**G. Andrew Mickley**
**Zana Hoxha**
**Anthony DiSorbo**
**Gina N. Wilson**
**Jennifer L. Remus**
**Orion Biesan**
**Kyle D. Ketchesin**
**Linnet Ramos**
**Joseph R. Luchsinger**
**Suzanna Prodan**
**Morgan Rogers**
**Nathanael R. Wiles**
**Nita Hoxha**

*The Neuroscience Program*
*Baldwin Wallace University*
*275 Eastland Rd., Berea, OH 44017*
*E-mail: amickley@bw.edu*

# Latent Inhibition of a Conditioned Taste Aversion in Fetal Rats

***ABSTRACT:*** *The etiology of schizophrenia's cognitive symptoms may have its basis in prenatal alterations of glutamate N-methyl-D-aspartate (NMDA) receptor functioning. Therefore, the current study investigated the effects of ketamine (an NMDA receptor blocking drug) on both a conditioned taste aversion (CTA) and latent inhibition (LI; a model of attentional capacity) in rat fetuses. We first sought to determine if a CTA could be diminished by nonreinforced preexposure to a CS in fetal rats (i.e., LI). We injected E18 pregnant Sprague–Dawley rats with 100% allicin (garlic taste) or an equal volume of saline. Some of the pregnant dams also received ketamine (100 mg/kg, i.p.). One day later (E19), the dams received a second injection of the CS, followed by either lithium chloride (the US) or saline. Finally, on E21 pups received oral lavage with allicin and observations of ingestive orofacial motor responses 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. Preexposure to the garlic taste on E18 produced a LI of this CTA. Ketamine significantly disrupted the formation of the CTA and had some impact on LI. However, the direct effect of ketamine on LI is less certain since the drug also blocked the original CTA. © 2013 Wiley Periodicals, Inc.* Dev Psychobiol 56: 435–447, 2014.

***Keywords:*** *fetal; learning; memory; conditioned taste aversion; CTA; latent inhibition; ketamine; NMDA; N-methyl-D-aspartate*

## INTRODUCTION

Schizophrenia is a multifaceted disorder that likely has multiple genetic, biological, and environmental origins (for reviews, see Koenig, Kirkpatrick, & Lee, 2002; Picard, 2011). A variety of studies also point to vulnerable periods during fetal development during which stress, toxins, or inflammatory reactions may enhance the appearance of positive schizophrenic symptoms much later in life (Opler & Susser, 2005; Rapoport, Addington, Frangou, & Psych, 2005; Rehn & Rees, 2005; Stefan & Murray, 1997). Therefore, the study of fetal cognitive capacities and how they may be influenced by drugs or toxins may better reveal the origins of schizophrenia.

One fundamental symptom of schizophrenia, dysfunction of attentional selectivity (i.e., a reduced ability to filter relevant from irrelevant information), has been modeled by a learning phenomenon called latent inhibition (LI) (Weiner & Arad, 2009). LI refers to the observation that organisms that receive nonreinforced preexposure to a to-be conditioned stimulus (CS) are less efficient in generating a conditioned response to that stimulus when it is subsequently paired with reinforcement (Gaisler-Salomon & Weiner, 2003). Essentially an animal can learn to disregard a stimulus, or particular dimension of a stimulus, when it predicts no important or unusual event (Best, 1975). A deficit in LI is seen in acute schizophrenics (Boutros, Belger, Campbell, D'Souza, & Krystal, 1999; Gray, Feldon, Rawlins, Hemsley, & Smith, 1991; Lubow, Kaplan, Abramovich, Rudnick, & Laor, 2000) and this paradigm has been employed experimentally to tap into attentional factors that are important in adaptive processing of information (Lubow, 1989, 2005). A variety of nonhuman animal studies have confirmed the

Manuscript Received: 16 September 2012
Manuscript Accepted: 18 February 2013
*Correspondence to*: G. Andrew Mickley

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 26 March 2013
DOI 10.1002/dev.21110 • © 2013 Wiley Periodicals, Inc.

usefulness of the LI paradigm in modeling both attentional and associative properties of schizophrenia (Katz, Rogers, & Steinmetz, 2002; Lewis & Gould, 2004; Lubow, 2005; Weiner & Arad, 2009) although there is some controversy about the extent to which the same underlying biological processes mediate LI deficits in humans and nonhuman animals (Schmidt-Hansen & Le Pelley, 2012).

The conditioned taste aversion (CTA) procedure has been an especially useful means of investigating LI (Lubow & De la Casa, 2005). A CTA may be established through the pairing of a novel gustatory CS with a malaise-inducing unconditioned stimulus (US) (Garcia, Kimeldorf, & Koelling, 1955; Gruest, Richer, & Hars, 2004; Welzel, Adamo, & Lipp, 2001). Further, LI of a CTA may be induced by preexposure to a CS without any US—resulting in a reduced potency of the taste aversion (Bethus, Lemaire, Lhomme, & Goodall, 2005; Lubow & De la Casa, 2005).

Given that the etiology of schizophrenia may have its origins during the prenatal period and that LI has been identified as a useful model of attentional and/or associative capabilities, we decided to evaluate the ability of rat fetuses to display LI of a CTA. The ability of perinatal animals to develop a CTA has been demonstrated in rats from E17 to P0 (Gruest et al., 2004; Mickley et al., 1998; Mickley, Remmers-Roeber, Dengler, Kenmuir, & Crouse, 2001; Smotherman & Robinson, 1985). LI of a CTA has been shown to be a reliable phenomenon in adult rats (Archer, Mohammed, & Jarbe, 1986), as well as in juvenile rats as early as age P18 (Yap & Richardson, 2005). However, to the best of our knowledge there is no published literature examining LI, or the neurochemical substrate of LI, in fetal rats.

As the central nervous system develops, *N*-methyl-D-aspartate (NMDA) glutamate receptors undergo dynamic changes in population and physiology (Ben-Ari, Cherubini, & Krnjevic, 1988; Hestrin, 1992; Sircar, 2000) and stimulation of these receptors is important for cell survival, differentiation and plastic responses to environmental pressures (Deutsch, Mastropaolo, & Rosse, 1998). Further, perinatal blockade of NMDA receptors produces several schizophrenic-like symptoms in animal models (Abekawa, Ito, Nakagawa, & Koyama, 2007; Behrens & Sejnowski, 2009). Our laboratory has also reported that, in an age-dependent fashion, NMDA receptor blockade can also affect the potency of CTA formation and detection of gustatory novelty by rat fetuses (Mickley et al., 1998; Mickley, Remmers-Roeber, Crouse, & Peluso, 2000; Mickley, Remmers-Roeber, Crouse, Walker, & Dengler, 2000; Mickley et al., 2001). Therefore, we became interested in the role that NMDA receptors might play in establishing LI of a CTA in fetal rats.

It is known that NMDA receptors play an important role in CTA formation in adults (for review, see, Jimenez & Tapia, 2004) and this literature has been extended to LI. For example, several studies have indicated that the NMDA-receptor blocking drugs MK-801 and ketamine impair attention to CS preexposure and thereby attenuate LI of conditioned fear (Davis & Gould, 2005; Lewis & Gould, 2007; Razoux, Garcia, & Léna, 2007) and LI of a CTA (Aguado, San Antonio, Perez, del Valle, & Gomez, 1994; Gallo, Bielavska, Roldan, & Bures, 1998; Traverso, Ruiz, & De la Casa, 2003). However these data are controversial and other experiments indicate that, in adult animals, timing of the NMDA-receptor blocking treatment can determine whether LI attenuation or potentiation is observed (Gaisler-Salomon & Weiner, 2003; Klamer et al., 2005; Palsson et al., 2005; Traverso et al., 2003; Weiner & Arad, 2009). Therefore, in addition to determining if fetal rats can exhibit LI of a CTA, we also investigated the impact that the NMDA receptor blocking drug ketamine would have on LI of a CTA in fetal rats if it was given before CS exposure.

The data we report here indicate that fetal rats can exhibit behaviors that are consistent with the phenomenon of LI. Moreover, ketamine-induced blockade of NMDA receptors can alter behavioral markers of CTA and LI in fetal rats.

