Neurobiology of Learning and Memory **76,** 151–182 (2001)
doi:10.1006/nlme.2000.3994, available online at http://www.idealibrary.com on IDEAL®

Fear Conditioning-Induced Alterations of Phospholipase C-β1a Protein Level and Enzyme Activity in Rat Hippocampal Formation and Medial Frontal Cortex

Edwin J. Weeber,*,†,[1] Daniel D. Savage,* Robert J. Sutherland,*,‡ and Kevin K. Caldwell*

**Department of Neurosciences, †Department of Biology, and ‡Department of Psychology, University of New Mexico, Albuquerque, New Mexico 87131-5223*

Published online May 8, 2001

We investigated the effects of one-trial fear conditioning on phospholipase C-β1a catalytic activity and protein level in hippocampal formation and medial frontal cortex of untreated control rats and rats prenatally exposed to ethanol. One hour following fear conditioning of untreated control rats, phospholipase C-β1a protein level was increased in the hippocampal cytosolic fraction and decreased in the hippocampal membrane and cortical cytosolic and cortical membrane fractions. Twenty-four hours after fear conditioning, phospholipase C-β1a protein level was reduced in the hippocampal cytosolic fraction and elevated in the cortical nuclear fraction; in addition, 24 h after conditioning, phospholipase C-β1a activity in the cortical cytosolic fraction was increased. Rats that were exposed prenatally to ethanol displayed attenuated contextual fear conditioning, whereas conditioning to the acoustic-conditioned stimulus was not different from controls. In behavioral control (unconditioned) rats, fetal ethanol exposure was associated with reduced phospholipase C-β1a enzyme activity in the hippocampal nuclear, cortical cytosolic, and cortical membrane fractions and increased phospholipase C-β1a protein level in the hippocampal membrane and cortical cytosolic fractions. In certain cases, prenatal ethanol exposure modified the relationship between fear conditioning and changes in phospholipase C-β1a protein level and/or activity. The majority of these effects occurred 1 h, rather than 24 h, after fear conditioning. Multivariate analysis

This research was supported by the University of New Mexico Research Endowment (K.K.C.) and National Institute on Alcohol Abuse and Alcoholism Grants AA12635 (K.K.C.) and AA06548 (D.D.S). A Minority Biomedical Research Support Training Grant from the National Institute of Health (GM52576) provided a stipend for E.J.W. We thank Buz Tyler for assistance in the quantification of optical densities, Andrea Allan for assistance with the statistical analysis and interpretation of the data, and Michael Wilson for thoughtful comments during the preparation of the manuscript.

Address correspondence and reprint requests to Kevin K. Caldwell, Department of Neurosciences, BMSB 145, University of New Mexico Health Sciences Center, Albuquerque, NM 87131-5223. Fax: (505) 272-8082. E-mail: kcaldwell@salud.unm.edu.

[1] Current address: Division of Neuroscience, Baylor College of Medicine, Houston, TX.

1074-7427/01 $35.00

of variance revealed interactions between fear conditioning, subcellular fraction, and prenatal ethanol exposure for measures of phospholipase C-β1a protein level in hippocampal formation and phospholipase C-β1a enzyme activity in medial frontal cortex. In the majority of cases, fear conditioning-induced changes in hippocampal phospholipase C-β1a protein level were augmented in rats prenatally exposed to ethanol. In contrast, fear conditioning-induced changes in cortical phospholipase C-β1a activity were, often, in opposite directions in prenatal ethanol-exposed compared to diet control rats. We speculate that alterations in subcellular phospholipase C-β1a catalytic activity and protein level contribute to contextual fear conditioning and that learning deficits observed in rats exposed prenatally to ethanol result, in part, from dysfunctions in phospholipase C-β1a signal transduction.

Key Words: phospholipase C; fear conditioning; fetal alcohol syndrome; learning; hippocampal formation; cerebral cortex.

INTRODUCTION

Despite a growing body of evidence identifying the circuitry and synaptic physiology that play critical roles in learning and memory, the intracellular signaling events that participate in these processes have not been well defined. It is our goal to elucidate the role of phosphatidylinositol signaling reactions in learning and memory, as well as in various cognitive dysfunctions, including those resulting from prenatal exposure to ethanol. In the present studies, we investigated the relationships among one-trial fear conditioning, hippocampal formation, and medial frontal cortical phosphatidylinositol-specific phospholipase C-β1a (PLC-β1a) protein level and enzyme activity and prenatal exposure to moderate levels of ethanol.

In order to assess the temporal associations among learning, memory formation, and brain neurochemistry, it is necessary to identify the exact moment when an animal is exposed to the learning event. This can be achieved using one-trial fear conditioning (Bevins & Ayres, 1994; Fanselow & Bolles, 1979; Silva et al., 1996). In this form of classical conditioning, the unconditioned stimulus (UCS) is of sufficient strength that only a single trial, or pairing of the UCS and the conditioned stimulus (CS), is needed in order for the test subject to learn to fear, or prepare for, the approach of the UCS in subsequent trials. In addition to learning to fear the explicit CS, animals learn the association between the shock experience and the constellation of environmental cues that form the training context. Fear responses to the CS and to the context are separable and can be measured as the duration of conditioned "freezing" (Bouton & Bolles, 1980; Fanselow, 1980).

