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Ketamine Blocks a Taste-Mediated Conditioned Motor Response in Perinatal Rats

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MICKLEY, G. A., D. R. REMMERS-ROEBER, C. CROUSE AND R. PELUSO. *Ketamine blocks a taste-mediated conditioned motor response in perinatal rats.* PHARMACOL BIOCHEM BEHAV **66**(3) 547–552, 2000.—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 in perinatal rats. Dams pregnant with E19 rat fetuses were injected with 0, 50, or 100 mg/kg ketamine HCl (IP). One-half hour later, a reversible spinal block was performed on the dam, and fetuses received an oral injection of 10 μl 0.3% Saccharin (SAC) or water while in utero. After the oral injection, fetuses received either saline or LiCl (81 mg/kg, IP). The uterus was replaced and, 2 days later (E21), rats received oral lavage with SAC. Rats in other litters were born via a normal vaginal delivery and were exposed to SAC on postnatal 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, both E21 and P3 pups exhibited a conditioned suppression of orofacial movements (compared to controls). Both doses of ketamine significantly attenuated this taste-mediated conditioned motor response. These data reinforce the current conception of the fetus and neonate as sophisticated sensors and responders to the uterine and extrauterine environment. Further, our findings indicate a role for NMDA receptors in the formation of a conditioned motor response in fetal rats.

Conditioned taste aversion Fetus Neonate Rat Memory Saccharin Lithium chloride Development Ketamine NMDA Learning

CONDITIONED taste aversions (CTAs) may be formed when an animal consumes a novel taste (conditioned stimulus = CS) and then experiences the symptoms of poisoning (unconditioned stimulus = US) (12). When later given a choice between the poisoned taste and some more-familiar taste (typically water), the organism will avoid the taste that it previously associated with malaise. This taste aversion memory is notable for its potency and the apparent biological preparedness of animals to acquire it. The CTA association may be acquired after only one CS–US pairing (5,13), with a long interval between the taste and the malaise (28) and under a variety of circumstances in which awareness of the relevant stimuli is degraded (5). In fact, the association of a gustatory trace with poisoning can proceed even under deep anesthesia (16,27).

Perinatal rats may also acquire conditioned aversions. Oral presentation of a chemical stimulus before an intraperitoneal injection of lithium chloride (LiCl) on embryonic day 17 (E17) created a conditioned suppression of rat fetal activity when subjects were reexposed to the same stimulus 2 days later (31). Pairing of a taste/odor with LiCl on either E18 (20,30) or E20 (34,35) created a CTA that was observed even when the taste test was conducted as long as 2.5 weeks postnatally. Further, Rudy and Chealate (29) have demonstrated olfactory aversions in 2-day-old rat pups.

Although little research has focused on the neuropharmacological substrate of CTA formation in perinatal rats, a role for glutaminergic neurons in adult CTA has been proposed. For example, Yamamoto and Fujimoto (40) reported that the NMDA antagonist DL-aminophosphovaleric acid (APV) disrupts CTA formation when it is injected into the amygdala. These data have been corroborated by other labs (1,37) that have documented ketamine-induced antagonism of conditioned aversions. Ketamine is a well-known noncompetitive

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glutamate NMDA receptor antagonist (23,36). These findings offer an obvious parallel with the more extensive data base indicating that NMDA antagonists block hippocampal long-term potentiation (LTP) and prevent some forms of learning [(15,38,39; see (25) for review]. Further, recent experiments indicate that intracortical injections of the NMDA receptor antagonist CPP [-3(-2 carboxipiperazin-4-yl)-proply-l-phosphonic acid] impairs both CTA and LTP recordings in the portion of the insular cortex implicated in the storage of CTA memories (9).

In addition to the role that NMDA receptors play in adult learning, these receptors also mediate early neuronal development. Evidence that a mechanism very similar to LTP might operate during refinement of the retinotectal projection in fish and frogs has been offered by Reh and Constantine-Paton (24). Experiments involving cell cultures have shown that NMDA exerts a trophic influence on hippocampal (7) and cerebellar neurons (3,4). Glutamate decreases dendritic growth and causes pruning of hippocampal cells in culture (19). Conversely, NMDA receptor antagonists block synapse elimination during brain development (2), promote axonal elongation (7), increase total dendritic length, and reduce the branch loss normally seen in granule cells (6).

