Ketamine enhances positive contrast effects (PCEs) and up-regulates N-methyl-D-aspartate (NMDA) NR2B receptors in fetal rats.

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Abstract

Rats exhibit an increase in ingestive consummatory responses if they experience an upward shift in the intensity of a gustatory stimulus (e.g., saccharin: SAC) (Mickley et al., *Dev. Psychobiol.* 44, 2004,176-188). These PCEs do not represent a direct sensory-motor reaction to a gustatory cue, but rather reflect a change in responding based on the memory of a previous taste. Ketamine, an NMDA-receptor antagonist, has an amnesic effect on conditioned taste aversion (CTA) formation in adult rats (Welzl et al., *Psychobiol.* 18, 1990, 43-47). However, we have shown that pre-treatment with ketamine enhances associative learning in E18 rats (Mickley et al., *Dev. Brain Res*., 127, 2001, 71-76). Here we sought to determine if ketamine also facilitates non-associative learning (i.e., PCEs) in fetuses. E18 rat fetuses received ketamine followed by oral lavage with either 0.15% or 0.30% SAC. Twenty-four hours later, we recorded observations of orofacial movements during, and immediately following, oral lavage with 0.30% SAC. E18 fetuses exhibited stronger evidence of PCEs (following an upward shift in SAC concentration) if they had received treatment with ketamine before the first taste exposure. Since NMDA NR2B receptors have been implicated in memory formation (Tang et al., *Nature.* 401, 1999, 63-69), we used western blot analyses to explore NR2B receptor populations in the gustatory neocortex (GNC) of the fetuses. Ketamine administered on E18 produced a discernable increase in GNC NR2B receptors 24-hours later. These data suggest that ketamine's ability to enhance associative memories in E18 rat fetuses may be extended to non-associative memories as well. Further, the up-regulation of NMDA NR2B receptors in response to ketamine treatment make them a viable target of further investigation as mediators of ketamine-induced potentiation of memory in E18 rats.

Introduction

NMDA receptor blockade and fetal learning:

- NMDA receptor blockade impairs memory formation in E19 fetuses (Mickley et al., *Dev. Brain Res*. 127, 2001, 71-76), neonates (Mickley et al., *Physiol. Behav.* 64, 1998, 381-390) and adult rats (Welzl et al., *Psychobiol*. 18, 1990, 43-47).
- However, we have demonstrated that N-methyl-D-aspartate (NMDA) receptor blockade with ketamine does not impair (and may even potentiate) memory formation in E18 rat fetuses (Mickley et al., *Dev. Brain Res*. 127, 2001, 71-76).
- Thus far, our studies have been confined to an associative memory task (conditioned taste aversion; CTA).
- The current study aimed to discover if ketamine has similar effects on E18 fetuses engaged in **non-associative** learning.
- Gustatory contrast can serve as an effective measure of non-associative memory over time (Grigson et al., *Am. J. Physiol.* 273, 1997, R479-R486).
- Gustatory positive contrast is represented by a change in consummatory behaviors (drinking or ingestive behaviors such as mouthing or licking) as a result of an upward shift in the hedonic value of a taste stimulus. (Weinstein, *J. Gen. Psychol*. 98, 1978, 225-240; Flaherty, *Animal Learn. Behav*. 10, 1982, 409-440).
- The increased responding by the "shifted" animal is presumably based on the memory of the previous taste experience (Grigson et al., *Am. J. Physiol*. 273, 1997, R479-R486).

The role of NMDA receptor subtypes in fetal development and learning:

- NMDA receptors are heteromeric glutamate ion-gated channels composed of two classes of subunits (NRs): NR1 and NR2. The NR2 subunit is characterized by four receptor subtypes NR2A, NR2B, NR2C and NR2D, and their distribution and expression in the rat brain depends on the stage of perinatal development (Mori & Mishina, *Neuropharmacol*. 34, 1995, 1219-1237).

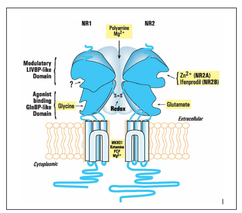

Figure 1: NMDA receptor. Modified from Kemp & McKernan, *Nature Neurosci*. 5, 2003, 1039-1042.