The neuroanatomical structures which play indispensable roles in conditioned fear include the amygdala and the hippocampal formation (Davis, 1997; Fanselow & LeDoux, 1999; LeDoux, 2000). The amygdala plays an essential role in the acquisition and consolidation of information about both the elemental CS and the training context, as well as the expression of both CS fear and contextual fear responses (Cousens & Otto, 1998; Desmedt, Garcia, & Jafard, 1998; Maren, 1999; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999). The hippocampal formation, which creates and stores configural representations of the environment (Sutherland & Rudy, 1989), plays a critical role in the consolidation of contextual fear memory and the expression of contextual fear (Chen,

Kim, Thompson, & Tonegawa, 1996; Kim, Rison, & Fanselow, 1993; Maren, Aharonov, & Fanselow, 1997; Phillips & LeDoux, 1992; Sacchetti et al., 1999). It should be noted, however, that there are other viable hypotheses about the role of the hippocampal formation in contextual fear conditioning (McNish, Gewirtz, & Davis, 1997; McNish, Gewirtz, & Davis, 2000). The hippocampal formation may not be essential for the acquisition, consolidation, or expression of CS conditioning (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Sacchetti et al., 1999; however, see McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Maren et al., 1997; Richmond, Yee, Pouzet, Veenman, Rawlins, Feldon, & Bannerman, 1999). In addition to the amygdala and hippocampal formation, several cortical regions, including the medial frontal cortex (Doyère, Burette, Rédini-Del Negro, & Laroche, 1993; Frysztak & Neafsey, 1991; Morgan & LeDoux, 1995; Morrow, Elsworth, Inglis, & Roth, 1999), have been reported to play a role in fear conditioning. In general, there is agreement that the medial frontal cortex plays a modulatory, rather than primary, role in conditioned fear (see Morrow et al., 1999, for discussion).

Identification of the neurochemical mechanisms that underlie fear conditioning and memory and the expression of fear responses lags behind our understanding of the circuitry that is critical for fear conditioning. A variety of neurotransmitter receptors have been implicated, including (1) ionotropic glutamate receptors (Davis, Falls, Campeau, & Kim, 1993; Fanselow & Kim, 1994; Kim, Fanselow, DeCola, & Landeira-Fernandez, 1992; Lee & Kim, 1998; Lu & Wehner, 1997; Maren, Aharonov, Stote, & Fanselow, 1996; McKernan & Shinnick-Gallagher, 1997, Rogan, Stäubli, & LeDoux, 1997), (2) metabotropic glutamate receptors (Aiba, Chen, Herrup, Rosenmund, Stevens, & Tonegawa, 1994; Frohardt, Guarraci, & Young, 1999; Lu, Jia, Janus, Henderson, Gerlai, Wojtowicz, & Roder, 1997; Christoffersen, Christensen, Harrington, Macphail, & Riedel, 1999), (3) glucocorticoid receptors (Cordero & Sandi, 1998; Pugh, Fleshner, & Rudy, 1997; Pugh, Tremblay, Fleshner, & Rudy, 1997), (4) monoamine receptors (Kim, Shih, Chen, Chen, Bao, Maren, Anagnostaras, Fanselow, De Maeyer, Seif, & Thompson, 1997), and (5) GABAergic receptors (Helmstetter & Bellgowan, 1994; Muller, Corodimas, Fridel, & LeDoux, 1997). The intracellular signaling systems that mediate fear conditioning have been reported to include (1) cAMP-dependent protein kinase (Bourtchouladze, Abel, Berman, Gordon, Lapidus, & Kandel, 1998; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999), (2) mitogen-activated protein kinase (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Schafe et al., 1999; Selcher, Atkins, Trzaskos, Paylor, & Sweatt, 1999), (3) microtubule-associated protein-2 (Woolf, Young, Johnson, & Fanselow, 1994), (4) growth-associated protein 43 (GAP-43; Young, Owen, Meiri, & Wehner, 2000), (5) a pertussis toxin-sensitive G-protein(s) (Melia, Falls, & Davis, 1992; Davis et al., 1993), (6) Ca^{2+}-calmodulin kinase II (Silva et al., 1996), and (7) protein kinase C (PKC; Fordyce, Clark, Paylor, & Wehner, 1995). These latter three observations suggest that one or more isozymes of PLC-β, such as PLC-β1a (Bahk, Lee, Lee, Seo, Ryu, & Suh, 1994), which are regulated by G-protein subunits (Exton, 1996; Rhee & Bae, 1997) and produce the Ca^{2+}-mobilizing and PKC-activating messengers, inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$, "IP_3"; Berridge, 1993) and 1,2-diacylglycerol (Hodgkin, Pettitt, Martin, Michell, Pemberton, & Wakelam, 1998), respectively, are a component of the signaling system involved in fear conditioning. This conclusion is supported by a study of Lynch and colleagues (Laroche, Rédini-Del Negro, Clements, & Lynch, 1990) demonstrating increased turnover rates of inositol

phosphates and 1,2-diacylglycerol in the dentate gyrus and areas CA1 and CA3 of the hippocampus following fear conditioning.

