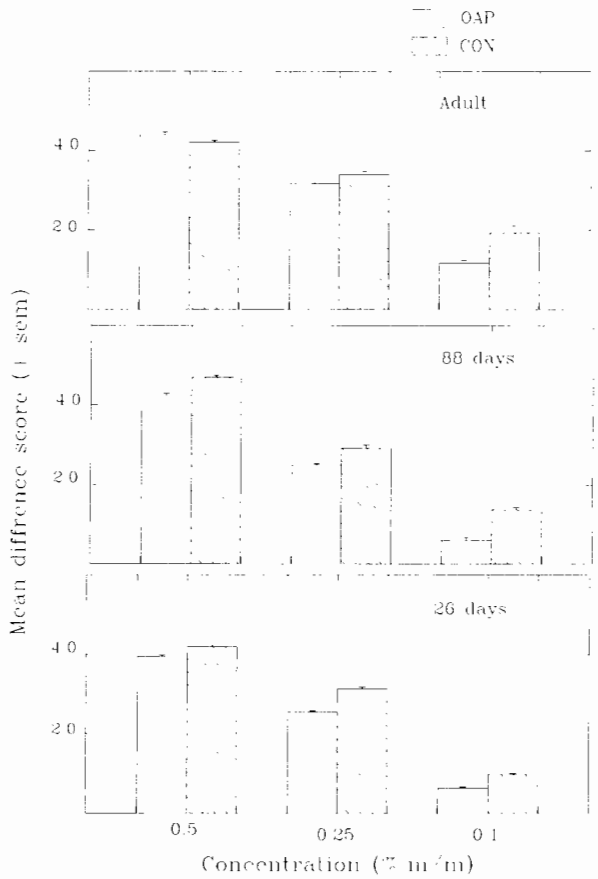

FIG. 1. Difference scores for three cohorts (adults, 88 days, 26 days) of mice exposed to either 0.1% ortho-aminoacetophenone (OAP) emulsions or water (control) and tested for their acceptance of 1 of 3 OAP test concentrations (.5%, .25%, .1%) in 1-choice tests. High scores indicate a relative avoidance of the stimuli while scores that approach zero indicate an indifference.

RESULTS

All subcohorts within an age cohort ingested similar amounts of water during the pretreatment period. At 26 days of age, pups born to mothers that ingested OAP during gestation ingested more OAP treated water than did offspring from mothers given water ($F = 11.2070$, df 1,84, $P = 0.0016$). Further, regardless of exposure, there was an inverse relationship between intake and OAP concentration ($F = 259.249$, df 2,84, $P < 0.0001$). There was not an interaction between exposure and test concentration ($F = 0.703$, df 2,84, $P > 0.35$).

Offspring tested at 88 days of age responded similarly to those tested at 26 days of age. Prior experience with OAP enhanced their intake during the treatment period ($F = 8.555$, df 1,83, $P = 0.0047$), mice restricted their intake with increasing test concentrations ($F = 113.701$, df 2,83, $P < 0.0001$) and no interaction between exposure and concentration occurred ($F = 0.352$, df 2,83, $P > 0.35$).

Adult experiences with OAP, however, did not enhance their intake during subsequent trials ($F = 0.733$, df 1,35, $P > 0.35$). As with the other cohorts, there was an OAP test concentration effect ($F = 30.310$, df 2,35, $P < 0.0001$) but no exposure by concentration interaction ($F = 0.884$, df 2,35, $P > 0.35$).

DISCUSSION

Mice restricted their intake of all concentrations of OAP regardless of treatment. This result is consistent with other studies showing that OAP is avoided by mice at concentrations ranging from 0.25–1.0% (23). Nevertheless, the data also demonstrate that prenatal experience with OAP was associated with increased ingestion of OAP.

The reasons for this effect are unclear. Simple exposure to OAP in the fetal environment could have increased tolerance for this chemical. The available evidence suggests that exposure can increase tolerance, particularly in no-choice situations. For example, mice will ingest more fennel flavored food if previously exposed to this aversive flavor (16). Likewise, guinea pigs (*Cavia porcellus*) will ingest more sucrose octaacetate (SOA) if they are reared on SOA-flavored water (33). Adult mice exposed to OAP for a similar duration, however, responded no differently than their respective controls. Further, simple exposures rarely induce persistent changes in dietary selection (6,7,10,33).

Alternatively, there may have been a hedonic shift in relation to OAP (i.e., OAP may have been perceived as relatively more palatable as a consequence of exposure). Again, there are data consistent with this notion. Rats develop temporary preferences for chili peppers if previous experiences with peppers have been paired with recovery from thiamine deficiency (27). Likewise, rats develop a preference for the flavor of morphine once it is associated with its psychogenic consequences (35). In the present experiment, OAP was being associated with the sole source of nourishment for the fetuses. Arguably, this association might promote preferences akin to those described for recovery from thiamine deficiency or morphine ingestion. Adults restricted to water treated with OAP for a similar duration, however, did not demonstrate a subsequent increased acceptance of OAP.

Another possibility is that fetal exposures to OAP decreased the offspring's sensitivity to OAP. In utero experiences may influence the structural and functional development of the taste system (5). The gustatory sensory system of the altricial rodents develops late in gestation though it is not fully functional until after parturition (20). Olfactory synapses are also present in appreciable numbers after gestational day 15, however, they too are not fully functional until after birth (13). Other stimuli were not tested, therefore, it is difficult to assess whether a decrease in sensitivity would be specific to OAP or a general reduction to similar stimuli. Capsaicin injections in neonatal rats, however, severely reduces their avoidance response to capsaicin and other strong trigeminal stimuli (28).

The inverse relationship between intake and concentration was probably a concentration effect rather than an effect related to the concentration of OAP exposed to the animals. This result is consistent with other studies that show intake of OAP treated water declines with increasing concentrations (23). Most flavors become aversive at some concentration level (14). Further, while it is true that 0.1% OAP was the concentration presented to mothers throughout gestation, this may not have been the concentration presented to fetuses. Chemical concentrations may become more dilute (8,9) or even increase by the time they reach the fetus (2,19).

SUMMARY

Fetal animals may encounter food flavors from the maternal diet (24). The present experiment demonstrates that prenatal experiences with an otherwise aversive flavor can increase ingestion of that flavor postparturition. The reason for this increase in ingestion remains obscure, although the most likely possibilities are increased acceptance through simple exposure, enhanced preference as a consequence of pairing with nutrient delivery via the placenta, or a decreased sensitivity through fetal experiences. The latter possibility may best explain the persistent enhanced acceptance of OAP by mice after fetal experiences, while there were no differences detected in adults given similar experiences.

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