Blockade of NMDA receptors in perinatal rats has produced age-dependent alterations in classically conditioned behavioral responding. A recent study from our laboratory indicated that ketamine administration (0.1 or 10 mg/kg, IP) can block CTA formation in neonatal (P0) rats (21). This finding is similar to that previously reported in adult rats (1,37). However, ketamine pretreatment can potentiate CTA formation in younger (E18) animals (20). Thus, there may be windows in the developmental process when NMDA receptor blockade can produce very different effects on learning. In an attempt to further sort out the ontogeny of perinatal learning, the current study investigated the ability of ketamine to modulate a taste-mediated conditioned motor response in E19 fetuses.

METHOD

Subject

The subjects were fetal and neonatal Sprague–Dawley rats (male and female) obtained from timed-pregnant rats supplied by Zivic-Miller Laboratories (Zelienople, PA). The date of conception (i.e., the first day that a vaginal plug was detected) was designated as "embryonic day 0" (E0). Pregnant animals (from which our subjects were derived) were individually housed in plastic "shoe box" cages (44.45 cm long × 21.59 cm wide × 20.32 cm high). Home cage temperature was maintained at 23–26°C under a 12 L:12 D cycle (lights on at 0600 h).

Drug Treatments

One-half hour before rat fetal injections began (see below), pregnant dams received an injection of either 0, 50, or 100 mg/kg ketamine HCl (Sigma Chemical Company), IP. These doses were selected based on previous HPLC studies documenting the amount of ketamine present in the brain of fetuses 0.5 h after maternal injections with this NMDA receptor antagonist (21). A maternal dose of 100 mg/kg ketamine, IP produces fetal brain levels of approximately 14 μg/g. Similar brain levels have been shown to be sufficient to block the formation of conditioned taste aversions in neonatal rats (21). Following this drug treatment, the dam was placed into a holding cage with absorbent paper towels until the conditioning procedures began.

Fetal Injections

Pregnant rat dams carrying the E19 fetuses were briefly anesthetized with Isoflurane™ before they underwent a reversible spinal block procedure. A 30-gage needle was used to inject Lidocaine HCl 2% and Epinephrine 1:100,000 (in a volume of 100 μl) between the first and second lumbar vertebrae. This procedure is effective in producing (a) a complete abdominal and hind limb paralysis, (b) consistently long periods of spinal anesthesia (>45 min), and (c) complete recovery after the anesthesia. There is no indication that litters are adversely affected by this procedure (30,32).

The analgesic dam was restrained in a plastic holding apparatus, and her vision of the fetal injection procedure restricted. Uterine horns were exposed through a midline laparotomy, and the hind legs and lower abdomen immersed in a warm bath (37.5 ± 1°C) containing isotonic saline (Locke's solution) (11). Both horns of the uterus were exteriorized through the abdominal incision and the horns allowed to float freely in the bath. Rat fetuses that are E18 or older can be seen through the walls of the uterus and positioned for accurate placement of injections. All fetuses in a particular litter received oral lavage of either the conditioned stimulus (CS = 10 μl of 0.3% saccharin, SAC) or a control vehicle injection (10 μl distilled water). Ten to 15 min following CS administration, rats received an IP injection of the unconditioned stimulus (US = 81.0 mg/kg lithium chloride, LiCl) or a control vehicle injection of an equal volume of saline. Thus, three combinations of injections defined our main treatment groups: (a) SAC + LiCl: this is the main taste aversion conditioning group. (b) SAC + Sal: this group allowed us to observe the effects of exposing fetuses/neonates to SAC alone and, therefore, controlled for the nonconditioned effects of the CS. (c) H_2O + LiCl: this group controlled for the nonconditioned effects of the US. The H_2O + LiCl treatment allowed us to determine if the malaise following exposure to LiCl alone produced a change in motor responding. Without this control group we would be uncertain about whether the alteration in orofacial movements we observed were due to the animals associating SAC and LiCl or merely a residual effect of the LiCl itself. Following the injections, the uterus was replaced, the abdominal wall and the skin of the pregnant rat sutured, and the wounds infused with a local anesthetic (Bupivicaine; 0.25%) to produce postsurgical analgesia.