## METHODS

### Subjects

The subjects were fetal male and female Sprague–Dawley rats obtained from timed pregnant dams (Charles River Laboratories, Wilmington, MA), whose initiation of pregnancy was designated as "embryonic day 0" (E0). The dams were housed individually in plastic wire-topped cages (44.45 cm long × 21.59 cm wide × 20.32 cm high) filled with "Bed-o-Cobs" corncob bedding (Andersons Industrial Products, Maumee, OH) and allowed ad libitum access to food (Purina Rodent Chow, No. 5001, PMI Nutrition International, Brentwood, MO) and tap water. Cage temperature was maintained at 23–26°C with 30% humidity, ±5%. The animals were maintained on a 12:12-hr light/dark cycle. Lights came on at 0600 hr and went off at 1800 hr.

The animal care and use procedures employed were approved by the Baldwin Wallace University Institutional Animal Care and Use Committee. Animals were procured and cared for according to the recommendations in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and in compliance with the Animal Welfare Act.

### Tastants and Drugs

The CS in this study was allicin (ALL; 100% 2-propene-1-sulfinothioic acid S-2-propenyl ester; the molecular taste

component of garlic; Amagase, 2006; Shadkchan et al., 2004). Allicin passes through the placental barrier unmodified and may be detected by fetuses (Gruest et al., 2004; Nolte, Provenza, Callan, & Panter, 1992). This tastant was obtained from Allimax (Chicago, IL) and filtered (.2 μm pore size) before injection into pregnant rats (2 ml/100 g, i.p.). To reduce the volume/injection, the allicin was divided between two injections separated by a 15-min interval to achieve the target concentration in the amniotic fluid (see below). The taste of garlic has been reported previously to be neither attractant nor repellant to rats before conditioning (Capretta & Rawls, 1974; Maruniak, Madon, & Kostelc, 1983). However, our pilot research (see below) confirmed a slight taste preference for .1% allicin in adult rats. Therefore, this concentration was approximated in both the amniotic fluid and subsequent oral lavage during fetal behavioral testing.

Lithium Chloride (LiCl; Sigma-Aldrich Chemical Co., St. Louis, MO) was utilized as the US. It was made weekly (81 mg/ml in .9% physiological saline), stored at 4°C to avoid degradation, and administered via the maternal circulation at 81 mg/kg, i.p. Ketamine HCl (Ketathesia™; Butler Schein Animal Health, Dublin, OH; 100 mg/ml) was administered to the fetuses through the maternal circulation via an i.p. injection of 100 mg/kg. All control injections consisted of .9% physiological saline.

### Pilot Studies

We performed two pilot studies aimed at determining: (1) the concentration of allicin/garlic taste that rats prefer, and (2) the concentration of allicin that is achieved in the amniotic fluid following a 2 ml/100 g, i.p. maternal injection.

Briefly, the first study employed five male Sprague–Dawley rats (300–400 g) that were placed on a 23 hr fluid deprivation schedule (water only from 1200 to 1300 hr) 2 days prior to their first consumption test. This schedule was maintained throughout the pilot study. Animals were given a total of five, 3-day sets of consumption testing during which a single bottle containing an allicin solution was presented from 1200 to 1230 hr. We used a 3-day exposure to each allicin/water solution to reduce the impact of gustatory neophobia (Lin, Amodeo, Arthurs, & Reilly, 2012). During each of these 3-consecutive-day tests, animals were given access to a single bottle of water, .10%, 1.00%, 5.00%, or 20% allicin dissolved in distilled water (%v/v). To avoid dehydration, animals were given supplemental water for 30 min/day 15 min following the preference test (12:45-13:15 hr). Following the third day of testing of each allicin concentration, animals were given one rest day on which they received access to water only for an hour. After this rest day the animals were again exposed to a different concentration of allicin for 3 consecutive days, followed by another rest day. The order of presentation of the different concentrations of allicin was counterbalanced among the five animals to account for the effects of taste preexposures on drinking levels. A preference index was calculated (ml allicin solution consumed/ml water consumed) for each animal and the two most preferred allicin solutions, with preference scores greater than one, for all animals were .1% and 5.0% allicin. Therefore, to more accurately determine the rats' preference, we did a simultaneous two-bottle preference test between 5.0% and .1% allicin solutions in the same group of animals. The two-bottle preference testing lasted a total of 3 days (and followed the final rest day mentioned above). This test consisted of simultaneously placing two bottles (.1% allicin and 5.0% allicin) on the animals' cages for 30 min. Since fluid-deprived rats tend to drink voraciously from the first source of liquid they encounter, bottle positions were switched at 1, 5, and 10 min into testing to force the animals to sample from each bottle. Following this 30 min of preference testing, animals were given 30 min access to water. A preference index was calculated (ml .1% allicin/ml 5.0% allicin) and showed that all animals preferred .1% allicin over 5.0% allicin. Therefore, for the fetal studies, the target concentration chosen for amniotic fluid was .1% allicin (see below) and the same concentration was used for oral lavage during fetal behavioral testing.

The second pilot study aimed to estimate the concentration of allicin that should be injected into a pregnant rat in order to produce a .1% allicin concentration in the amniotic fluid. Seven timed-pregnant Sprague–Dawley dams (E17 or E18), supplied by Charles River Laboratories, and a total of 54 fetuses were used for analysis of allicin concentrations in amniotic fluid via reversed phase high performance liquid chromatography (HPLC) within 30–90 min following an intraperitoneal injection of 100% allicin (2 ml/100 g rat weight). The time points chosen for sample collection were based on previous work of Pushpendran, Devasagayam, Chintalwar, Banerji, and Eapen (1980) who detected garlic metabolites in the rodent liver optimally between 30 and 120 min after an i.p. injection. Gruest et al. (2004) also used similar time points for their fetal taste exposure procedures with garlic. In our pilot study, dams were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) 20 min prior to a laparotomy procedure used to extract amniotic fluid. Using a 30 gauge needle, 200 μl amniotic fluid samples were taken from each individual amniotic sac. The samples were centrifuged and the supernate was extracted and used for HPLC analysis. The pH of each sample (and standards) was adjusted with trifluoroacetic acid to 1. A prosphere HP C18 AQ column (5 μl, 15 cm long) with a 50 μl sample loop was used for samples and pressure (Ar gas) was maintained between 1,550 and 1,680 psi. The solvent system was 30% acetonitrile and 70% deionized water. Flow rate was set at 1.0 ml/min and run time was 10 min. Based on runs with standard solutions, we determined that the expected elution time of allicin was 7–8 min under the given conditions.

This HPLC analysis revealed that amniotic fluid allicin concentration following a 100% allicin, 2 ml/100 g i.p. injection to the dam was about 1–2% of the total injection. This procedure produced an amniotic fluid concentration of allicin equal to .163 ± .006% (mean ± SEM) after 60 min in both E17 and E18 animals. Volumes of amniotic fluid in fetal sacs at E17 and E18 were based on values reported by Robinson and Smotherman (1992) and used to make these calculations. It was through these studies that we determined that a maternal i.p. injection of 100% allicin (2 ml/100 g, i.p.) approximated the desired .1% allicin concentration (i.e., the preferred

tastant concentration of adult rats) in amniotic fluid at ~60 min postinjection (i.p.).

## Experimental Design, Procedural Overview, and Group Nomenclature

We employed a 3-way factorial design: Drug treatment (ketamine or saline) × Tastant pretreatment (ALL or saline) × Conditioning treatment (ALL + LiCl or ALL + saline). During phase 1 of the study, pregnant dams (E18) were treated with ketamine (i.p.) or received a control saline injection. On this same day, rat fetuses were either preexposed to the taste of garlic (allicin, i.p.) through the maternal circulation or the dams received a saline control injection. One day later (E19; experimental phase 2), dams received an injection of allicin followed by either LiCl or saline. Finally, on E21 the pregnant dam was given a spinal block and fetuses were removed from the uterus and allicin was placed in their mouth while we recorded orofacial (mouthing and licking) responses to the tastant (phase 3).

The following group nomenclature was employed (see also Tab. 1): treatment designations before the "+" sign indicate dam treatments that occurred on E18 [either an i.p. injection of saline (SAL) or ALL, accompanied by either a ketamine or saline control injection]. Group designations that follow the "+" indicate treatments on E19 (either an ALL and saline pairing or an ALL and LiCl pairing, i.p.). Therefore a group designated as SAL + ALL(LiCl) assessed the existence of a CTA to ALL, while a group designated ALL + ALL(LiCl) assessed the existence of a LI of a CTA.

## Procedures

***Experiment Phase 1—Preexposure.*** Fetal learning has historically been difficult to study due to the immaturity of sensory and motor systems in neonates. However, the gustatory and olfactory systems are somewhat functional in mammals during late gestation, thus providing an opportunity to study learning through measures that are dependent on these systems (Teicher & Blass, 1977). Late in gestation, fetal rats consume amniotic fluid, thus allowing exposure to tastes within the womb (Lev & Orlic, 1972). Further, drugs like ketamine cross the placental barrier and move freely into the fetal circulation (Mickley et al., 1998).