In addition to the proposed role for PLC-β1a in fear conditioning, several lines of evidence suggest that PLC-β1a may participate in other forms of learning and synaptic plasticity. First, spatial learning in the Morris water maze has been correlated with PLC-β1a protein levels (Nicolle, Colombo, Gallagher, & McKinney, 1999) and altered turnover rates and levels of inositol phosphates in rat hippocampal formation (Chouinard, Gallagher, Yasuda, Wolfe, & McKinney, 1995; de Bruin, Facchinetti, Tóth, Balázs, & Joosten, 1996; Lynch, Voss, & Gower, 1994; Nicolle et al., 1999; Tandon, Mundy, Ali, Nanry, Rogers, & Tilson, 1991). Second, decreased PLC-β1 protein levels in the hippocampus, frontal cortex, and striatum have been proposed to play a role in the cognitive losses that occur with aging (Nicolle et al., 1999; Undie, Wang, & Friedman, 1995). Third, roles for $Ins(1,4,5)P_3$-dependent mobilization of intracellular Ca^{2+} in long-term potentiation (LTP; Berridge, 1993; Komatsu, 1996) and associative learning (Alkon, Nelson, Zhao, & Cavallaro, 1998) have been proposed. Fourth, LTP in the dentate gyrus is associated with an increased rate of formation of phosphatidylinositols (Kusuki, Imahori, Fujii, Inokuchi, Kimura, & Ueda, 1998). Fifth, cholinergic receptor and metabotropic glutamate receptor agonists, which are known to facilitate LTP, alter the turnover rates or levels of phosphatidylinositols and inositol phosphates in brain (Ayyagari, Gerber, Joseph, & Crews, 1998; Hwang, Bredt, & Snyder, 1990; Lynch, Clements, Errington, & Bliss, 1988; McGahon & Lynch, 1998; Nicolle et al., 1999; Schoepp, Salhoff, Wright, Johnson, Burnett, Mayne, Belagaje, Wu, & Monn, 1996). Finally, rats that were exposed prenatally to ethanol display deficits in various learning tasks (Berman, & Hannigan, 2000; Furuya, Aikawa, Yoshida, & Okazaki, 1996; Gianoulakis, 1990; Kim, Kalynchuk, Kornecook, Mumby, Dadgar, Pinel, & Weinberg, 1997; Reyes, Wolfe, & Savage, 1989; Westergren, Rydenhag, Archer, & Conradi, 1996; Zimmerberg, Mattson, & Riley, 1989), as well as deficits in PLC-β1a basal enzyme activity (Allan, Weeber, Savage, & Caldwell, 1997) and metabotropic glutamate receptor-stimulated inositol phosphate metabolism (Queen, Sanchez, Lopez, Paxton, & Savage, 1993)

The physiologic function of a number of signal transduction proteins, including PLC-β1a (Matteucci, Faenza, Gilmour, Manzoli, Billi, Peruzzi, Bavelloni, Rhee, & Cocco, 1998), has been shown to be dependent upon intracellular localization (Feliciello, Gallo, Mele, Porcellini, Troncone, Garbi, Gottesman, & Avvedimento, 2000; Mochly-Rosen, 1995; Yin, 1999). Thus, when assessing the effects of a behavioral activation paradigm, such as one-trial fear conditioning, on a signal-transducing enzyme, such as PLC-β1a, it is important to determine whether the catalytic activity of the enzyme, as well as the amount of enzyme protein, in various subcellular compartments has been altered. In the case of PLC-β1a, plasma membrane-associated enzyme is believed to transduce signals from G-protein-coupled receptors into intracellular signals (Berridge, 1993), while nuclear PLC-β1a may play a role in gene transcription (Zini, Sabatelli, Faenza, Ognibene, & Maraldi, 1996; Marmiroli, Zini, Bavelloni, Faenza, Ognibene, & Maraldi, 1996). Although a specific cellular role for cytosolic PLC-β1a has not been identified, Cockcroft and Thomas (1992) have proposed that it may act as a reservoir of enzyme that is in equilibrium with other subcellular compartments, such as the plasma membrane and nucleus.

In the present studies, we addressed the following questions: (1) Are there changes in

hippocampal formation and/or medial frontal cortical PLC-β1a enzyme activity or PLC-β1a protein level that occur following one-trial fear conditioning? (2) Is prenatal exposure to ethanol associated with altered fear responses following one-trial fear conditioning? and (3) If the answer to both of the previous questions is yes, then, are there differences in the effects of one-trial fear conditioning on PLC-β1a in fetal ethanol-exposed and diet control rats?