Even et al. (10) have reported that steroids present in one amniotic sac may diffuse, across the fetal membranes, to other fetuses in the uterus. Although our injections were aimed toward the mouth of the fetus, SAC or LiCl almost certainly also spilled into the amniotic fluid, and may have moved into adjacent uterine compartments. If different pups in a litter had different injections, this could have confounded our conditioning procedure. For this reason, we did not mix different taste injections within litters. This procedure necessitated special data analysis techniques (see Statistical Analysis below).

Behavioral Testing

Smotherman and Robinson (31,33) have shown that LiCl produces a suppression of motor responding in rat fetuses. When a taste/odor has been paired with LiCl, rats will later suppress their spontaneous movements in response to this CS. In an attempt to determine the limits on retention of these conditioned motor responses, our subjects were tested as neonates on either E21 (2 days after training) or P3 (5 days after training). Throughout this article, the groups of animals are

designated by the subject's age during the conditioning procedure and their age at time of the behavioral test. Therefore, the age groups in these experiments were E19–E21 and E19–P3. The number of subjects/litters in each treatment group were: (a) SAC + LiCl: E19–E21, 50 mg/kg ketamine pretreatment: $n = 15$ pups from four litters; 100 mg/kg ketamine pretreatment: $n = 18$ pups from four litters; nondrugged controls: $n = 21$ pups from six litters; E19–P3, 50 mg/kg ketamine pretreatment: $n = 13$ pups from four litters; 100 mg/kg ketamine pretreatment: $n = 14$ pups from three litters; nondrugged controls: $n = 11$ pups from three litters. (b) SAC + Sal: E19–E21, 50 mg/kg ketamine pretreatment: $n = 20$ pups from four litters; 100 mg/kg ketamine pretreatment: $n = 20$ pups from five litters; nondrugged controls: $n = 15$ pups from three litters; E19–P3, 50 mg/kg ketamine pretreatment: $n = 15$ pups from three litters; 100 mg/kg ketamine pretreatment: $n = 17$ pups from four litters; nondrugged controls: $n = 10$ pups from two litters. (c) H_2O + LiCl: E19–E21, 50 mg/kg ketamine pretreatment: $n = 10$ pups from three litters; 100 mg/kg ketamine pretreatment: $n = 14$ pups from three litters; nondrugged controls: $n = 11$ pups from two litters; E19–P3, 50 mg/kg ketamine pretreatment: $n = 20$ pups from four litters; 100 mg/kg ketamine pretreatment: $n = 14$ pups from four litters; nondrugged controls: $n = 15$ pups from five litters.

Neonatal Behavioral Testing

If rats had not been born 4 h before the scheduled behavioral test on E21, they were removed by Cesarean section. Twenty-four of the 34 litters experienced this procedure. Cesarean section was accomplished while the dam was provided analgesia via an irreversible spinal block (0.1 ml 100% ethanol) using the injection procedure described above. If rat pups had been born via a normal vaginal delivery they were separated from the dam 20 min before the behavioral test. While awaiting testing, pups were placed, with littermates, in a small plastic container sitting on a warm (38.5 ± 0.5°C) heating pad. This container was covered with gauze and maintained in a temperature-controlled incubator (ambient temperature = 28 ± 1°C) until immediately before testing of the litter began. For the behavioral observations, neonates were placed in a warm (ambient temperature = 28 ± 1°C), high-humidity chamber on a glass plate warmed (via constantly circulating water) to 36 ± 1°C. Pups received oral lavage with 10 μl SAC through a blunt/smooth 18-gage stainless steel infusion needle. Subjects were then placed (ventral side down) on the glass plate. Using a mirror, behavior was videotaped from below the animal for 1 min before (baseline) and after oral injection.