In this initial phase of our study, E18 pregnant dams received ketamine (100 mg/kg, i.p.) or a control injection consisting of an equal volume of physiological saline. The timing of the ketamine treatment (before CS administration) was consistent with two previous studies in adult rats showing that ketamine or MK-801, injected before or just after preexposure to a CS, disrupted LI in a conditioned taste-aversion paradigm whereas no effect was obtained when these compounds were injected during the conditioning stage (Aguado et al., 1994; Traverso et al., 2003). One hour following this

**Table 1. Summary of Group names, Treatments, Procedures, and Ns/Group**

| | Experimental Phase 1–3 | | | |
|---|---|---|---|---|
| | 1, CS Preexposure (i.p. Injections)[a] | 2 CTA Training Day (i.p. Injections)[a,b] | 3 Fetal Behavioral Tests (Oral infusions)[c] | |
| | Fetal Age | | | |
| Groups and Group Nomenclature | E18 | E19 | E21 | Number of Subjects (Litters/Fetuses) |
| *Saline, pretreatment* | | | | |
| CTA [SAL + ALL(LiCl)] | Saline (SAL) | Allicin + LiCl | Allicin | 5/21 |
| Latent inhibition [ALL + ALL(LiCl)] | Allicin | Allicin + LiCl | Allicin | 4/19 |
| Nonconditioned control (one CS exposure) [SAL + ALL(SAL)] | Saline | Allicin + SAL | Allicin | 4/17 |
| Nonconditioned control (two CS exposures) [ALL + ALL(SAL)] | Allicin | Allicin + SAL | Allicin | 4/17 |
| *Ketamine, pretreatment* | | | | |
| CTA [SAL(KET) + ALL(LiCl)] | Saline | Allicin + LiCl | Allicin | 4/21 |
| Latent inhibition [ALL(KET) + ALL(LiCl)] | Allicin | Allicin + LiCl | Allicin | 4/20 |
| Nonconditioned control (one CS exposure) [SAL(KET) + ALL(SAL)] | Saline | Allicin + SAL | Allicin | 3/18 |
| Nonconditioned control (two CS exposures) [ALL(KET) + ALL(SAL)] | Allicin | Allicin + SAL | Allicin | 5/20 |

[a]Pregnant dam received a 2 ml/100 g i.p. injection of 100% filtered allicin, which diluted to a .1% concentration of allicin in amniotic fluid. Controls received equal volume of physiological saline (SAL). Dams also received either 100 mg/kg ketamine (i.p.) or equal volume of SAL.

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

[c]Fetus received oral infusion of 10 μl of .1% allicin.

ketamine/saline treatment, the two groups were further subdivided into pregnant dams receiving allicin (2 ml/100 g, i.p., 100% concentration) or dams receiving an equal volume of physiological saline (see Tab. 1).

***Experiment Phase 2—CTA Training.*** The following day (E19) pregnant dams received a CS injection of allicin (100% concentration; 2 ml/100 g, i.p.) or saline followed by a US injection of LiCl (81 mg/kg, i.p.) or a saline control injection.

***Experiment Phase 3—Fetal Behavioral Testing.*** Taste recognition and the hedonic value of tastes experienced within the womb can be evaluated by observing the orofacial movements (e.g., mouths or licks) of fetuses during initial exposure or re-exposure to a gustatory stimulus (Shwartz & Grill, 1985; Soussignan, Schaal, & Marlier, 1998). The licking and mouthing movements we observed have been closely associated with the ingestion of preferred sweet tastants like sucrose and saccharin (Ganchrow, Steiner, & Canetto, 1986). Further, the rhythmic oral responses following infusion of sweet liquids are likened to consummatory taste preference measures (like spout-licking) in several respects. Both are emitted in the same frequency range, organized in burst/pause patterns, and serve the function of oral transport of fluid into position for swallowing (Kaplan, Roitman, & Grill, 1995). Other investigators have shown that licking frequency increases in direct proportion to the concentration of sucrose (Hsiao & Fan, 1993). Thus, the motor responses measured in the current study are, in many ways, similar to other measures of taste preference or intake. The orofacial motor analysis we report here is identical to the well-established procedure used by Smotherman and Robinson (1985) as an indication of CTAs (for reviews see Smotherman & Robinson, 1988a, 1988b, 1988c, 1989, 1990). Ingestive mouthing and licking movements indicate acceptance of a taste (Shwartz & Grill, 1985; Soussignan et al., 1998), whereas a reduction of mouthing and licking movements indicate disgust or aversion (Berridge, 2000; Grill & Norgren, 1978a, 1978b; Mickley et al., 2001).

Fetal behavioral testing commenced 48 hr after CTA conditioning. On E21 dams were briefly anesthetized with isoflurane while an irreversible spinal block was performed by an injection of .1 cc of 100% ethanol between the first and second lumbar vertebrae using a 30 gauge needle. This produced total abdominal and hindlimb paralysis and analgesia (Mickley, Remmers-Roeber, Crouse, & Peluso, 2000; Mickley, Remmers-Roeber, Crouse, et al., 2000; Smotherman, Richards, & Robinson, 1984) that allowed fetuses to be tested without the influence of general anesthesia. The dam was held on a plastic platform and a visor was secured over her head to restrict her vision of the procedure while a midline abdominal incision was made. The dam was then immersed in a bath of Locke's solution at $37.5 \pm 1^{\circ}C$ (Galigher & Kozloff, 1971; Mickley et al., 2001) and the horns of the uterus were exposed. After a 15-min recovery period to allow the removal of isoflurane from the maternal/fetal system, fetuses were removed one-by-one and tested. Care was taken to maintain the umbilical cord connections (and thereby blood/oxygen supplies) to the fetuses. Individual fetuses, while continuously immersed in the bath, were placed on an observation platform and received oral lavage of 10 μl of .1% allicin solution via a blunt 30 gauge needle. Their behavior was recorded for 30 s during the injection period using Windows Media Player™ (Microsoft, Inc., Redmond, WA).

## Behavioral Scoring

Using the Observer® computer program (Noldus Information Technology, Wageningen, Netherlands) video recordings of fetal behavior were viewed and scored according to the number of mouthing and licking movements made during a 30-s period of allicin infusion. A ''mouth'' was scored when the observer saw an opening and closing of the fetus' mouth. A protrusion of the tongue was scored as a ''lick.'' The scoring system is a reliable one. Within our laboratory, inter-rater reliability correlations (i.e., Pearson's product moment **r** values) ranged from .67 to .99 with a mean of .91.

## Statistical Analysis

In the current experiment it was impossible to assign subjects at random to particular treatment conditions since all rats in each litter received the same dam treatment (ALL + Saline or ALL + LiCl), preexposure treatment (ALL or Saline), and drug treatment (Ketamine or Saline). Thus, in this hierarchical design these experimental treatments are ''nested'' within litters. There are a variety of methodological and statistical measures that have been suggested to evaluate and deal with this type of potential confound (see, Denenberg, 1976; Holson and Pearce, 1992; Zorrilla, 1997). Here, we followed the procedure specified by Denenberg (1976) who allows the use of multiple pups from each litter (in order to decrease the variation among litters) and recommends a preliminary evaluation regarding the extent to which the nested variable (litter) should be retained as part of additional statistical analyses. Although it is often the case that there is a significant effect due to litters, there are still many instances in the literature in which the variation among litter is no greater than the variation within litters (see, e.g., Mickley et al., 2001). This may be due to pups from large litters developing in slightly different environments from each other (for review, see Ryan & Vandenbergh, 2002). To evaluate the contribution of litter variance to our findings, we performed an initial 3-way ANOVA [Dam treatment (ALL + Saline or ALL + LiCl) × Preexposure treatment (ALL or Saline) × Drug Treatment (Ketamine or Saline)] with litter nested within these factors. The number of litters/group in our study ranged from 3 to 5 (see Tab. 1) and so, in order to avoid a Type II error (indicating that there is no litter effect when there may indeed be one; Denenberg, 1976) we adopted a high $\alpha$ (.15) for this preliminary analysis. The IBM SPSS statistics™, v. 20 (Chicago, IL) software was used to perform these analyses. As described below, our initial examination of the data failed to reveal a significant litter effect. Therefore, subsequent analyses were run without consideration of litter.