METHODS

Fetal Ethanol Exposure Paradigm

All of the procedures involving rats were approved by the University of New Mexico Health Sciences Center Laboratory Animal Care and Use Committee. Four- to 7-month-old Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were housed in a constant (22°C) temperature room on a 16-h dark/8-h light schedule (lights off from 1730 to 0930 h). Except during the experimental diet feeding procedures described below, all rats were provided *ad lib* access to standard rat chow and tap water.

Prenatal exposure to moderate levels of ethanol was performed as described previously (Allan et al., 1997; Sutherland, McDonald, & Savage, 1997). On Day 1 of gestation, rat dams were assigned to one of three diet groups. Dams assigned to the "5% ethanol diet" group received 110 mL of BioServ (Frenchtown, NJ) each day at 1730 h, according to the following schedule: Gestational Days (GD) 1 and 2, BioServ alone (0% ethanol); GD 3 and 4, BioServ containing 2% (v/v) ethanol; GD 5 and 6, BioServ containing 3% (v/v) ethanol; and, GD 7 through 21, BioServ containing 5% (v/v) ethanol (26% ethanol-derived calories). The feeding tubes were removed 16 h later, at the start of the daily light cycle (0930 h) and replaced with water bottles. A second group of dams, serving as the "pair-fed" controls, received an ethanol-free BioServ diet isocalorically equivalent to the 5% ethanol diet at 1730 each day, throughout gestation, for 16 h per day with water for the remaining 8 h. The third group of rat dams was given *ad lib* access to Purina breeder chow and water throughout gestation. At birth, offspring were cross-fostered to surrogate, untreated rat dams. Offspring were weaned at 24 days of age and then housed in groups of two or three. Female rat offspring, 130 to 170 days of age, were used in all of the experiments described in the present report.

The effects of the 5% ethanol liquid diet on rat dams and offspring were similar to the results reported previously using the same paradigm (Allan et al., 1997; Sutherland et al., 1997). Consumption of the 5% ethanol liquid diet produced a mean peak maternal blood ethanol concentration of 83 mg/dL. No differences were observed among the three diet groups in litter size, offspring birth weights, or brain weights of adult offspring at sacrifice (data not shown).

One-Trial Fear Conditioning Paradigm

Offspring were randomly assigned to one of three behavioral groups: (1) the one-trial fear conditioning group, (2) the "unpaired" control (UPC) group, or (3) the naïve, unhandled (UH) group. All behavioral conditioning sessions occurred between 1000 and 1300 h. Rats assigned to the one-trial fear conditioning group were individually housed in standard

plastic cages for 6 to 9 days prior to fear conditioning. On the conditioning day, each rat was separately transported in its home cage to the behavioral room, adjacent to the home room, and immediately placed in the conditioning chamber (a 40 cm wide × 56 cm deep × 28 cm high wooden box with a stainless-steel rod floor and a 7.5-w light bulb in the ceiling). After a 3-min acclimation period in the chamber, a 30-s-long tone (the conditioned stimulus) was initiated. Twenty-eight seconds into the CS, a 1 mA foot shock (the unconditioned stimulus) was administered for a period of 2 s through the stainless-steel rods which make up the floor of the chamber. Two minutes after delivery of the foot shock, the rat was returned to its home cage and immediately returned to the home room.

Rats assigned to the UPC group were individually housed in plastic-walled cages with modified floors, constructed of the same stainless-steel rod flooring as was used in the conditioning chamber described above. These cages rested on sawdust bedding, the same as that placed in the cages of the one-trial fear conditioning and UH rats. The rats acclimated to these modified cages for 6 to 9 days prior to the behavioral session. UPC rats received all three of the experiences (context, tone, and shock) separately, with a minimum interval of 60 min between exposure to the context, then tone, and finally foot shock. Specifically, each rat was transferred, in its modified home cage, to the behavioral room and placed in the context chamber for a period of 5.5 min (without tone or shock). Then, the rat was returned to its home cage and remained in the behavioral room. Sixty to one hundred-twenty minutes after exposure to the context, the tone was given for a period of 30 s. Five minutes later, the rat was returned to the home room. Sixty minutes later, the rat received a 2-s, 1 mA foot shock in its home cage.

A third group or rats was designated as UH controls. These rats were housed individually in plastic cages for 6 to 9 days prior to sacrifice and received no experimental manipulation aside from the routine cage cleaning procedures twice weekly.

Assessment of Fear Conditioning

In order to determine whether associative learning occurred as a result of the one-trial fear conditioning paradigm, we measured contextual and auditory conditioning essentially as described by Fanselow and colleagues (Kim & Fanselow, 1992; Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991). Briefly, 24 h following one-trial fear conditioning, rats were returned for 8 min to the conditioning chamber. The tone and the foot shock were not delivered during this test session. The animal's behavior was videotaped and context conditioning was assessed using a time-sampling procedure by an individual who was uninformed about the subject's prenatal exposure, or not, to ethanol. Approximately 1 h later, the fear response elicited by the tone CS was determined by measuring freezing during an 8-min tone presented in a novel context (an opaque, circular, plexiglas test chamber in a different room). Again, a time-sampling procedure was employed and freezing was determined by an individual who was blinded to the experimental history of the rat.

