Presented at the 1999 meeting of the Society for Neuroscience:

KETAMINE BLOCKS ASSOCIATIVE AND NON-ASSOCIATIVE LEARNING IN FETAL RATS. G. A. Mickley*, D. R. Remmers-Roeber, C. Crouse, R. Peluso and C. Walker. Department of Psychology, Baldwin-Wallace College, Berea, OH 44017-2088

Saccharin (SAC) evokes different orofacial responses in neonatal rats depending on whether the taste is novel, familiar, or if it had been previously associated with malaise *in utero*. Here we report that Ketamine [an N-methyl-D-aspartate (NMDA) glutamate receptor blocker] can attenuate the formation/expression of both a taste recognition memory and a conditioned taste aversion in perinatal rats. Dams pregnant with E19 rat fetuses were injected with 0, 50, or 100 mg/kg Ketamine HCl (i.p.). One-half hour later, a reversible spinal block was performed on the dam and fetuses received an oral injection of 10µl, 0.3% SAC or water while *in utero*. After the oral injection, fetuses received either saline or LiCl (81 mg/kg, i.p.). Following a normal vaginal delivery, these animals were exposed to SAC on post-natal day 3 (P3). Observations of motor responses were recorded immediately after the oral lavage of SAC. Familiar SAC evoked more mouth/lick movements than did novel SAC. Further, if SAC had been paired with LiCl in utero, neonates exhibited a conditioned suppression of orofacial movements. Both doses of ketamine significantly attenuated these indicators of associative and non-associative learning. These data reinforce the current conception of the fetus and neonate as sophisticated sensors and responders to the uterine and extra-uterine environment. Further, our findings indicate a role for NMDA receptors in the formation/expression of a taste recognition memory and conditioned taste aversion in fetal rats.

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Presented at the 2000 meeting of the Society for Neuroscience:

PARADOXICAL EFFECTS OF KETAMINE ON THE MEMORY OF FETUSES OF DIFFERENT AGES. G.A. Mickley*, D.R. Remmers-Roeber, C. Dengler, C. Kenmuir, and C. McMullen. Department of Psychology and the Neuroscience Program, Baldwin-Wallace College, Berea, OH 44017-2088.

Brain N-methyl-D-aspartate (NMDA) glutamate receptors have been implicated as important mediators of both learning and neuronal development. The current study investigated how ketamine HCl (a well-known NMDA-receptor blocking drug) would influence taste-mediated conditioned motor responses (CMRs) in perinatal rats. Dams pregnant with either E18 or E19 rat fetuses were injected with 0, 50, or 100 mg/kg Ketamine HCl (i.p.). One-half hour later, a reversible spinal block was performed on the dam and fetuses received oral lavage with 10µl, 0.3% Saccharin (SAC) or water (control) while *in utero*. After the oral injection, fetuses received either saline (control) or LiCl (81 mg/kg, i.p.). The uterus was replaced and, two days later (E20 or E21), some of the rats received oral lavage with SAC. Rats in other litters were born via a normal vaginal delivery and were exposed to SAC on post-natal day 3 (P3). Observations of motor responses were recorded immediately after the oral lavage of SAC. If SAC had been paired with LiCl *in utero*, pups exhibited a conditioned suppression of orofacial movements (as compared to controls). In a dose-dependent manner, ketamine significantly attenuated this taste-mediated CMR of animals conditioned on E19. However, the same ketamine treatments did not disrupt (and in some cases potentiated) the CMRs of rats treated with the drug before CS-US pairing on E18. Our findings indicate an age-dependent role for NMDA receptors in the formation of CMRs in perinatal rats.

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Presented at the 2001 meeting of the Society for Neuroscience:

A ROLE FOR GUSTATORY NEOCORTEX (GNC) IN THE DETECTION OF A FAMILIAR TASTE AND THE EXTINCTION OF A CONDITIONED TASTE AVERSION (CTA).