We hypothesized that a conditioned suppression of orofacial responding to our CS would be curtailed by preexposure to that gustatory stimulus (i.e., LI effect) and that ketamine

would disrupt these phenomena. We evaluated these hypotheses via selective *t*-tests with the resulting *p*-values adjusted using the Holm–Sidak procedure (see Abdi, 2010) to reduce the likelihood of a Type I error (see specific group comparisons in the Results Section). The Holm–Sidak procedure is a more-powerful modification of the Bonferroni approach that still keeps under control the inflation of the Type I error. This is a sequential method in which the calculated *p*-value for each pairwise comparison is adjusted for each test separately as more tests are performed. The *p*-values indicated below correspond to the *t*-statistics as originally calculated but we report only those that were also $\leq .05$ once the Holm–Sidak correction was applied.

Although not one of our original predictions, in order to assess possible ketamine-induced alterations in gustatory habituation or action as a US (see the Discussion Section), we employed an additional post hoc *t*-test to evaluate the orofacial responses of ketamine-treated rat pups that did not receive an ALL + LiCl pairing (i.e., nonconditioned controls) following either allicin or saline preexposure. This was a 3rd *t*-test that incorporated the same Holm–Sidak procedure as described above (Abdi, 2010).

## RESULTS

We observed a CTA (i.e., a suppression of the fetal ingestive behaviors of mouthing and licking) in our fetal rats but preexposure to the CS reversed this suppression, indicating LI of this conditioned response. Ketamine treatment on the day of CS preexposure blocked the CTA. Since a significant CTA was not detectable in these rats, it was difficult to fully evaluate the occurrence of LI in the ketamine-treated pups.

We performed an initial 3-way ANOVA [Dam treatment (ALL + Saline or ALL + LiCl) × Preexposure treatment (ALL or Saline) × Drug Treatment (Ketamine or Saline)] with litter nested within these factors in order to determine the extent to which the nested variable (litter) should be retained as part of additional statistical analyses (see Denenberg, 1976 and Statistical Analysis Section). This analysis did not reveal a significant effect of litter. Therefore, the 3-way ANOVA was run without litter as a factor. This analysis did not uncover main effects but did reveal a significant 3-way interaction [$F(1,140) = 8.00$, $p = .005$].

In order to further investigate these groups differences, subsequent Holm–Sidak corrected *t*-tests (Abdi, 2010) were conducted to perform a limited number of ad hoc pair-wise group comparisons of interest (see the Statistical Analysis Section). These analyses indicated that saline-control rats with an ALL + LiCl pairing on E19 later demonstrated evidence of a CTA by suppressing their mouthing and licking as compared to fetuses that had an ALL + Saline pairing [$t(36) = 2.13$; $p = .02$] (see Fig. 1). Further, if fetuses had an

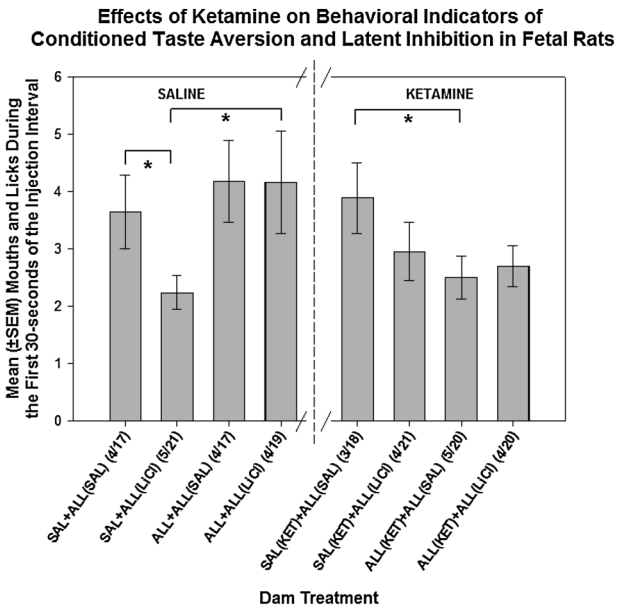


**FIGURE 1** Mouthing and licking behaviors exhibited by E21 rat fetuses during an oral infusion of allicin (ALL). A previous pairing of ALL + LiCl produced a suppression of mouthing and licking. But this aversive reaction was eliminated if fetuses had been preexposed to allicin (LI effect). Ketamine blocked the CTA. Further, ketamine-treated rats in the latent inhibition group preexposed to the CS [ALL(KET) + ALL(LiCl)] did not differ from the CTA group [SAL(KET) + ALL(LiCl)] in their orofacial responding to allicin. This finding is consistent with ketamine-induced blockade of LI. However, this conclusion is obfuscated by the lack of a CTA in the rats exposed to the NMDA-receptor blocking drug. Group nomenclature (see also Tab. 1): treatment designations before the "+" sign indicate dam treatments that occurred on E18 [either an i.p. injection of saline or allicin, accompanied by either ketamine (right side of figure) or saline control injection (left side of figure)]. Group designations that follow the "+" indicate treatments on E19 (either an allicin and saline pairing or an allicin and LiCl pairing, i.p.). The numbers in parentheses after each dam treatment designation indicate the number of litters and (following the slash) the total number of fetuses tested; $^{*}p < .05$.

ALL + LiCl pairing but were preexposed to allicin, this treatment produced a LI as indicated by a statistically significant reversal of the CTA effect [$t(38) = -2.06$; $p = .025$].

Unlike the saline-injected controls, rats treated with ketamine on E18 did not exhibit a CTA. Inspection of the descriptive statistics (including overlapping SEMs; see Fig. 1) revealed that orofacial responses of nonconditioned rat pups in the SAL(KET) + ALL(SAL) group were not reliably different than the mouthing and licking movements of animals in the SAL(KET) + ALL(LiCl) CTA-treatment group. Further, ketamine-treated animals that had been preexposed to allicin before

they received an ALL + LiCl pairing [ALL(KET) + ALL(LiCl) group] showed levels of mouthing and licking similar to those of rats that had not been preexposed to the CS [SAL(KET) + ALL(LiCl) group]. This finding would normally suggest that this NMDA-receptor-blocking drug altered LI. However, this conclusion must be tempered by the fact that a CTA was not detectable in the animals treated with ketamine.

Ketamine-treated rat pups that did not receive an ALL + LiCl pairing (i.e., nonconditioned controls) but were preexposed to allicin [ALL(KET) + ALL(SAL) group] showed a suppression of mouthing and licking as compared to animals that were not preexposed to allicin on E18 [SAL(KET) + ALL(SAL); $t(36) = 2.10$; $p = .02$; see Fig. 1].

## DISCUSSION

Our data revealed that rat fetuses can acquire a conditioned suppression of mouthing and licking in response to oral lavage with allicin if it had been paired with LiCl. This outcome may be reversed if the rats had been preexposed to allicin (LI effect). To the best of our knowledge, this is the first demonstration of the LI effect in rat fetuses. Ketamine treatment eliminated the taste aversion effect and rats in the LI group preexposed to the CS [ALL(KET) + ALL(LiCl)] did not differ from the CTA group [SAL(KET) + ALL(LiCl)] in their orofacial responding to allicin. This is consistent with ketamine-induced blockade of LI but this conclusion is obfuscated by the lack of a CTA in the rats exposed to the NMDA-receptor blocking drug. Our findings are consistent with other studies conducted in fetal (Mickley et al., 2001) and adult rats (Traverso, Ruiz, Camino, & De la Casa, 2008), indicating that NMDA receptor blockade can impair CTA memories and affect LI (Davis & Gould, 2005; Gaisler-Salomon & Weiner, 2003; Gallo et al., 1998; Turgeon, Auerbach, & Heller, 1998; Razoux et al., 2007). However, the literature suggests that the timing of NMDA receptor blockade (i.e., before/after CS preexposure/conditioning) may influence these behavioral outcomes (Aguado et al., 1994; Klamer et al., 2005; Moser, Hitchcock, Lister, & Moran, 2000; Palsson et al., 2005; Traverso et al., 2003; Turgeon et al., 1998; Weiner & Arad, 2009).