Tissue Preparation and Subcellular Fractionation

Rats were taken directly from the room in which they were housed to an adjacent room and sacrificed by decapitation. Brains were rapidly removed and the hippocampal formation

and medial frontal cortex (identified as all areas rostral to the fornix and optic chiasm with the olfactory region previously removed) were dissected on a Tris-buffered saline-soaked filter paper, mounted on a glass platform, resting on ice. Tissues were homogenized as described in Allan et al. (1997), except the volume of homogenization buffer was reduced to 1.0 mL. Homogenates were centrifuged (1000g_{max}, 6 min, 4°C) to separate crude soluble ("S1") and particulate ("P1") fractions (see Fig. 1). The P1 fraction was suspended and washed once with 1.0 mL homogenization buffer. The S1 and washed P1 fractions were rapidly frozen in liquid nitrogen and stored at −80°C until further fractionation.

Nuclear, cytosolic, and membrane fractions were isolated as follows. The P1 fraction was thawed and Triton X-100, NaCl, and KCl were added to give final concentrations of 16 mM (1%, v/v), 75 and 75 mM, respectively. While setting in an ice bath, the mixture was sonicated (Kontes Micro Ultrasonic Cell Disrupter, 20% maximal setting) twice for 5 s each with a 2-min interval between sonications. The sonicated P1 was left standing in ice for 15 min. Then, the soluble material, which was designated as the "nuclear" fraction, was collected following centrifugation (200,000g_{max}, 30 min, 4°C). In unpublished studies, we have found that greater than 90% of the total anti-PLC-β1a immunoreactivity associated with the nuclear fraction copurified with nuclei which were isolated from the P1 fraction as described by Graham (1993), except that the $MgCl_2$ concentration used was 1 mM. The S1 fraction was thawed and centrifuged (200,000g_{max}, 30 min, 4°C) to isolate S2 (soluble) and P2 (particulate) fractions (Fig. 1). The S2 and P2 fractions were extracted as described in Allan et al. (1997), using 16 mM (1%, v/v) Triton X-100, 75 mM NaCl, and 75 mM KCl, and then centrifuged at 200,000g_{max} for 30 min (4°C). The "cytosolic" (Triton X-100-soluble material isolated from the S2) and "membrane" (Triton X-100-soluble material isolated from the P2) fractions were collected. It should be noted that this membrane fraction contains particulate material derived from all subcellular compartments other than the nucleus. In preliminary studies, we determined that the Triton X-100-insoluble material which was isolated from the P2 fraction contained less than 1% of the anti-PLC-β1a immunoreactivity present in the membrane fraction (data not shown) and, thus, this material was routinely discarded. The nuclear, cytosolic and membrane

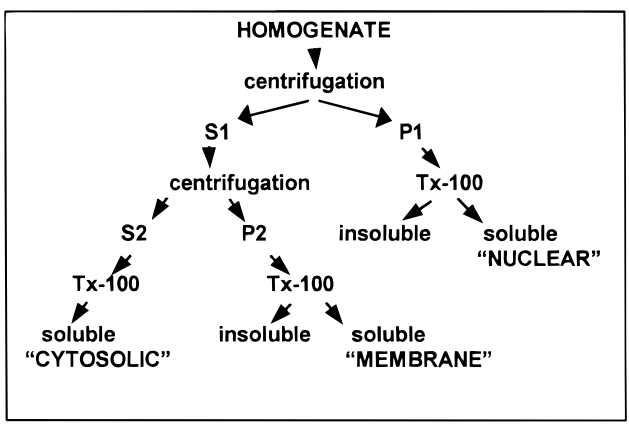


FIG. 1. Diagram illustrating the centrifugation steps that were employed for the preparation of nuclear, membrane, and cytoplasmic fractions. The pellets from the first (P1) and second (P2) centrifugations, along with the supernatant from the second centrifugation (S2), were treated with Triton X-100 to extract PLC-β1a.

samples were rapidly frozen in liquid nitrogen and stored at −80°C until assayed for PLC enzyme activity and anti-PLC-β1a immunoreactivity. The total protein concentration of each of the subcellular fractions was determined by the method of Bradford (1976), using the Bio-Rad (Richmond, CA) protein assay kit; bovine serum albumin (Fraction V) served as the protein standard for these determinations.