- The predominant expression of NR2B receptors in the fetal brain suggests that they are important physiologically and functionally during neural development, but their function is not fully explained (Loftis & Janowsky, *Pharmacol. Therapeut*. 97, 2003, 55-85).
- Tang et al. (*Neuropharmacol*. 41, 2001, 779-790) reported that transgenic mice that over-express NR2B subunits showed longer recognition memory than did control mice. Enhancement of NR2B receptors correlate with enhanced learning and memory.

Hypotheses

- **Ketamine given on E18 will potentiate positive contrast effects in E19 fetuses. This phenomenon will not be observed in rats dosed 24-hours later (on E19).**
- **A single dose of ketamine will cause an up-regulation of NR2B receptors in the gustatory cortex if given on E18 - but not if given on E19.**

Methods

Subjects

Fetal Sprague Dawley rats (male and female) obtained from timed-pregnant dams supplied by Zivic Laboratories (Zelienople, PA).

Table 1: Treatment Groups, Numbers of Subjects per Group and Group Nomenclature.

Drug Treatment (administered to pregnant dam)	Experimental Condition	Fetal Age at First Taste Exposure (TE1)	Subject Age at Second Taste Exposure (TE2)	Number of Subjects (Number of litters)	Group Nomenclature [1]
Ketamine[2]	Shifted	E18	E19	36(7)	E18-E19-Ket-S
Ketamine	Not shifted	E18	E19	43(7)	E18-E19-Ket-NS
Saline[3]	Shifted[4]	E18	E19	23(5)	E18-E19-Sal-S
Saline	Not shifted[5]	E18	E19	20(5)	E18-E19-Sal-NS
Ketamine[2]	Shifted	E19	E20	24(5)	E19-E20-Ket-S
Ketamine	Not Shifted	E19	E20	30(5)	E19-E20-Ket-NS
Saline[3]	Shifted[4]	E19	E20	29(5)	E19-E20-Sal-S
Saline	Not Shifted[5]	E19	E20	17(5)	E19-E20-Sal-NS

[1] Groups are identified by 2 numbers representing subject age at TE1 and TE2 (E18, E19 = Embryonic Days 18, 19, respectively); the drug treatment
(S saline control; Ket = ketamine HCL) and then the Shifted (S) or Non-Shifted (NS) treatment condition.
[2] Ketamine-treated dams received 100mg/kg, ketamine HCL, i.p., 30-min before the TE1 procedure.
[3] Saline-treated dams received a volume of saline equal to that of the animals receiving ketamine.
[4] Shifted rats received oral lavage with 10µl of 0.15% Saccharin (SAC) during TE1 and then 10µl of 0.30% SAC during TE2.
[5] Non-shifted rats received oral lavage with 10µl of 0.30% SAC as their first taste and then 10µl of 0.30% SAC as their second taste.

Tastants: Saccharin was mixed in deionized water to create solutions of 0.15% and 0.30%.

Experimental Treatments:
Drugs: Pregnant dams received either 100 mg/kg ketamine HCL, i.p., or an equal volume of saline 30-min before the first taste exposure.

Taste exposures:
Taste exposure 1 (TE1): Following a transient spinal block of the dam, fetal rats in the same litter received oral lavage with either 10µl of 0.15% or 0.30% saccharin (SAC) on E18 or E19.

All animals in the right or left uterine horn were randomly assigned to either the shifted or non-shifted condition. All fetuses in the opposite horn received the alternate treatment. Thus, both treatment conditions were represented in each litter.

Taste exposure 2 and behavioral testing:
Twenty-four hours after TE1 the pregnant dam received an irreversible spinal block. Fetal behavioral observations (with the help of The Observer™ computer program developed by Noldus Information Technology) followed oral lavage with 10µl of 0.30% SAC.

Behavioral observation periods:
- 1-minute and 10-second baseline before SAC lavage
- 15-second injection period
- 1-minute after SAC lavage

We counted fetal mouth movements and licks (using a modification of the methods described by Smotherman et al., *Dev. Psychobiol*. 17, 1984, 661-674).