The effects of ketamine on CTA and LI reported here may also be dependent on the drug kinetics/metabolism and the dose of the drug administration. Other studies have indicated that ketamine works at NMDA receptors for ~95 min after i.p. administration (Lannes, Micheletti, Warter, Kempfl, & Di Scala, 1991). The actual levels of ketamine that reached the fetal brain were likely quite low. A previous HPLC analysis from our lab indicated that a 100 mg/kg, i.p. dose of ketamine administered to a pregnant rat on E18 produced fetal concentrations of ~14 μg/g of brain tissue 30–60 min postinjection (Mickley et al., 1998). Therefore, it is unlikely that ketamine effects carried over from E18 (when allicin preexposure occurred) to E19 (when allicin was paired with the US) or to E21 (when behavioral testing was conducted). Since ketamine was effectively out of the fetal system at the time of conditioning and test, this reduces concerns about state-dependent learning (Overton, 1983). However, it also raises questions about how the drug influenced CTA formation when conditioning occurred 24-hr later. It should be noted, however, that the duration of ketamine's effects may be further lengthened through the action of active metabolites (such as norketamine; for review, see White, Way, & Trevor, 1982).

Ketamine has been reported to impede formation of a gustatory trace memory (Traverso et al., 2008) but this effect should be distinguished from ketamine's effects on gustatory capacity or sensation. Evidence suggests that ketamine and other NMDA-receptor blocking drugs have minimal ability to modulate taste sensations (Mickley et al., 2002) and behavioral phenomena such as habituation (Aguado et al., 1994; Mickley et al., 1998) and neophobia (Robinson, Crooks, Shinkman, & Gallagher, 1989) that depend on intact gustatory capability. In fact, on E18 (but not later in perinatal development) ketamine-induced NMDA receptor blockade heightens the intensity of a CTA (Mickley, Lovelace, Farrell, & Chang, 1995; Mickley et al., 1998; Mickley et al., 2001)—suggesting that fetuses do not have an impaired ability to taste. If it is the case that ketamine has a limited ability to alter gustatory sensation itself, then the data reported here may reflect ketamine's ability to disrupt CTA and possibly nonassociative taste recognition memories (i.e., LI) through ketamine's pharmacological effects on the NMDA receptor.

An unanticipated finding from our study was the apparent suppression of the mouthing and licking response of rats in the ALL(KET) + ALL(SAL) group as compared to the SAL(KET) + ALL(SAL)-treated animals. The first of these two treatment groups was exposed to allicin twice whereas the second was exposed only once. These data are consistent with gustatory habituation to the taste of allicin in these ketamine-treated rats—a phenomenon not observed in control fetuses that received saline instead of ketamine. Alternatively, it may be hypothesized that ketamine acted as a US to eventually cause the suppression of orofacial responses during behavioral testing on E21.

Similar phenomena have been described following NMDA receptor blockade in adult animals (Aguado, Valle, & Pérez, 1997; Turgeon, Auerbach, Duncan-Smith, George, & Graves, 2000). The taste of allicin on E18 was our fetuses' initial exposure to this novel substance. Adult rats tend to avoid novel tastes (''neophobia''; Lin et al., 2012) and novel tastes are especially effective CSs within CTA paradigms (Franchina & Slank, 1988). Perhaps this novel taste potentiated CTA acquisition in the ALL(KET) + ALL(SAL) group? Although some of our results seem to indicate that ketamine may have acted as a US, this explanation is not consistent with much of the adult rat literature reporting that NMDA receptor blockade impairs CTA or LI (Aguado et al., 1994; Gallo et al., 1998). We have seen similar age-dependent disruptions of CTA formation in fetal and neonatal rats (Mickley et al., 1998, 2001). We acknowledge that the results of our study do not allow a clear conclusion regarding the means by which ketamine caused the suppression of mouthing and licking in our ALL(KET) + ALL(SAL) group.

The detrimental effects of neonatal administration of NMDA-receptor blocking agents on rat learning and memory and brain development have been well documented (Lyall, Swanson, Liu, Blumenthal, & Turner, 2009; Olney, 2002; Turner et al., 2012). However, the effects of such drugs, like ketamine, on fetal learning and brain growth are less well understood. Data from our laboratory suggest that there are transitional periods late in rat gestation when ketamine administration can either enhance or impair formation of a CTA (Mickley et al., 2001). The neural mechanisms of this phenomenon remain unclear but ketamine has been reported to alter proliferation and increase differentiation in rat cultured E17 cortical stem cells (Dong, Rovnaghi, & Anand, 2012). LI of CTAs have been modulated by manipulation of adult rat gustatory neocortex (Gallo et al., 1998; Roman & Reilly, 2007; Rosenblum, Meiri, & Dudai, 1993), hippocampus (Gerdjikov, Rudolph, Mohler, Feldon, & Yee, 2008; Purves, Bonardi, & Hall, 1995), medial septum (Turgeon, Kegel, & Davis, 2001), and striatum (Turgeon & Reichstein, 2002) making these brain areas likely targets of future studies aimed at discerning the neural mechanisms that subserve the learning phenomena reported here.

LI represents a task that is thought to assess the ability of an organism to ignore irrelevant information and has been proposed as a model of the attention deficits associated with schizophrenia (Davis & Gould, 2005). The LI phenomenon has been studied most extensively in mature animals, despite the fact that several schizophrenic symptoms may have prenatal origins. Therefore, behavioral studies during fetal development represent one of the significant gaps in the LI literature. Although some work has been done using amphibian embryonic models (Ferrari & Chivers, 2011), up to this point LI experiments in mammals have only investigated this phenomenon following birth (see, e.g., Peterschmitt, Meyer, & Louilot, 2007). The studies reported here extend this literature to include both a prenatal neuropharmacological manipulation with ketamine and a prenatal behavioral assessment of LI. Unfortunately, since ketamine also disrupted the formation of the CTA itself, it is difficult to unequivocally determine the effects of this drug on LI.

There is a continuing search for early biomarkers of psychosis and it has been reported that the disruption of the developing immune system and the brain's glutamatergic systems may contribute to the production of schizophrenic symptoms later in life (Freedman et al., 2005). Several of the genes associated with schizophrenia are involved with the formation of glutamatergic synapses (Harrison & Owen, 2003) suggesting that the development of these synapses may be a final common pathway for several genetic risk factors for this disease (Freedman et al., 2005). Some of the most interesting experiments aimed at determining the etiology of schizophrenic-like symptoms have reported schizophrenic-like behavioral outcomes in adults following challenges to the immune system during pregnancy (Zuckerman, Rehavi, Nachman, & Weiner, 2003; Zuckerman & Weiner, 2005) or disruption of fetal neurogenesis (Flagstad, Glenthøj, & Didriksen, 2005). The future use of techniques like those applied in the current study will allow the expansion of this kind of work by testing a variety of other early neurotoxic alterations and performing fetal behavioral assessments that may have implications for the etiology of schizophrenia.

To the best of our knowledge there is no published evidence indicating that a specific impairment in LI early in a person's life is a potential vulnerability factor for later schizophrenia. However, several studies indicate that performance on other vigilance or sustained attention tasks is consistently impaired throughout late childhood in those who go on to develop schizophrenia (for reviews, see Seidman et al., 2012; Owens & Johnstone, 2006). In particular, deficits in attentional selectivity (e.g., poor performance on tasks that require focus and execution during distraction) in children are among the best predictors of later diagnosis of schizophrenia spectrum disorder. Relevant to the current study, ''errors-under-distraction scores'' collected in younger children (age 11) were better predictors of a diagnosis of schizophrenia than were the same test scores 6 years later when the participants were 17 years of age (Mirsky, Ingraham, & Kugelmass, 1995). Thus,

the study of the origins of early attention deficits such as LI may be useful as we meet the challenging task of discovering the etiology of schizophrenia.

## NOTES

This study was supported by NSF Award: 9514799. The authors would like to thank the following research associates and staff for their contributions to this work: Sean Anderson, Islam Ayad, Stephanie Bacik, Haley Bartholomew, Kenneth Bisson, Benjamin Brown, Armand DeAsis, Jennifer Duman, Sarah Frischmann, Kara Gawelek, Janace Gifford, Sara Gombash, Jen Graebert, Chris Gutoskey, Jenna Hardwick, Natalie Hogan, Sarah Hummel, Ashlee Jeter, Bruce Kinley, Nicholas McGinty, Aaron McNair, Kim Parkinson, Doug Placko, Ginger Portman, Dan Petersen, Cliff Raymond, David Revta, Marcial Rodriguez, James Romanchik, Hayleigh Sanders, Taylor Shreve, Logan Sirline, Diana Slawski, Katie Stiles, Andi Sulovari, Agata Szwaykowska, Stephen Toth, Kate Tylicki, Mark Warman, and Rachel Zacharias.