C.L. Kenmuir, C.A. McMullen, C.M. Dengler, D.R. Remmers-Roeber, and G.A. Mickley. Department of Psychology and the Neuroscience Program, Baldwin-Wallace College, Berea, OH 44017-2088.

We investigated brain *c-fos* protein expression during 3 stages of extinction of a CTA (Nolan et al., 1997) as well as following the taste of a novel *vs* familiar gustatory stimulus. Brain *c-fos* expression was compared among: (a) rats that had acquired a CTA [through pairings of 0.3% oral saccharin, (SAC) and i.p. Lithium Chloride, 81mg/kg, (LiCl)] and then received extinction training (i.e., subsequent non-reinforced SAC exposures), (b) rats that had acquired a CTA but received no extinction training (i.e., water exposure), (c) rats given SAC & LiCl explicitly unpaired (i.e., no CTA formed) and extinction training, (d) rats given SAC & LiCl explicitly unpaired followed by no extinction training, and (e) rats exposed only to oral, novel, SAC. The data suggest that extinction is not represented by a simple reversal of the *c-fos* activity evoked by CTA conditioning. Rather, our results identify a series of brain nuclei along the taste pathway that are sequentially activated as the CTA becomes extinguished. In particular, during initial and intermediate stages of extinction, expression of *c-fos* in the GNC is no different from that seen in non-extinguished controls. However, as rats achieve total reacceptance of SAC, *c-fos* expression becomes very prominent in the GNC. Likewise, *c-fos* expression in the GNC is more prominent following familiar *vs* novel SAC. These data suggest a role for the GNC in both the consolidation of extinction learning and in the detection of familiarity. *Supported by NIMH.*

Presented at the 1999 meeting of the International Behavioral Neuroscience Society:

KETAMINE BLOCKS TASTE RECOGNITION MEMORY (TRM) IN PERINATAL RATS. G.A. Mickley, D. Remmers-Roeber, R. Peluso, C. Crouse, and K. Bryan. Departments of Psychology, and Biology. Baldwin-Wallace College, Berea OH 44017 USA

Decisions about novelty/familiarity are critical to determining whether or not information should be attended to, and possibly encoded, for long-term storage. We have reported that fetal and neonatal rats exhibit an increase in orofacial movements (e.g., gapes, mouth movements, or licks) upon tasting saccharin (SAC) if it was experienced previously. E19 rat fetuses can acquire this "implicit" TRM and retain it for at least 5 days (P3) (Mickley et al., *Soc. Neurosci. Abstr.*, Vol. 24/1, p 177, 1998). In the current study we sought to determine the role of N-methyl-D-aspartate (NMDA) receptors in establishing a TRM. Pregnant Sprague-Dawley rats received ketamine (NMDA receptor antagonist) (doses: 0, 50 or 100 mg/kg, i.p.). One-half hour later we performed a reversible spinal block on each pregnant dam and E19 fetuses received an oral injection of 10 μl, 0.3% Saccharin (SAC) or water (control) while *in utero*. The uterus was replaced and the pups were later born via a normal vaginal delivery. On P3, all pups experienced oral lavage of 10 μl, 0.3% SAC and motor responses were recorded. As expected, control neonates tasting familiar SAC exhibited significantly more total mouth movements than did pups tasting novel SAC. However, this TRM response was not observed in rats exposed to ketamine *in utero*. The data suggest that early taste memories may be disrupted by NMDA receptor blockade. Although the mechanisms of this disruption have not been fully explored, previous data indicate that ketamine has a limited ability to alter sensory experiences of adult rats (Mickley et al., *IBNS Abstr*. p. 41, 1998). Thus, these findings may suggest a role for NMDA receptors in the formation of implicit memories during the fetal period. *Supported by NSF Award 9514799.*

Presented at the 2000 meeting of the International Behavioral Neuroscience Society:

DEVELOPMENT OF POSITIVE CONTRAST GUSTATORY LEARNING IN FETAL RATS. C.M. Dengler, F. Haarmann and G.A. Mickley, Department of Psychology, Baldwin-Wallace College, Berea, OH 44017 USA

