Latent Inhibition of a Conditioned Taste Aversion (CTA) In Fetal Rats Is Age-dependent

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Abstract

Conditioned taste aversions (CTAs) may be acquired when an animal consumes a novel taste (Conditioned Stimulus = CS) and then experiences the symptoms of poisoning (Unconditioned Stimulus = US) (Pavlov, 1927; Garcia et al., 1955). When later re-exposed to the CS, the animal will avoid the taste or reduce consummatory oral-facial movements (Grill &Norgren, 1978a,b). In the current studies we sought to determine if a CTA could be diminished by non-reinforced pre-exposure to a CS (i.e., latent inhibition; LI) in fetal rats. We injected E17 or E18 pregnant Sprague-Dawley rats with 100% allicin (pure garlic extract; i.p.) or an equal volume of physiological saline. The taste/smell of garlic has been shown to cross the placental barrier (Gruest et al., 2004) and we were able to measure it (via HPLC) in the amniotic fluid during pilot studies. One day later the pregnant dams received a second injection of the CS, allicin (i.p.) followed by either LiCl (81 mg/kg, i.p.; the US) or a control injection of saline. Forty-eight hours later (either E20 or E21) a spinal block was performed on the dam producing complete abdominal analgesia while pups were removed (still attached via umbilical cord), and tested in a temperature-controlled isotonic saline bath. Pups received oral lavage with 10µl, 0.1% allicin (i.e., similar to the concentration experienced in the amniotic fluid days earlier). Observations of ingestive orofacial motor responses (mouthing and licking) were recorded during the oral lavage of the garlic taste. If allicin had been paired with LiCl in utero, E21 fetuses exhibited a conditioned suppression of orofacial movements, indicative of an aversion to this taste (Grill &Norgren, 1978a,b). However, pre-exposure to the garlic taste on E18 produced a latent inhibition of this CTA. Rats one day younger during conditioning (E18) did not exhibit signs of a CTA when they were tested *ex utero* on E20. LI of a CTA is a non-associative form of learning that requires the animal to remember the non-reinforced CS if it is going to be effective in diminishing the CTA acquisition. Thus, our data provide the first demonstration that fetal rats can acquire a LI. Our data also suggest that this ability emerges when pre-exposure to the CS occurs on E18 but not E17.

Introduction

- In a conditioned taste aversion (CTA) paradigm, an animal is given a novel flavor (CS), which is paired with an unconditioned stimulus (US) that has aversive affects. The animal associates US-induced malaise with the CS and forms an aversion to the taste (Pavlov, 1927; Garcia et al. 1955).
- Latent Inhibition (LI) is a phenomenon by which pre-exposure to a CS, prior to subsequent pairings of that same CS with a US, results in decreased conditioned responding to the CS (Manrique et al., 2004).
- Late-term (E18, E19) fetal rats can acquire a CTA (Smotherman & Robinson, 1985; Mickley et al., 2001) but the capacity of an animal at this age to exhibit a latent inhibition of the CTA has not been evaluated.
- Here, we used a garlic taste, which is known to cross the placental barrier (Gruest et al., 2004; Nolte et al., 1992) to evaluate the ability of fetal rats to acquire a latent inhibition of a CTA.

Methods

Subjects:

Sprague-Dawley rat fetuses (male and female) obtained from timed-pregnant dams supplied by Charles-River Laboratories; Wilmington, MA.

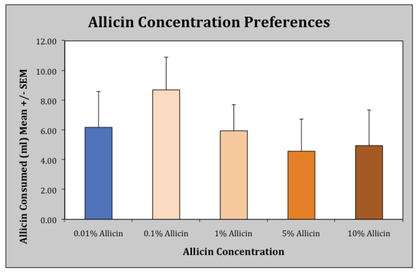
Materials:

CS = Allicin (100% 2-propene-1-sulfinothioic acid S-2-propenyl ester; the molecular taste component of garlic) was obtained from Allimax; Chicago, IL, and filtered (0.2 µm pore size) before injection into pregnant rat dams (2ml/100g i.p.). Allicin passes through the amniotic barrier unmodified and may be detected by fetuses (Gruest, et al., 2004; Nolte et al. 1992).

US = Lithium Chloride (LiCl; 81mg/kg, i.p.) was injected as the Unconditioned Stimulus (US)

Pilot Studies:

Garlic preference tests: Adult male Sprague-Dawley rats prefer 0.1% Allicin (dissolved in distilled water) over0.01%, 1.0%, 5.0% and 10.0% Allicin.



HPLC analyses of amniotic fluid: When pregnant Sprague-Dawley rat dams (E17 and E18) receive an injection of 100% Allicin (2ml/100g, i.p.) the amniotic fluid concentration of Allicin is 0.163% ± 0.006% (Mean ± SEM) after 60 minutes.