## REFERENCES

Abdi, H. (2010). Holm's sequential Bonferroni procedure. In N. Salkind (Ed.), Encyclopedia of research design (pp. 1–8). Thousand Oaks, CA: Sage Press.

Abekawa, T., Ito, K., Nakagawa, S., & Koyama, T. (2007). Prenatal exposure to NMDA receptor antagonist MK-801 reduces density of parallbumin-immunoreactive GABAergic neurons in the medial prefrontal cortex and enhances phencyclidine-induced hyperlocomotion but not behavioral sensitization to methamphetamine in postpubertal rats. Psychopharmacology, 192, 303–316. DOI: 10.1007/s00213-007-0729-8

Aguado, L., del Valle, R., & Pérez, L. (1997). The NMDA-receptor antagonist ketamine as an unconditioned stimulus in taste aversion learning. Neurobiology of Learning and Memory, 68, 189–196. DOI: 10.1006/nlme.1997.3773

Aguado, L., San Antonio, A., Perez, L., del Valle, R., & Gomez, J. (1994). Effects of the NMDA receptor antagonist ketamine on flavor memory: Conditioned aversion, latent inhibition, and habituation of neophobia. Behavioral and Neural Biology, 61, 271–281. DOI: 10.1016/S0163-1047(05)80010-X

Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. Journal of Nutrition, 136(3 Suppl.), 716S–724S.

Archer, T., Mohammed, A. K., & Jarbe, T. U. (1986). Context-dependent latent inhibition in taste aversion learning. Scandinavian Journal of Psychology, 27, 277–284. DOI: 10.1111/j.1467-9450.1986.tb01205.x

Behrens, M. M., & Sejnowski, T. J. (2009). Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? Neuropharmacology, 57, 193–200. DOI: 10.1016/j.neuropharm.2009.06.002

Ben-Ari, Y., Cherubini, E., & Krnjevic, K. (1988). Changes in voltage dependence of NMDA currents during development. Neuroscience Letters, 94, 88–92. DOI: 10.1016/0304-3940(88)90275-3

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: Microstructure of affective taste reactivity patterns. Neuroscience and Biobehavioral Reviews, 24, 173–198. DOI: 10.1016/S0149-7634(99)00072-X

Best, M. R. (1975). Conditioned and latent inhibition in taste-aversion learning: Clarifying the role of learned safety. Journal of Experimental Psychology: Animal Behavioral Processes, 104, 97–113. DOI: 10.1037/0097-7403.1.2.97

Bethus, I., Lemaire, V., Lhomme, M., & Goodall, G. (2005). Does prenatal stress affect latent inhibition? It depends on the gender. Behavioural Brain Research, 158, 331–338. DOI: 10.1016/j.bbr.2004.09.013

Boutros, N. N., Belger, A., Campbell, D., D'Souza, C., & Krystal, J. (1999). Comparison of four components of sensory gating in schizophrenia and normal subjects: A preliminary report. Psychiatry Research, 88, 119–130. DOI: 10.1016/S0165-1781(99)00074-8

Capretta, P. J., & Rawls, L. H. (1974). Establishment of flavor preference in rats: Importance of nursing and weaning experience. Journal of Comparative and Physiological Psychology, 86, 670–673. DOI: 10.1037/h0036158

Davis, J. A., & Gould, T. J. (2005). Rolipram attenuates MK-801-induced deficits in latent inhibition. Behavioral Neuroscience, 119, 595–602. DOI: 10.1037/0735-7044.119.2.595

Denenberg, V. H. (1976). Statistics and experimental design for behavioral and biological researchers: An introduction. New York: John Wiley & Sons.

Deutsch, S. I., Mastropaolo, J., & Rosse, R. B. (1998). Neurodevelopmental consequences of early exposure to phencyclidine and related drugs. Clinical Neuropharmacology, 21, 320–332.

Dong, C., Rovnaghi, C. R., & Anand, K. J. (2012). Ketamine alters the neurogenesis of rat cortical neural stem cells. Critical Care Medicine, 40, 2407–2416. DOI: 10.1097/CCM.0b013e318253563c

Ferrari, M. C., & Chivers, D. P. (2011). Learning about non-predators and safe places: The forgotten elements of risk assessment. Animal Cognition, 14, 309–316. DOI: 10.1007/s10071-010-0363-4

Flagstad, P., Glenthøj, B. Y., & Didriksen, M. (2005). Cognitive deficits caused by late gestational disruption of neurogenesis in rats: A preclinical model of schizophrenia. Neuropsychopharmacology, 30, 250–260. DOI: 10.1038/sj.npp.1300625

Freedman, R., Ross, R., Leonard, S., Myles-Worsley, M., Adams, C. E., Waldo, M., ... Stevens, K. E. (2005). Early biomarkers of psychosis. Dialogues in Clinical Neuroscience, 7, 17–29.

Franchina, J. J., & Slank, K. L. (1988). Salience and the effects of CS preexposure on aversion conditioning. Behavioral and Neural Biology, 50, 367–373. DOI: 10.1016/S0163-1047(88)91114-4

Gaisler-Salomon, I., & Weiner, I. (2003). Systemic administration of MK-801 produces an abnormally persistent latent inhibition which is reversed by clozapine but not haloperidol. Psychopharmacology, 166, 333–342. DOI: 10.1007/s00213-002-1311-z

Galigher, A. E., & Kozloff, E. N. (1971). Essentials of practical microtechnique. Philadelphia, PA: Lea & Febiger.

Gallo, M., Bielavska, E., Roldan, G., & Bures, J. (1998). Tetrotoxin inactivation of the gustatory cortex disrupts the effect of the *N*-methyl-D-aspartate antagonist ketamine on latent inhibition of conditioned taste aversion in rats. Neuroscience Letters, 240, 61–64. DOI: 10.1016/S0304-3940(97)00897-5

Ganchrow, J. R., Steiner, J. E., & Canetto, S. (1986). Behavioral displays to gustatory stimuli in newborn rat pups. Developmental Psychobiology, 19, 163–174. DOI: 10.1002/dev.420190303

Garcia, J., Kimeldorf, D. J., & Koelling, R. A. (1955). Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science, 122, 157–158. DOI: 10.1126/science.122.3179.1089

Gerdjikov, T. V., Rudolph, U., Keist, R., Mohler, H., Feldon, J., & Yee, B. K. (2008). Hippocampal alpha 5 subunit-containing GABA A receptors are involved in the development of the latent inhibition effect. Neurobiology of Learning & Memory, 89, 87–94.

Gray, J. A., Feldon, J., Rawlins, J. N. P., Hemsley, D. R., & Smith, A. D. (1991). The neuropsychology of schizophrenia. Behavioural and Brain Science, 14, 1–84. DOI: 10.1017/S0140525X00065055

Grill, H. J., & Norgren, R. (1978a). The taste reactivity test I: Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263–279. DOI: 10.1016/0006-8993(78)90568-1

Grill, H. J., & Norgren, R. (1978b). Chronically decerebrate rats demonstrate satiation but not bait shyness. Science, 201, 267–269. DOI: 10.1126/science.663655

Gruest, N., Richer, P., & Hars, B. (2004). Emergence of long-term memory for conditioned aversion in the rat fetus. Developmental Psychobiology, 44, 189–198. DOI: 10.1002/dev.20004

Harrison, P. J., & Owen, M. J. (2003). Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet, 361, 417–419.

Hestrin, S. (1992). Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. Nature, 357, 686–689. DOI: 10.1038/357686a0

Holson, R. R., & Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicology and Teratology, 14, 221–228.