Western blot analysis of NMDA NR2B receptors in fetal brains:
Timed-pregnant Sprague-Dawley rats received either Ketamine HCl (100 mg/kg, i.p.) or saline 24-hours before they were sacrificed and the brains of their fetuses dissected for western blot analyses of NMDA NR2B receptors.

NR2B subunit receptors are composed of 1456 amino acids with an approximate molecular mass of 170-180 kilo Daltons (kDa) (Loftis & Janowsky, *Pharmacol. Therapeut*. 97, 2003, 55-85).

Since gustatory neocortex (GNC) has been implicated in taste learning, we measured NR2B protein subunit expression in this, and closely adjacent areas of the rat cortex.

Western blot analyses of NMDA NR2B receptors were performed using procedures described by (Curras-Collazo & Dao, *Molecular Brain Research 70*, 1999, 187-196; with modification).

Table 2: Age of fetuses, number of subjects, drug treatments and group names in the NMDA NR2B receptor analysis

	E18-E19*	E19-E20
Ketamine (100 mg/kg, i.p.)	18 pups from 3 litters (E18-E19-K)	18 pups from 3 litters (E19-E20-K)
Saline (0.9%)	18 pups from 3 litters (E18-E19-S)	18 pups from 3 litters (E19-E20-S)

* = The first fetal age represents that age of the drug treatment; the second fetal age represents the age at sacrifice, 24-hours later.

Results

Behavioral Tests

E18-E19 fetuses exhibited stronger evidence of Positive Contrast Effects (PCEs) following an upward shift in SAC concentration, if they had received treatment with ketamine before the first taste exposure. This effect was not seen in E19-E20 rats.

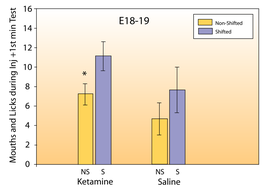

Figure 2: Mean number of mouthing and licking movements exhibited by E19 fetuses during, and 1 minute after, oral lavage with 0.3% SAC. NS = non-shifted rats; S = shifted rats.

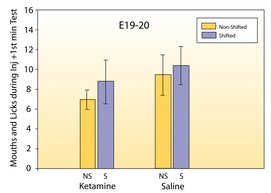

Figure 3: Figure 3: Mean number of mouthing and licking movements exhibited by E20 fetuses during, and 1 minute after, oral lavage with 0.3% SAC. NS = non-shifted rats; S = shifted rats.

Western Blot Analysis

Ketamine causes an up-regulation of NMDA NR2B receptors in the gustatory neocortex of E18-E19 rat fetuses. This phenomenon is not seen in E19-E20 fetuses.

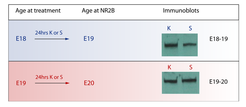

Figure 4: NMDA NR2B receptor subunit expression (170 kDa bands) in the gustatory neocortex (GNC) of fetal rats on E19 or E20 (i.e., 24-hours after treatment with ketamine or saline). (Note: K = ketamine treatment, S = saline control).

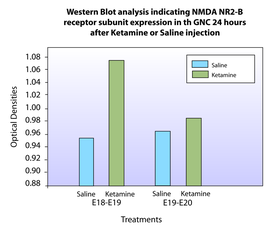

Figure 5: Quantitative analysis of NMDA NR2B receptor subunit expression in the gustatory neocortex of E19 or E20 fetal rats, 24 hours after a single ketamine (100mg/kg, i.p.) or saline injection. Bars represent the optical density of the band detected with the NMDA NR2B antibody. Values were obtained using NIH image software after the background and noise was subtracted from the film.

Summary & Conclusions

- Fetuses can form non-associative memories *in utero*.
- Ketamine treatment enhances the potency of a gustatory positive contrast effect in E18-E19 fetuses, but not E19-E20 fetuses.
- Within 24 hours, ketamine causes an up-regulation of NMDA NR2B receptors in gustatory neocortex (GNC) in fetuses receiving the drug on E18 – an effect not observed in older rats.
- This work complements other studies (Mickley et al., *Dev. Brain Res*. 127, 2001, 71-76) indicating that ketamine can produce age-dependent enhancements in classically conditioned responses in E18 rat fetuses.
- Age-dependent, ketamine-induced up-regulation of NMDA NR2B receptors in GNC may help explain these phenomena.

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