Ketamine Blocks Latent Inhibition of a Conditioned Taste Aversion in Fetal Rats

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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). When later re-exposed to the CS, the animal will avoid the taste or reduce consummatory oral-facial movements. In the current study we sought to determine if a CTA could be diminished by non-reinforced pre-exposure to a CS in fetal rats (i.e., latent inhibition; LI). We injected E18 pregnant Sprague-Dawley rats with 100% allicin (the taste component of garlic that crosses the placental barrier; i.p.) or an equal volume of physiological saline. On this day some of the pregnant dams also received the N-Methyl-D-Aspartate (NMDA)-receptor blocking drug ketamine (100mg/kg, i.p.). One day later (E19), the pregnant dams received a second injection of the CS, 100% allicin (i.p.) followed by either LiCl (81 mg/kg, i.p.; the US) or saline. Finally, on E21 pups received oral lavage with 10µl, 0.1% allicin and observations of ingestive orofacial motor responses (mouthing and licking) were recorded. When allicin had been paired with LiCl *in utero*, E21 fetuses exhibited a conditioned suppression of orofacial movements, indicative of an aversion to this taste. Pre-exposure to the garlic taste on E18 produced a latent inhibition of this CTA. Ketamine significantly disrupted the formation of both the CTA and LI. Our data provide the first demonstration that fetal rats can acquire a LI. Moreover, NMDA receptor blockade in E18 fetuses impairs the acquisition of these gustatory memories.

Introduction

- In a conditioned taste aversion (CTA) paradigm, an animal is given a novel tastant (CS), which is paired with an unconditioned stimulus (US) that induces malaise. 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 (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 an LI of the CTA has not been evaluated.
- In the current study, we used the molecular taste component of garlic (allicin) that 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 LI of a CTA.
- We also used an N-methyl-D-aspartate (NMDA) receptor blocking drug, ketamine (Aguado et al., 1994), to evaluate the extent to which these receptors are involved in CTA and LI learning at this stage of fetal development.

Methods

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

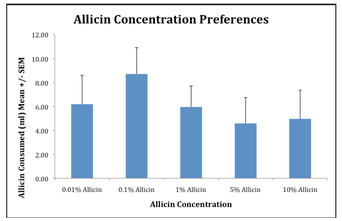
Materials:

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 is detected by fetuses (Gruest, et al., 2004; Nolte et al. 1992).

US = Lithium Chloride (LiCl; 81mg/kg, i.p. to dam) was delivered to fetuses through the maternal circulation.

Ketamine = Ketamine HCL (100mg/kg, i.p. to dam) was delivered to fetuses through the maternal circulation.

Pilot Studies: *Garlic preference tests:* Adult male Sprague-Dawley rats prefer 0.1% allicin (dissolved in distilled water) over 0.01%, 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 E18:
  - 100 mg/kg (i.p.) ketamine HCL or an equal volume of physiological saline as a control.
  - One hour later, pregnant dams received 2ml/100g, i.p. injection of allicin OR an equal volume injection of physiological saline (SAL).
- **Phase 2 - CTA training:** The following day (E19) pregnant dams received a CS injection of allicin (in doses equivalent to previous day) or saline followed by a US injection of LiCl (81 mg/kg, i.p.) or a saline control injection.

Experiment Phase 3: CTA Testing

- Two days later (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. 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 fetus and video recorded their orofacial reactions for 30 sec during the injection period.

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
SALINE	Fetal Age			
PRE-TREATMENT	E18	E19	E21	
CTA	Saline (SAL)	Allicin+LiCl	Allicin	5/21
Latent Inhibition	Allicin	Allicin+LiCl	Allicin	4/19
Non-conditioned Control (One CS exposure)	Saline	Allicin+SAL	Allicin	2/10
Non-conditioned Control (Two CS exposures)	Allicin	Allicin+SAL	Allicin	3/11
KETAMINE	Fetal Age			
PRE-TREATMENT	E18	E19	E21	
CTA	Saline	Allicin+LiCl	Allicin	2/12
Latent Inhibition	Allicin	Allicin+LiCl	Allicin	2/10
Non-conditioned Control (One CS exposure)	Saline	Allicin+SAL	Allicin	2/11
Non-conditioned Control (Two CS exposures)	Allicin	Allicin+SAL	Allicin	2/20

[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). Dams also received either 100mg/kg Ketamine (i.p.) or equal volume of SAL.

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

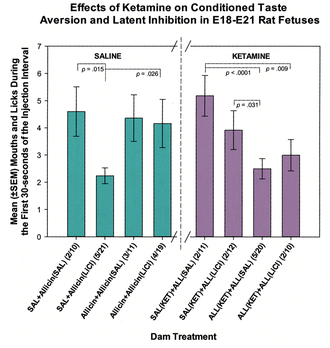
[3] Fetus received oral infusion of 10 µL of 0.1% allicin.

Data Analysis

- Video recordings were later replayed 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).

Results

- **A CTA is reflected as a suppression of fetal ingestive behaviors (mouthing & licking) but pre-exposure to the CS reversed this suppression, indicating latent inhibition (LI) of this conditioned response.**
- **Ketamine treatment on the day of CS pre-exposure attenuated orofacial indicators of both a CTA and LI measured 3 days later.**



Summary & Conclusions

- Rat fetuses conditioned on E19 (and tested on E21) exhibited a CTA as evidenced by a suppression of mouthing and licking following the taste of allicin.
- Pre-exposure to the allicin CS on E18 inhibited the formation of the CTA in fetuses conditioned on E19 – indicating a latent inhibition.
- Ketamine significantly disrupted the formation of both the CTA and LI.
- **Our data provide the first demonstration that fetal rats can acquire a latent inhibition of a CTA. Moreover, ketamine-induced blockade of NMDA receptors in E18 fetuses may impair memory formation or act as a US to suppress fetal mouthing and licking following the taste of allicin. These data are consistent with findings derived from studies of adult rats (Gallo et al., 1998).**

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