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

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Abstract

Twenty-four hours later, we recorded observations of associative learning in E18 rats (Mickley et al., 1990). Conditioned taste aversion (CTA) formation in adult rats, NMDA-receptor antagonist, has an amnesic effect on intensity of a gustatory stimulus (e.g., saccharin: SAC). The current study aimed to discover if ketamine, a NMDA receptor antagonist, can act as a positive contrast agent and increase NR2B receptor expression in developing fetal brains. Ketamine enhances positive contrast effects (PCEs) following an upward shift in SAC concentration. Ketamine-induced exposure with ketamine before the first taste exposure. This effect was not seen in E19-E20 fetuses.

Hypotheses

• Ketamine given on E18 will potentiate positive contrast effects in E19 fetuses. This phenomenon will not be observed until rats dosed 24-hours later (on E19).

• A single dose of ketamine will cause an up-regulation of NR2B receptors in the gustatory cortex given on E18 but not if given on E19.

Methods

1. Subject Age at Exposure: The mean age of the fetuses at the time of exposure to ketamine was 18.9 days (SD = 0.3 days). The mean age at the time of the measurement of NR2B expression was 19.5 days (SD = 0.3 days).

2. Behavioral Tests:

   a. Ear flicking (15-second injection period; 1-minute after SAC lavage).

   b. Licking movements: observed 30 minutes after SAC lavage.

3. Western Blot Analysis: NMDA NR2B receptor expression was determined by Western Blot analysis indicating NMDA NR2-B receptor subunit expression (170 kDa bands) in fetal brains. The bands were quantified using ImageJ software. The expression of NR2B subunits was normalized to GAPDH.

Results

Western Blot Analysis: Ketamine causes an up-regulation of NMDA NR2B receptors in the gustatory cortex of E18 rats.

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

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 (GCC), increasing the magnitude of the positive contrast effect.

• This work complements other studies (Mickley et al., 2012, 2015) 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 GCC may explain these phenomena.

Acknowledgements

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Introduction

The role of NMDA receptors in the development of taste perception and later associative learning is well documented (Grigson et al., 1999). NMDA receptors are known to play a crucial role in the development of taste perception, and later associative learning is well documented (Grigson et al., 1999). The rat neocortex, including the gustatory neocortex (GNC) of the rat brain, depends on the stage of prenatal development, and the role of NMDA NR2B receptors in the development of taste perception and later associative learning is well documented (Grigson et al., 1999). The role of NMDA NR2B receptors in the development of taste perception and later associative learning is well documented (Grigson et al., 1999).

Figure 1: NMDA receptor. Modified from Kemp & McKernan, 1999, 187-196; with modification.

Table 1: Age of fetuses, number of subjects, drug treatments and group names in the NMDA NR2B Western Blot Analysis.

<table>
<thead>
<tr>
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<th>Groups</th>
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| 10µl of 0.30% SAC. E18 fetuses exhibited stronger evidence of Positive Contrast Effects (PCEs) following an upward shift in SAC concentration. Ketamine-induced exposure with ketamine before the first taste exposure. This effect was not seen in E19-E20 fetuses.

Table 2: Age of fetuses, number of subjects, drug treatments and group names in the NMDA NR2B Western Blot Analysis.

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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: 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 5: Quantitative analysis of NMDA NR2B receptor subunit expression in the gustatory neocortex of E19 or E20 total 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.