Hsiao, S., & Fan, R. J. (1993). Additivity of taste-specific effects of sucrose and quinine: Microstructural analysis of ingestive behavior in rats. Behavioral Neuroscience, 107, 317–326. DOI: 10.1037/0735-7044.107.2.317

Jimenez, B., & Tapia, R. (2004). Biochemical modulation of NMDA receptors: Role in conditioned taste aversion. Neurochemical Research, 29, 161–168. DOI: 10.1023/B:NERE.0000010445.27905.aa

Kaplan, J. M., Roitman, M. F., & Grill, H. J. (1995). Ingestive taste reactivity as licking behavior. Neuroscience and Biobehavioral Reviews, 19, 89–98. DOI: 10.1016/0149-7634(94)00023-T

Katz, D. B., Rogers, R. F., & Steinmetz, J. E. (2002). Novel factors contributing to the expression of latent inhibition. Behavioral Neuroscience 116, 824–836. DOI: 10.1037/0735-7044.116.5.824

Klamer, D., Palsson, E., Wass, C., Archer, T., Engel, J. A., & Svensson, L. (2005). Antagonism of the nitric oxide synthase inhibitor, L-NAME, of the effects of phencyclidine on latent inhibition in taste aversion conditioning. Behavioural Brain Research, 161, 60–68. DOI: 10.1016/j.bbr.2005.01.008

Koenig, J. L., Kirkpatrick, B., & Lee, P. (2002). Glucocorticoid hormones and early brain development in schizophrenia. Neuropsychopharmacology, 27, 309–318. DOI: 10.1016/S0893-133X(01)00396-7

Lannes, B., Micheletti, G., Warter, J. M., Kempfl, E., & Di Scala, G. (1991). Behavioural, pharmacological and biochemical effects of acute and chronic administration of ketamine in the rat. Neuroscience Letters, 128, 177–181. DOI: 10.1016/0304-3940(91)90255-R

Lev, R., & Orlic, D. (1972). Protein absorption by the intestine of the fetal rat in utero. Science, 177, 522–524. DOI: 10.1126/science.177.4048.522

Lewis, M. C., & Gould, T. J. (2004). Latent inhibition of cued fear conditioning: An NMDA receptor-dependent process that can be established in the presence of anisomycin. European Journal of Neuroscience, 20, 818–826. DOI: 10.1111/j.1460-9568.2004.03531.x

Lewis, M. C., & Gould, T. J. (2007). Signal transduction mechanisms within the entorhinal cortex that support latent inhibition of cued fear conditioning. Neurobiology of Learning and Memory, 88, 359–368. DOI: 10.1016/j.nlm.2007.04.011

Lin, J. Y., Amodeo, L. R., Arthurs, J., & Reilly, S. (2012). Taste neophobia and palatability: The pleasure of drinking. Physiology and Behavior, 106, 515–519. DOI: 10.1016/j.physbeh.2012.03.029

Lubow, R. E. (1989). Latent inhibition and conditional attention theory. New York, NY: Cambridge University Press.

Lubow, R. E. (2005). Construct validity of the animal latent inhibition model of selective attention deficits in schizophrenia. Schizophrenia Bulletin, 31, 139–153. DOI: 10.1093/schbul/sbi005

Lubow, R. E., & De la Casa, L. G. (2005). There is a time and a place for everything: Bidirectional modulations of latent inhibition by time-induced context differentiation. Psychonomic Bulletin and Review, 12, 806–821. DOI: 10.3758/BF03196774

Lubow, R. E., Kaplan, O., Abramovich, P., Rudnick, A., & Laor, N. (2000). Visual search in schizophrenia: Latent inhibition and novel pop-out effects. Schizophrenia Research, 45, 145–156. DOI: 10.1016/S0920-9964(99)00188-7

Lyall, A., Swanson, J., Liu, C., Blumenthal, T. D., & Turner, C. P. (2009). Neonatal exposure to MK-801 promotes

prepulse-induced delay in startle response time in adult rats. Experimental Brain Research, 197, 215–222. DOI: 10.1007/s00221-009-1906-2

Maruniak, J. A., Madon, J. R., & Kostelc, J. G. (1983). Conditioned aversions to an intravascular odorant. Physiology and Behavior, 30, 617–620. DOI: 10.1016/0031-9384(83)90230-5

Mickley, G. A., Lovelace, J. D., Farrell, S. T., & Chang, K. S. (1995). The intensity of a fetal taste aversion is modulated by the anesthesia used during conditioning. Developmental Brain Research, 85, 119–127. DOI: 10.1016/0165-3806(94)00202-B

Mickley, G. A., Remmers-Roeber, D., Crouse, C., & Peluso, R. (2000). Ketamine blocks a taste recognition memory in fetal rats. Pharmacology, Biochemistry and Behavior, 67, 575–581.

Mickley, G. A., Remmers-Roeber, D., Crouse, C., Walker, C., & Dengler, C. (2000). Detection of novelty by perinatal rats. Physiology and Behavior, 70, 217–225. DOI: 10.1016/S0031-9384(00)00229-8

Mickley, G. A., Remmers-Roeber, D., Dengler, C., Kenmuir, C., & Crouse, C. (2001). Paradoxical effects of ketamine on memory of fetuses of different ages. Developmental Brain Research, 127, 71–76. DOI: 10.1016/S0165-3806(01)00119-5

Mickley, G. A., Remmers-Roeber, D. R., Dengler, C. M., McMullen, C. A., Kenmuir, C. L., Girdler, B., ... Walker, C. (2002). Simple behavioral methods to assess the effect of drugs or toxins on sensory experience. Journal of Neuroscience Methods, 115, 85–92.

Mickley, G. A., Schaldach, M., Snyder, K., Balogh, S., Len, T., Neimanis, K., ... Yamamoto, B. (1998). Ketamine blocks a conditioned taste aversion (CTA) in neonatal rats. Physiology and Behavior, 64, 381–390. DOI: 10.1016/S0031-9384(98)00097-3

Mirsky, A. F., Ingraham, L. J., & Kugelmass, S. (1995). Neuropsychological assessment of attention and its pathology in the Israeli cohort. Schizophrenia Bulletin 21, 193–204.

Moser, P. C., Hitchcock, J. M., Lister, S., & Moran, P. M. (2000). The pharmacology of latent inhibition as an animal model of schizophrenia. Brain Research Reviews, 33, 275–307. DOI: 10.1016/S0165-0173(00)00026-6

National Research Council. (1996). Guide for the care and use of laboratory animals. Washington, D.C.: National Academy Press.

Nolte, D. L., Provenza, F. D., Callan, R., & Panter, K. E. (1992). Garlic in the ovine fetal environment. Physiology and Behavior, 52, 1091–1093. DOI: 10.1016/0031-9384(92)90464-D

Olney, J. W. (2002). New insights and new issues in developmental neurotoxicology. Neurotoxicology, 23, 659–668.

Opler, M. G. A., & Susser, E. S. (2005). Fetal Environment and Schizophrenia. Environmental Health Perspectives, 113, 1239–1242.

Overton, D. A. (1983). State-dependent learning and drug discrimination. In L. L. Iverson, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of Psychopharmacology, Vol. 18 (pp. 59–127). New York, NY: Plenum Press.

Owens, D. G., & Johnstone, E. C. (2006). Precursors and prodromata of schizophrenia: Findings from the Edinburgh high risk study and their literature context. Psychological Medicine, 36, 1501–1514.

Palsson, E., Klamer, D., Wass, C., Archer, T., Engel, J. A., & Svensson, L. (2005). The effects of phencyclidine on latent inhibition in taste aversion conditioning: Differential effect of preexposure and conditioning. Behavioural Brain Research, 157, 139–146. DOI: 10.1016/j.bbr.2004.06.018

Peterschmitt, Y., Meyer, F., & Louilot, A. (2007). Neonatal functional blockade of the entorhinal cortex results in disruption of accumbal dopaminergic responses observed in latent inhibition paradigm in adult rats. European Journal of Neuroscience, 25, 2504–2513. DOI: 10.1111/j.1460-9568.2007.05503.x

Picard, B. (2011). Progress defining the biological causes of schizophrenia. Expert Reviews in Molecular Medicine, 13, e25. DOI: http://dx.doi.org/10.1017/S1462399411001955

Purves, D., Bonardi, C., & Hall, G. (1995). Enhancement of latent inhibition in rats with electrolytic lesions of the hippocampus. Behavioral Neuroscience, 109, 366–370.

Pushpendran, C. K., Devasagayam, T. P., Chintalwar, G. J., Banerji, A., & Eapen, J. (1980). The metabolic fate of [35S]-diallyl disulphide in mice. Cellular and Molecular Life Sciences, 36, 1000–1001. DOI: 10.1007/BF01953845

Rapoport, J. L., Addington, A. M., Frangou, S., & Psych, M. R. (2005). The neurodevelopmental model of schizophrenia: Update 2005. Molecular Psychiatry, 10, 434–449. DOI: 10.1038/sj.mp.4001642

Razoux, F., Garcia, R., & Lena, I. (2007). Ketamine at a dose that disrupts motor behavior and latent inhibition, enhances prefrontal cortex synaptic efficacy and glutamate release in the nucleus accumbens. Neuropsychopharmacol ogy, 32, 719–727. DOI: 10.1038/sj.npp.1301057

Rehn, A. E., & Rees, S. M. (2005). Investigating the neurodevelopmental hypothesis of schizophrenia. Clinical and Experimental Pharmacology & Physiology, 32, 687–696. DOI: 10.1111/j.1440-1681.2005.04257.x

Robinson, G. S., Crooks, G. B., Shinkman, P. G., & Gallagher, M. (1989). Behavioral effects of MK-801 mimic deficits associated with hippocampal damage. Psychobiology, 17, 156–164. Retrieved from http://psycnet.apa.org/psycinfo/1989-39380-001

Robinson, S. R., & Smotherman, W. P. (1992). Behavioral response of atricial and precocial rodent fetuses to acute umbilical cord compression. Behavioral and Neural Biology, 57, 93–102. DOI: 10.1016/0163-1047(92)90581-N

Roman, C., & Reilly, S. (2007). Effects of insular cortex lesions on conditioned taste aversion and latent inhibition in the rat. European Journal of Neuroscience, 26, 2627–2632.