Dependent Variables and Data Analysis

Rat behaviors were recorded on videotape and later reviewed and scored with the help of The Observer™ computer program developed by Noldus Information Technology. Using a modification of the methods described by Smotherman and Robinson (31–33), we sorted observed behaviors into 12 exclusive and exhaustive categories of spontaneous fetal movements (head, mouth, lick, gape, curl, stretch, twist, roll, hindlimb, forelimb, facewipe, and twitch movements). Because they seemed to be the most sensitive indicators of taste recognition, this article focuses on orofacial movements: mouth movements and licks. Neonates born via Cesarean section exhibited mouthing and licking responses that were statistically indistinguishable from pups that underwent a normal vaginal delivery. Therefore, the data from these animals were combined in all the analyses reported.

The data were analyzed via an analysis of covariance [ANCOVA: age (E19–E21, E19–P3) × drug (0, 50, 100 mg/kg ketamine) × treatment (Sac + LiCl, Sac + Sal, H_2O + LiCl)] using a linear model (SASTM, SAS Institute, Carey, NC) compensating for unequal n-values. Because all the rats in a particular litter received the same conditioning treatment, we included litter as an independent, random, and nested factor (within the conditioning treatments). This approach controls for litter effects and offers a direct statistical test of the significance of such effects (8,17). In the analyses conducted here, effects attributable to litter were not statistically significant, and therefore, subsequent analyses were run without this nested factor. Likewise, the motor responses of the animals in the two control groups (Sac + saline and H_2O + LiCl) were not significantly different, and therefore, they were combined in a subsequent analysis. Post hoc analyses employed Duncan's Multiple Range Test (18). An $\alpha = 0.05$ was adopted throughout these tests.

Our method of data analysis attempted to take into account some of the differences between the motor capabilities of different aged rats by employing an ANCOVA. Here we used, as a covariate, each animal's total activity (a total of head, mouth, lick, gape, curl, stretch, twist, roll, hindlimb, forelimb, facewipe, and twitch movements) during the 1-min baseline period immediately before oral lavage with SAC on the test day. Thus, the differing ability/motivation of different aged rats to move spontaneously was factored into our treatment of the data.

RESULTS

The data indicate that E19 fetuses can acquire a significant conditioned motor response to a gustatory stimulus, and that ketamine can block the formation of this CTA. The AN-

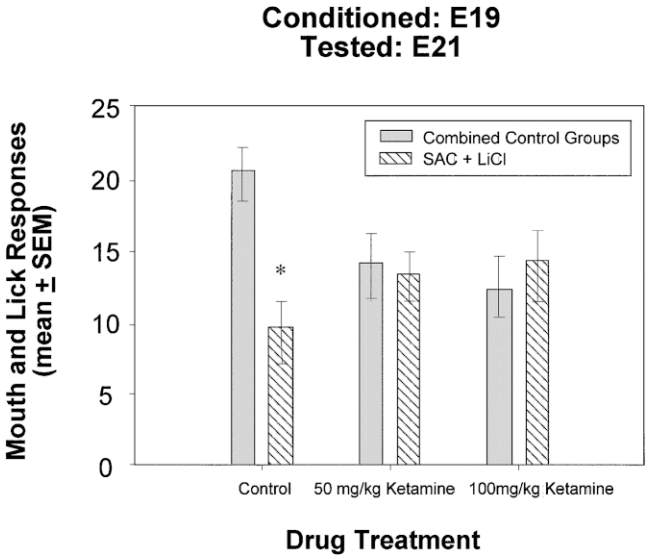

FIG. 1. Effects of ketamine on conditioned orofacial movements in E21 neonates. Fetal rats that received oral lavage with saccharin (SAC) and then an injection of Lithium Chloride (LiCl) on E19 exhibited significantly (*<0.05) fewer mouth movements and licks when reexposed to SAC on E21. Comparison groups are control rats that received SAC + saline or H_2O + LiCl injections on E19. This taste-mediated conditioned suppression of movement on E21 was not observed in rats treated with ketamine before the CS–US pairing on E19. Variance indicators are the standard error of the mean (SEM).