Immunoprecipitation of PLC-β1a

Subcellular fractions were incubated with protein A - Sepharose (30-μL packed volume) for 1 h at 4°C with mixing, and then centrifuged (11,100g_{max}, 2 min, 4°C) to remove the protein A - Sepharose and bound proteins. This process of preclearing the extracts with protein A - Sepharose helped to reduce the variability subsequently measured in PLC enzyme assays of immunoprecipitated PLC-β1a. The protein A - Sepharose used in the preclearing was assayed, but revealed no PLC enzyme activity (data not shown). The precleared extracts were incubated with or without 4 μg anti-PLC-β1a polyclonal antibodies for 1 h at 4°C with mixing. In unpublished studies, we have found no cross-reactivity of the anti-PLC-β1a antibodies with PLC-β2, -β3, or -β4 isozymes. Thirty microliters (packed volume) of protein A - Sepharose was added and mixing was continued at 4°C for 1 h. Immune complexes were collected by centrifugation (11,100g_{max} for 30 s) and washed twice with 1 mL wash buffer (35 mM sodium phosphate, pH 6.8, 70 mM KCl, 0.8 mM EGTA, 0.8 mM $CaCl_2$) prior to assay for PLC enzyme activity (see below). The amount of extract used for the immunoprecipitation was adjusted so that less than 10% of the substrate was hydrolyzed during the PLC enzyme activity assay.

PLC-β1a Enzyme Activity Assay

PLC-β1a enzyme activities were measured essentially as described in Allan et al., (1997). Briefly, immune complexes (30 μL) were assayed for 5 min at 37°C in the presence of 35 mM sodium phosphate, pH 6.8, 70 mM KCl, 0.8 mM EGTA, 0.8 mM $CaCl_2$, 0.20 mM [^{3}H]phosphatidylinositol 4,5-bisphosphate (Ptdlns(4,5)P_2, 10,000 cpm/nmol), and 2.86 mM (0.179%, v/v) Triton X-100 in a final volume of 50 μL. Enzyme activity was quantitated as the release of [^{3}H] Ins(1,4,5)P_3 measured by liquid scintillation spectroscopy. Isozyme-specific activity was calculated by subtracting background [^{3}H] Ins(1,4,5)P_3 (release present in no antibody control samples) from the activity measured in immune complexes. Data were calculated as nanomoles Ins(1,4,5)P_3 product formed per minute per milligram protein present in the fraction from which the enzyme was immunoprecipitated. All conditions were run in triplicate with the variability among the triplicates generally being less than 10% of the mean value.

Determination of PLC-β1a Protein Level

Tissue subcellular fractions were subjected to SDS-PAGE and then transferred to a polyvinylidene difluoride membrane with a Bio-Rad Trans-Blot apparatus. Membranes were blocked with 5% (w/v) nonfat milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 for 1 h at room temperature with shaking and then incubated with anti-PLC-β1a polyclonal antibodies (0.067 μg/mL) in the same solution for 1 h at room temperature.

Membranes were washed with 0.1% (v/v) Tween 20 in Tris-buffered saline and anti-PLC-β1a immunoreactivity was visualized using an amplified alkaline phosphatase immunoblot kit, as described by the manufacturer (Bio-Rad). The developed gels were scanned (Hewlett Packard Scan Jet 5P) and immunoreactivities were quantified by measurements of optical densities using SigmaGel gel analysis software (Jandel). Anti-PLC-β1a immunoreactivity determinations were performed in duplicate.

Each Western blot contained an internal standard consisting of increasing amounts (0.3–3.0 μg) of medial frontal cortical S1 fraction. The anti-PLC-β1a immunoreactivity measurements obtained from the internal standards were used to generate an optical density standard curve ($r^2 \geq 0.90$) from which one unit of immunoreactivity was defined as the amount of anti-PLC-β1a optical density measured for 1.0 μg of medial frontal cortical S1 protein. For experimental samples yielding optical densities within the linear range defined by the optical density standard curve, the amount of PLC-β1a in the sample was determined by dividing the optical density of the sample by the optical density of one "unit" of immunoreactivity. The PLC-β1a protein level in the sample was then calculated by dividing the number of "units" of PLC-β1a present in the sample by the number of micrograms of total protein loaded on the SDS-PAGE gel. The generation of a standard immunoreactivity curve for each blot corrected for any artifactual differences in blotting (e.g., efficiency of transfer) and immunoreactions (e.g., stain development, antibody titer) that may have occurred from one blot to the next. Gallagher and colleagues (Colombo, Wetsel, & Gallagher, 1997; Nicolle et al., 1999) employed essentially this same technique for the quantification of PKC isozymes and PLC-β1a in rat subcellular fractions. In addition, we have demonstrated the validity of this method in unpublished studies, in which we found that the relative "units" of anti-PLC-β1a, anti-PLC-β2, anti-PLC-β3, and anti-PLC-β4a immunoreactivities in hippocampal formation, medial frontal cortex, caudate nucleus, cerebellum, and olfactory tissue were comparable to the relative levels of immunoreactivities (Gerfen, Choi, Suh, & Rhee, 1988) and mRNA (Ross, MacCumber, Glatt, & Snyder, 1989; Watanabe, Nakamura, Sato, Kano, Simon, & Inoue, 1998) in these brain regions reported by other investigators.

Statistical Analyses

Statistical analyses were performed by one-way analysis of variance (ANOVA), two-way ANOVA, or multivariate analysis of variance (MANOVA), as noted in the text and figure legends. Post hoc analyses were performed using Tukey's multiple comparison test or, where appropriate, a one-sample *t* test, as noted in the text and figure legends.