Procedures:

Experiment Phases 1 and 2: Pre-exposure and CTA Training

- **Phase 1 – Pre-exposure:** Pregnant dams received pre-exposure treatments on E17 or E18: 2ml/100g i.p. injection of allicin OR an equal volume injection of physiological saline (SAL).
- **Phase 2 - CTA training:** The following day (E18 or E19) pregnant dams received a CS injection of allicin (in doses equivalent to previous day) followed by a US injection of LiCl (81 mg/kg, i.p.) or saline control injection.

Experiment Phase 3: CTA Testing

- **Two days later (E20 or E21), fetal behavioral tests were conducted**
  - An irreversible spinal block was performed by injection of 0.1cc of 100% ethanol between the first and second lumbar vertebrae of the pregnant dam. This ensured complete abdominal and hindlimb analgesia and paralysis (Smotherman et al., 1987; Mickley et al., 2000).
  - Fetuses were then exposed through an incision in the uterus.
  - One by one, we injected 0.1% allicin into the mouth of each of each fetus and recorded their orofacial reactions for 30 sec during the injection period.

Data Analysis:

- Video recordings were later replayed on the computer and scored using the Observer® computer program (Noldus Information Technology, Netherlands).
- We recorded mouths and licks as indicators of taste palatability. Previous studies involving rats or humans have reported that mouths and licks are ingestive movements, indicative of liking the taste, or familiarity with the taste (Schwartz & Grill, 1985; Soussignan et al., 1998). A reduction in mouthing and licking indicates conditioned disgust or aversion to the taste (Grill & Norgren, 1978a,b; Mickley, et al., 2001).

Table 1. Summary of Group names, Treatments, Procedures and Ns/group

Groups	Experimental Phase 1-3			Number of Subjects
	1 CS Pre-exposure (i.p. injections)[1]	2 CTA Training Day (i.p. injections)[1,2]	3 Retention Tests (oral infusions)[3]	Litters/fetuses
	Fetal Age			
	E17	E18	E20	
CTA	Saline	Allicin+LiCl	Allicin	2/4
Latent Inhibition	Allicin	Allicin+LiCl	Allicin	3/14
Control	SAL	Allicin+SAL	Allicin	2/8
	Fetal Age			
	E18	E19	E21	
CTA	Saline	Allicin+LiCl	Allicin	6/22
Latent Inhibition	Allicin	Allicin+LiCl	Allicin	6/23
Control	SAL	Allicin+SAL	Allicin	2/11

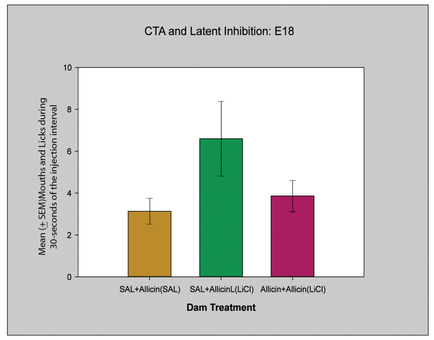
[1] Pregnant dam received a 2ml/100g i.p. injection of 100% filtered allicin, which diluted to a 0.1% concentration of allicin in amniotic fluid. Controls received equal volume of physiological saline (SAL).

[2] Dam received injection (i.p.) of 81mg/kg LiCl in a volume of 1ml/kg or equal volume of physiological saline.

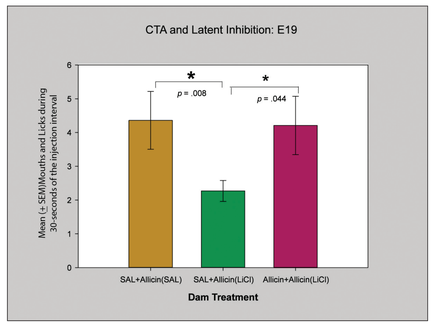
[3] Fetus received oral infusion of 10 μL of 0.1% allicin.

Results

Rat fetuses conditioned on <u>E18</u> did <u>not</u> acquire a CTA nor did they exhibit a latent inhibition



Rat fetuses conditioned on E19 acquired a CTA and exhibited a latent inhibition



Summary & Conclusions

- Rat fetuses conditioned on E19 (and tested on E21) exhibited a CTA, whereas those conditioned a day earlier on E18 (and tested on E20) did not.
- Pre-exposure to the Allicin CS on E18 inhibited the formation of the CTA in fetuses conditioned on E19 – indicating a latent inhibition.
- Using different procedures, previous studies have indicated that rats may form a CTA on E18 (Mickley et al., 2001). The very long CS exposure time in the current study may help explain the different finding reported here.
- **Our data provide the first demonstration that fetal rats can acquire a latent inhibition and also suggest that this ability emerges when pre-exposure to the CS occurs on E18 but not E17.**

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