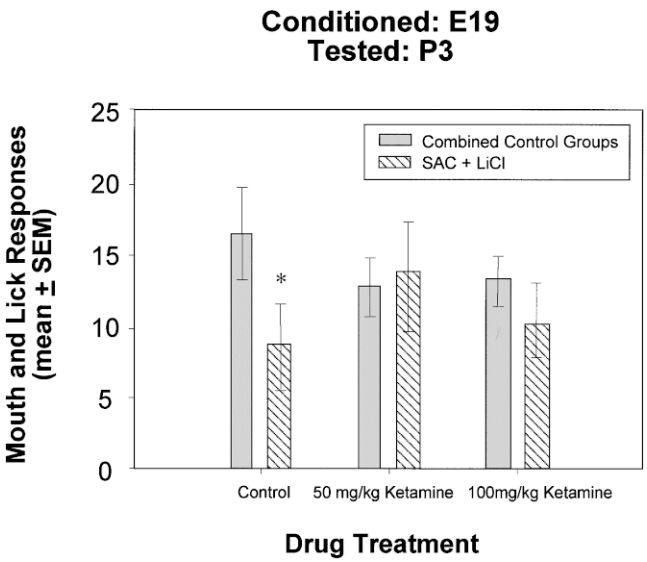

FIG. 2. Effects of ketamine on conditioned orofacial movements of P3 neonates. Fetal rats that received oral lavage with saccharin (SAC) and then an injection of Lithium Chloride (LiCl) on E19 exhibited significantly (*<0.05) fewer mouth movements and licks when reexposed to SAC on P3. Comparison groups are control rats that received SAC + saline or H_2O + LiCl injections on E19. This taste-mediated conditioned suppression of movement on P3 was not observed in rats treated with ketamine before the CS–US pairing on E19. Variance indicators are the standard error of the mean (SEM).

COVA revealed a significant treatment effect, $F(1, 260) = 5.30$, $p = 0.02$, and a significant drug × treatment interaction, $F(2, 260) = 4.43$, $p = 0.01$. Post hoc tests indicated that rat fetuses (from dams not pretreated with ketamine) receiving SAC + LiCl on E19 exhibited significantly fewer orofacial movements than did controls when they again tasted SAC on the test day (see Figs. 1 and 2). However, the ketamine-treated subjects did not show this SAC-induced suppression of orofacial movement. Both doses of ketamine seemed similarly effective in blocking the CTA.

The conditioned motor responses were prominent both 2 and 5 days after the CS–US pairing (see Figs. 1 and 2). Likewise, ketamine pretreatment was equally effective in producing blockade of CTAs in animals tested on E21 and P3. The total activity covariate did not achieve statistical significance. Further, there was not a reliable difference between the orofacial responses of different aged rats (E21 vs. P3) in the various drug or CS–US treatment groups.

Our statistical analysis did not reveal a significant main effect of drug administration. As Fig. 2 illustrates, rat pups treated with ketamine on E19 and tested on P3 did not consistently reduce, or enhance, orofacial responses to SAC. Likewise, the changes in motor responses 2 days after ketamine exposure (Fig. 1) were not generally depressed by the drug treatment. There was a significant main effect of treatment (CS–US) and a significant drug × treatment interaction—suggesting that decreases in oral responding depend on particular combinations of the stimuli presented and drug treatment. Post hoc tests indicated that the sole group of ketamine-treated subjects showing significantly reduced mouth and lick movements on E21, was the combined-control group treated with 100-mg/kg ketamine (i.e., the pups that received the largest dose of ketamine and were tested within 2 days).