Contrary to the classic view of limited fetal cognitive capabilities (W. James, *The Principles of Psychology*, 1890, 462), modern evidence suggests that fetuses can sense and respond to stimuli presented *in utero* (W.P. Smotherman, S.R. Robinson, *Psychol. Sci.*, 1:97-106, 1990). Here we sought to determine if fetal rats could (like adults) adjust their orofacial motor responses based on a memory of recent gustatory experience. The behavioral contrast paradigm has served as an effective measure of STM in adult rats (C.F. Flaherty, *Anim. Learn. Behav.*, 10:109-440, 1982) who will show an increase (i.e. positive contrast effect or PCE) in consummatory responses if they first experience a weak taste and later experience a stronger taste. This enhancement in responding is not observed in control rats that receive two exposures to only the stronger taste. Thus, PCE does not represent a direct sensory-motor reaction to a gustatory cue but rather it reflects a change in responding based on the memory of a previous taste. Pregnant dams carrying E18 or E19 fetuses received complete abdominal analgesia while pups were removed (still attached via umbilical cord), and tested in a temperature-controlled isotonic saline bath. Pups received 10 μl of either 0.15% or 0.3% SAC. Subsequent motor responses were videotaped. Approximately 50 minutes later, oral lavage of 10 μl of 0.3% SAC was administered and behavior was observed a second time. Videotapes were analyzed for orofacial movements. E19 fetuses shifted from 0.15% SAC to 0.3% SAC demonstrated a robust PCE whereas E18 fetuses did not. These data suggest that the PCE paradigm may be used to evaluate STM in fetal rats. Further, our data indicate that the ability to demonstrate a PCE becomes part of the rat's behavioral repertoire between E18 and E19. *Supported by NSF Award: 9514799*

Presented at the 2001 meeting of the International Behavioral Neuroscience Society:

CHANGES IN BRAIN C-FOS EXPRESSION DURING EXTINCTION OF A CONDITIONED TASTE AVERSION (CTA). C.L. Kenmuir, C.A. McMullen, C.M. Dengler, D.R. Remmers-Roeber, and G.A. Mickley. Department of Psychology and the Neuroscience Program, Baldwin-Wallace College, Berea, OH 44017-2088.

CTAs are produced by pairing a novel taste (conditioned stimulus; CS) with the symptoms of poisoning (unconditioned stimulus; US). The current study investigated the neural changes that accompany the extinction of a CTA. Rats received three pairings of a CS [0.15% saccharin, (SAC) orally] and US [Lithium Chloride; 81mg/kg, i.p.] in order to establish a robust CTA. The CTA was then extinguished by allowing the 23-hour/day fluid-deprived rats to drink SAC each day. The brains of these animals were collected and the number of *c-fos* nuclei counted following pre-determined levels of saccharin acceptance. These levels were based on three phases of extinction: *static*: <10% of baseline SAC consumption; *dynamic*: >10%<80% of baseline SAC consumption; *asymptotic*: 100% of baseline SAC consumption (Nolan et al., 1997). We measured *c-fos* protein immunoreactivity in several nuclei within the gustatory pathway: Nucleus tractus solitarius (NTS), pontine parabrachial nucleus (PBN), basolateral amygdala (BLA) and gustatory neocortex (GNC). Brain *c-fos* expression associated with each extinction phase was compared among the following treatment groups: (a) rats that had acquired a CTA and then received extinction training (i.e., subsequent SAC exposures), (b) rats that had acquired a CTA but received no extinction training, (c) rats given the CS & US explicitly unpaired (i.e., no CTA formed) and extinction training, (d) rats given the CS & US explicitly unpaired followed by no extinction training, and (e) rats exposed only to oral SAC. The data suggest a non-uniform pattern of c-fos expression in various brain nuclei throughout the course of extinction. Extinction is not represented by a simple reversal of the *c-fos* activity evoked by CTA conditioning. Rather, our results identify several nuclei activated by CTA training that remain activated throughout extinction. Concurrently, extinction evokes increased activity of additional nuclei in the rostral gustatory pathway (e.g., GNC), which were inactive during CTA training. *Supported by NIMH.*