Materials

Polyclonal antibodies to PLC-β1a were purchased from Santa Cruz Biotechnology, Inc. Ptdlns(4,5)P_2 and Triton X-100 were purchased from Boehringer-Mannheim. [^{3}H] Ptdlns(4,5)P_2 was purchased from NEN. Protein A-Sepharose CL4B was obtained from Pharmacia. Amplified alkaline phosphatase protein detection kit was purchased from Bio-Rad. All other chemicals and supplies were purchased from commercial sources.

RESULTS

One-Trial Fear Conditioning Alters PLC-β1a Enzyme Activity and PLC-β1a Protein Level

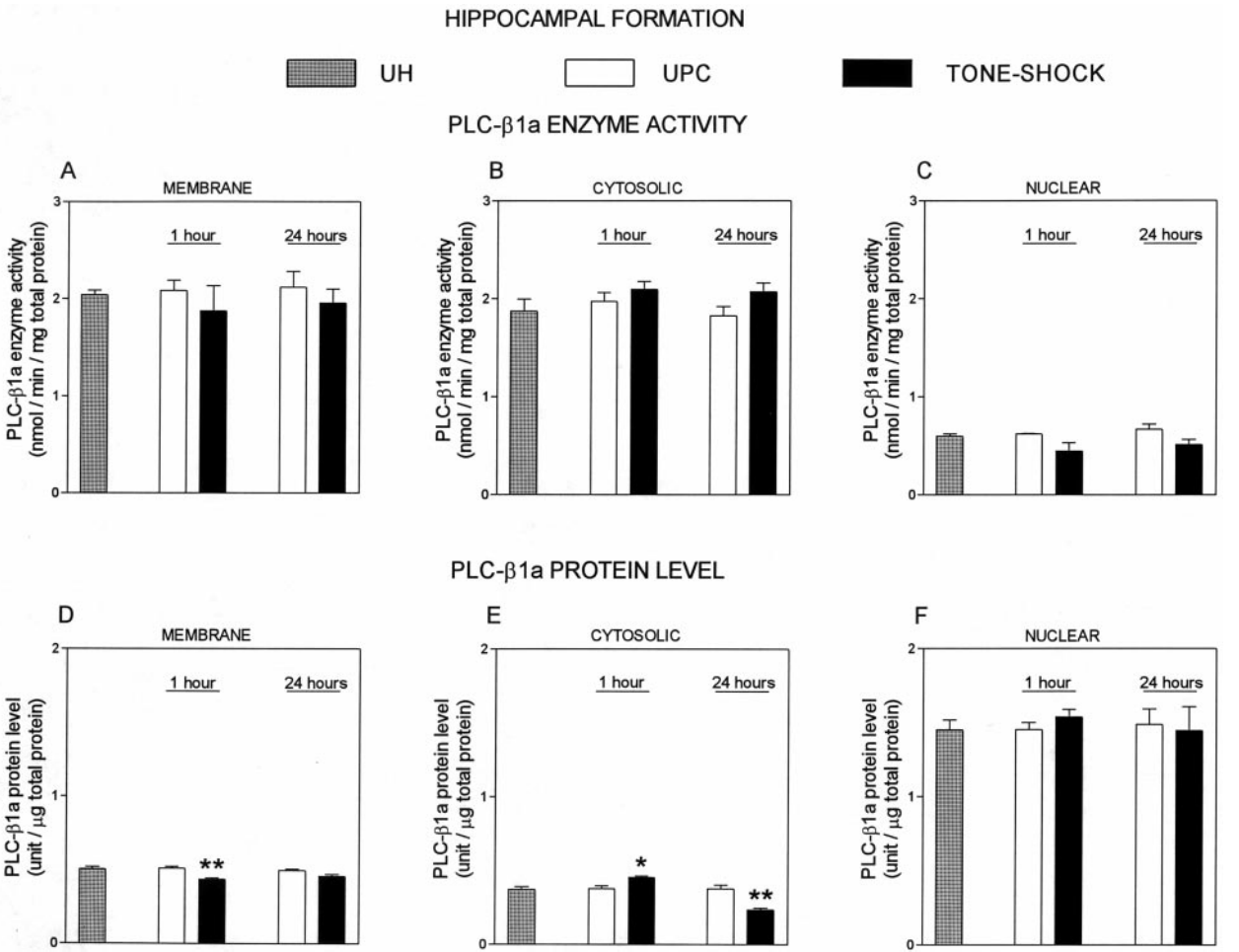
In order to ensure that the neurochemical changes that we observed following one-trial fear conditioning were correlated with conditioned learning, rather than unconditioned responses to the novel context, tone, foot shock, or the stress of handling, we developed a behavioral control paradigm. In this paradigm, which we call the "unpaired control" paradigm (see Methods), the context, tone, handling, and shock stimuli were temporally dissociated. Of particular note is the delivery of the foot shock in the home cage, which the rat should not associate with the shock experience because of maximal latent inhibition of the home cage context (Mackintosh, 1983). Under these conditions, in which there is not a positive association between the CS and the UCS, associative learning does not occur (Leahey & Harris, 1980; Mackintosh, 1983; Rescorla, 1988).