Rosenblum, K., Meiri, N., & Dudai, Y. (1993). Taste memory: The role of protein synthesis in gustatory cortex. Behavioral & Neural Biology, 59, 49–56.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. Neuroscience & Biobehavioral Reviews, 26, 665–678.

Schmidt-Hansen, M., & Le Pelley, M. (2012). The positive symptoms of acute schizophrenia and latent inhibition in humans and animals: Underpinned by the same process(es)? Cognitive Neuropsychiatry, 17, 473–505. DOI: 10.1080/13546805.2012.667202

Seidman, L. J., Meyer, E. C., Giuliano, A. J., Breiter, H. C., Goldstein, J. M., Kremen, W. S., . . . Faraone, S. V. (2012). Auditory working memory impairments in individuals at familial high risk for schizophrenia. Neuropsychology, 26, 288–303. DOI: 10.1037/a0027970

Shadkchan, Y., Shemesh, E., Mirelman, D., Miron, T., Rabinkov, A., Wilchek, M., & Osherov, N. (2004). Efficacy of allicin, the reactive molecule of garlic, in inhibiting Aspergillus spp. *in vitro*, and in a murine model of disseminated aspergillosis. Journal of Antimicrobial Chemotherapy, 53, 832–836. DOI: 10.1093/jac/dkh174

Shwartz, G. J., & Grill, H. J. (1985). Comparing taste-elicited behaviors in adult and neonatal rats. Appetite, 6, 373–386. DOI: 10.1016/S0195-6663(85)80005-2

Sircar, R. (2000). Developmental maturation of the NMDA acid receptor channel complex in postnatal rat brain. International Journal of Developmental Neuroscience, 18, 121–131. DOI: 10.1016/S0736-5748(99)00069-6

Smotherman, W. P., Richards, L. S., & Robinson, S. R. (1984). Techniques for observing fetal behavior in utero: A comparison of chemomyelotomy and spinal transection. Developmental Psychobiology, 17, 661–674. DOI: 10.1002/dev.420170608

Smotherman, W. P., & Robinson, S. R. (1985). The rat fetus in its environment: Behavioral adjustments to novel, familiar, aversive, and conditioned stimuli presented in utero. Behavioral Neuroscience, 99, 521–530. DOI: 10.1037/0735-7044.99.3.521

Smotherman, W. P., & Robinson, S. R. (1988a). Dimensions of fetal investigation. In W. P. Smotherman & S. R. Robinson (Eds.), Behavior of the fetus (pp. 19–34). Caldwell, NJ: Telford Press.

Smotherman, W. P., & Robinson, S. R. (1988b). The uterus as environment: The ecology of fetal behavior, Chapter 5. In E. M. Blass (Ed.), Developmental psychobiology and behavioral ecology, Vol. 9, Handbook of Behavioral Neurobiology (pp. 149–196). New York, NY: Plenum Press.

Smotherman, W. P., & Robinson, S. R. (1988c). Behavior of rat fetuses following chemical or tactile stimulation. Behavioral Neuroscience, 102, 24–34. DOI: 10.1037/0735-7044.102.1.24

Smotherman, W. P., & Robinson, S. R. (1989). Cryptopsychobiology: The appearance, disappearance and reappearance of a species-typical action pattern during early development. Behavioral Neuroscience, 103, 246–253. DOI: 10.1037/0735-7044.103.2.246

Smotherman, W. P., & Robinson, S. R. (1990). The prenatal origins of behavioral organization. Psychological Science, 1, 97–106. DOI: 10.1111/j.1467-9280.1990.tb00075.x

Soussignan, R., Schaal, B., & Marlier, L. (1998). Olfactory alliesthesia in human neonates: Prandial state and stimulus familiarity modulate facial and autonomic responses to milk odors. Developmental Psychobiology, 35, 3–14. DOI: 10.1002/(SICI)1098-2302(199907)35:1<3::AID-DEV2>3.0.CO;2-F

Stefan, M. D., & Murray, R. M. (1997). Schizophrenia: Developmental disturbance of brain and mind? Acta paediatrica. Supplementum, 422, 112–116.

Teicher, M. H., & Blass, E. M. (1977). First sucking response of the newborn albino rat: The roles of olfaction and amniotic fluid. Science, 198, 635–636.

Traverso, L. M., Ruiz, G., Camino, G., & De la Casa, L. G. (2008). Ketamine blocks the formation of a gustatory memory trace in rats. Pharmacology, Biochemistry and Behavior, 60, 553–558. DOI: 10.1016/j.pbb.2008.03.002

Traverso, L. M., Ruiz, G., & De la Casa, L. G. (2003). Latent inhibition disruption by MK-801 in a conditioned taste-aversion paradigm. Neurobiology of Learning and Memory, 80, 140–146. DOI: 10.1016/S1074-7427(03)00059-5

Turgeon, S. M., Auerbach, E. A., Duncan-Smith, M. K., George, J. R., & Graves, W. W. (2000). The delayed effects of DTG and MK-801 on latent inhibition in a conditioned taste aversion paradigm. Pharmacology, Biochemistry and Behavior, 66, 533–539. DOI: 10.1016/S0091-3057(00)00223-9

Turgeon, S. M., Auerbach, E. A., & Heller, M. A. (1998). The delayed effects of phencyclidine (PCP) disrupt latent inhibition in a conditioned taste aversion paradigm. Pharmacology, Biochemistry and Behavior, 90, 305–311. DOI: 10.1016/S0091-3057(98)00026-4

Turgeon, S. M., Kegel, G., & Davis, M. M. (2001). Electrolytic lesions of the medial septum enhance latent inhibition in a conditioned taste aversion paradigm. Brain Research, 890, 333–337.

Turgeon, S. M., & Reichstein, D. A. (2002). Decrease strialtal c-Fos accompanies latent inhibition in a conditioned taste aversion paradigm. Brain Research, 924, 120–123.

Turner, C. P., Gutierrez, S., Liu, C., Miller, L., Chou, J., Finucane, B., . . . Phillips, A. (2012). Strategies to defeat ketamine-induced neonatal brain injury. Neuroscience, 17, 384–392. DOI: 10.1016/j.neuroscience.2012.02.015

Weiner, I., & Arad, M. (2009). Using the pharmacology of latent inhibition to model domains of pathology in schizophrenia and their treatment. Behavioural Brain Research, 204, 369–386. DOI: 10.1016/j.bbr.2009.05.004

Welzel, H. D., Adamo, P., & Lipp, H.-P. (2001). Conditioned taste aversion as a learning and memory paradigm. Behavioural Brain Research, 125, 205–213. DOI: 10.1016/j.bbr.2009.05.004

White, P. F., Way, W. L., & Trevor, A. J. (1982). Ketamine—Its pharmacology and therapeutic uses. Anesthesiology, 56, 119–136. Retrieved from http://journals.lww.com/anesthesiology/Citation/1982/02000/Ketamine_Its_Pharmacology_and_Therapeutic_Uses.7.aspx

Yap, C. S. L., & Richardson, R. (2005). Latent inhibition in the developing rat: An examination of context-specific

effects. Developmental Psychobiology, 47, 45–55. DOI: 10.1002/dev.20074

Zorrilla, E. P. (1997). Multiparous species present problems (and possibilities) to developmentalists. Developmental Psychobiology, 30, 141–150

Zuckerman, L., Rehavi, M., Nachman, R., & Weiner, I. (2003). Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: A novel neurodevelopmental model of schizophrenia. Neuropsychopharmacology, 28, 1778–1789. DOI: 10.1038/sj.npp.1300248

Zuckerman, L., & Weiner, I. (2005). Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. Journal of Psychiatric Research, 39, 311–323. DOI: 10.1016/j.jpsychires.2004.08.008