DISCUSSION

Smotherman and Robinson (31) have reported that LiCl produces a suppression of motor responding in rat fetuses. When a taste/odor was paired with LiCl, rats later suppressed their spontaneous movements in response to this CS. The current data are consistent with this earlier work. Here we found that SAC + LiCl parings in E19 fetuses cause a relative decrease in mouthing and licking movements when the subjects again taste SAC on either E21 or P3. Further, our studies also indicate that this taste-mediated conditioned motor response may be blocked by an injection of ketamine administered 30 min before the original CS–US pairing.

The underlying mechanisms of the phenomenon described here have been explored to only a limited extent. Ketamine may block the conditioned suppression of orofacial movements by altering saccharin's gustatory sensation, by attenuating the effects of the LiCl US or by disrupting the CS–US association. It should be noted, however, that several lines of evidence suggest that the abilities to taste sweet substances or to experience LiCl-induced malaise are not significantly altered by ketamine.

Other studies from our lab (22) have investigated the ability of ketamine to impair gustation or reduce the effects of LiCl. We determined the initial taste preferences of young adult rats by measuring their consumption of 0.3 or 0.6% SAC. Rats drank significantly more of the 0.3% SAC. Ketamine did not significantly modify this preference, suggesting that the animals could still discriminate between the two concentrations based on taste/palatability factors. These data are consistent with those reported by Aguado et al. (1), who found that ketamine (25 mg/kg administered to adult rats) did not alter the process of habituation to novel sucrose. A similar failure to disrupt gustatory habituation was observed when the NMDA receptor antagonist MK-801 was used (26). Further, if ketamine was given before a CTA test, it did not block the retrieval of an already established taste aversion, nor did it impair the ability to recognize a saccharin solution (21,37).

Although the experiments cited above were performed in more mature animals, our belief that ketamine does not significantly impair taste sensation in fetuses is supported by the observation that ketamine (100 mg/kg administered through the maternal circulation—as in the current study) can actually enhance CTAs of E18 rat pups (20). Apparently, NMDA receptor blockade does not eliminate the ability of these younger fetuses to taste because they can associate SAC and LiCl on E18 and then exhibit a conditioned taste aversion when tested over 2 weeks later.

Does ketamine block conditioned taste aversions (CTAs) by antagonizing the malaise-inducing properties of the US? This proposed mechanism also seems unlikely. In other experiments from our laboratory (22), young adult rats received either ketamine or physiological saline (IP) followed by a second injection of either LiCl (81 mg/kg, IP) or saline. One half-hour later, rats each had access to a single bottle of either 0.3% or 0.6% SAC. Thus, this procedure provided information about the direct (nonconditioned) effects of ketamine and LiCl on SAC consumption. As expected, LiCl alone reduced drinking of SAC water. Likewise, ketamine (given in doses that disrupt CTA formation in neonatal rats) did not alter LiCl's ability to suppress consummatory responses (22).

Although there remains more work to be done in this area, the evidence cited suggest that ketamine-treated rats can still taste and sense the malaise associated with LiCl. If it is the case that ketamine has a limited ability to alter CS (saccharin) and US (LiCl) sensation, then our data may reflect ketamine's ability to disrupt the associative taste-mediated conditioned motor response we describe here.

Other investigators have reported a role for NMDA receptors in CTA formation in adult animals (1,9,14,38,40). Consistent with these data, NMDA receptor antagonists, like ketamine, have a well-known ability to block the formation/expression of a variety of associative memory tasks. Ketamine-induces learning/performance deficits in mature animals learning a water maze (38), a delayed alternation task (15), or undergoing classical fear conditioning (39). Further, ketamine administration (0.1–70 mg/kg, IP) can block conditioned taste aversion formation in adult (1,37) or neonatal (P0) rats (21). The findings reported here suggest that NMDA receptor blockade in E19 fetuses produces a disruption in memory formation that is in some ways similar to that seen in neonatal and adult rats. Paradoxically, ketamine does not have this same detrimental effect on the acquisition of conditioned aversive responses by E18 fetuses (20). In fact, ketamine potentiates CTA formation in these younger animals. The mechanism of this apparent paradox is under active investigation in our laboratory.

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