Presented at the 2002 meeting of the Society for Neuroscience:

LONG-TERM, AGE-DEPENDENT EFFECTS OF A SINGLE EPISODE OF FETAL N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE: THE KETAMINE PARADOX REVISITED. G.A. Mickley; C.L. Kenmuir; A.M. McConnell; C.A. McMullen; A.A. Snyder; D.G. Likins-Fowler. Department of Psychology and the Neuroscience Program, Baldwin-Wallace College, Berea, OH, USA

Embryonic day 18 (E18) rat fetuses pre-treated with ketamine and taught a conditioned taste aversion (CTA) learn and remember the CTA, whereas E19 fetuses do not (Mickley et al., Dev. Brain Res., 2001, 127, 71-76). The current studies determined if long-term behavioral effects could be detected in animals that received ketamine (100 mg/kg, i.p. through the dam) or a saline control injection on either E18 or E19. Behavior was evaluated on several measures, including spontaneous locomotion and water maze learning. Measures were collected during 2 periods: Test period 1 (P0-P21) or, Test period 2 (P60-P90). Prenatal ketamine improved performance in the water maze, especially during the initial training trials. On the first trial of Test 2, rats treated with ketamine on E18 reached the hidden platform faster than any other group including rats treated with ketamine on E19. Swim speeds of experimental and control rats were not significantly different. Since the enhanced maze performance of ketamine-treated rats (especially those treated on E18) is not easily attributable to faster swim times our data may reflect differences in learning ability depending on when, during the fetal period, an animal experiences NMDA receptor blockade. These data indicate that the paradoxical age-dependent effects of early ketamine treatment may also be detected later in young adult rats. *Supported by: NSF Award: 9514799*

Presented at the 2003 meeting of the Society for Neuroscience:

C-FOS PROTEIN EXPRESSION IN THE BRAIN DURING THE EXTINCTION OF A CONDITIONED TASTE AVERSION (CTA). G.A. Mickley, C.L. Kenmuir, C.A. McMullen, A.M. McConnell, E. Valentine, C.M. Dengler-Crish and B.A. Weber. Department of Psychology and the Neuroscience Program, Baldwin-Wallace College, Berea, OH 44017-2088.

This study investigated changes in brain activity during 3 different stages of CTA extinction (Nolan et al., 1997). Brain *c-fos* protein expression was analyzed in fluid-deprived rats that had acquired a CTA [3 pairings of 0.3% oral saccharin (SAC) and 81mg/kg i.p. Lithium Chloride (LiCl)] followed by extinction training (i.e., subsequent non-reinforced SAC exposures). The neuroanatomical expression of *c-fos* protein in these rats was compared to that from subjects in several additional control groups including animals that had acquired a CTA followed by subsequent water exposures (not extinguished), rats that experienced SAC & LiCl explicitly unpaired (no CTA was formed) followed by subsequent non-reinforced SAC exposures, rats that experienced SAC & LiCl explicitly unpaired followed by subsequent water exposures, rats that were exposed only to oral, novel, SAC, and rats that were sacrificed immediately following CTA conditioning. Throughout the extinction process, elevated levels of *c-fos* protein were evident in the brainstem nuclei along the taste pathway. This increased expression within the Solitary Tract Nuclei was independent of the stage of extinction. However, the increase in expression within the Parabrachial Nuclei declined as the CTA was further extinguished. Neurons in the Basolateral Amygdala expressed less c-fos protein during the intermediate stage of extinction than during the initial or final stages. As rats achieve total reacceptance of SAC, *c-fos* expression reached its peak in the Gustatory Neocortex. These data suggest that extinction is not represented by a simple reversal of the *c-fos* activity evoked by CTA conditioning. Rather, our results identify a series of brain nuclei along the taste pathway that are sequentially activated as the CTA becomes extinguished. *Supported by NIMH.*