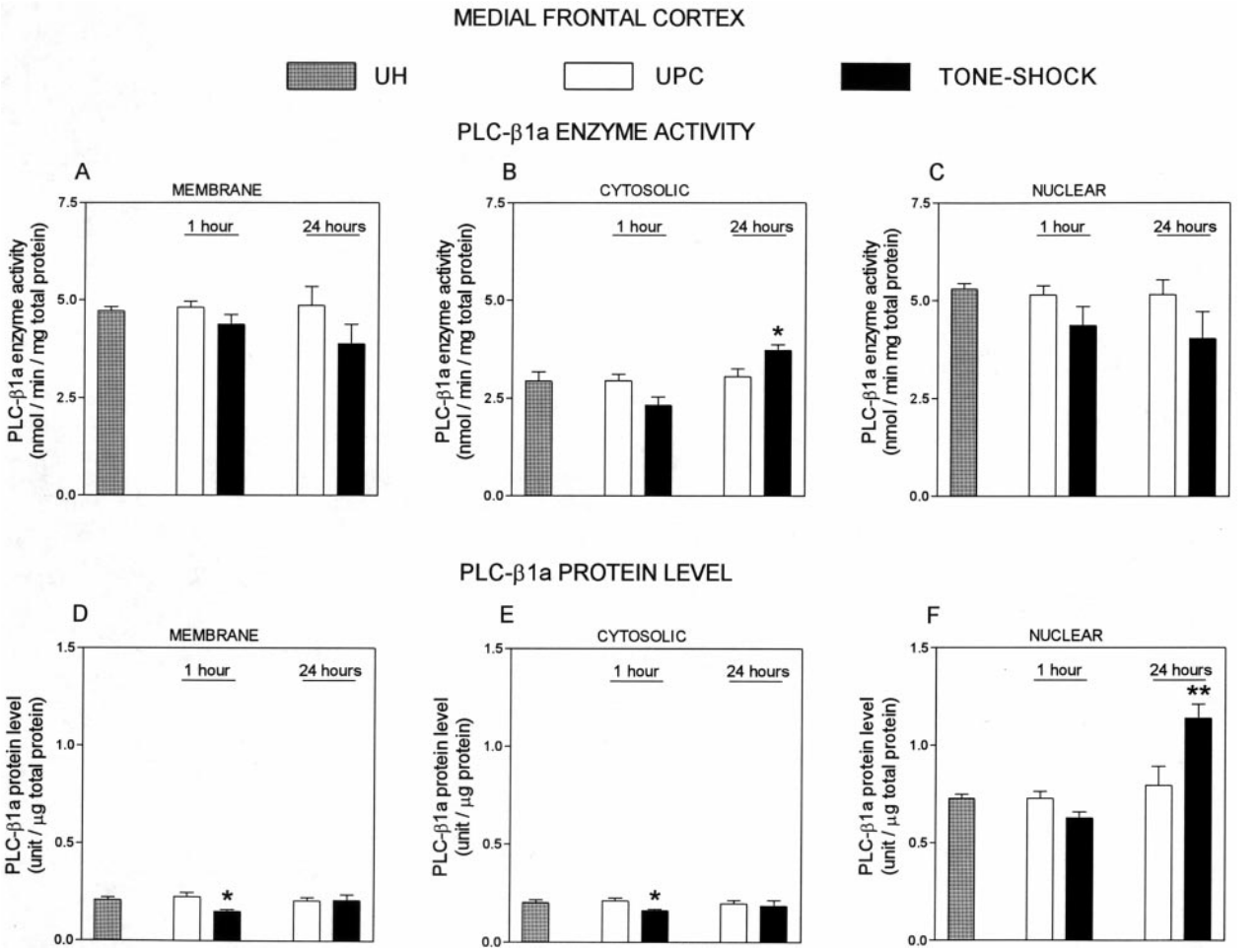
Figures 2 and 3 show PLC-β1a enzyme activity and PLC-β1a protein level in subcellular fractions derived from the hippocampal formation (Fig. 2) and medial frontal cortex (Fig. 3) of UPC rats, fear-conditioned rats, and naïve, unhandled control rats; all of these rats were the offspring of dams that received an *ad lib* diet throughout pregnancy. Both PLC-β1a enzyme activity and protein level were expressed relative to the total protein present in the fraction. Subcellular fractions were prepared from tissues isolated 1 or 24 h after delivery of the foot shock to the UPC and fear-conditioned rats. These two times were chosen in order to identify "early" and "late" biochemical changes that occur following fear conditioning and are consistent with studies performed by Sweatt and colleagues (Atkins et al., 1998), LeDoux and colleagues (Schafe et al., 1999), and Kandel and colleagues (Bourtchouladze et al., 1998).

Statistical comparisons (one-way ANOVA) made within a brain region and subcellular fraction revealed no significant differences between measures of PLC-β1a enzyme activity in samples derived from UH rats and samples derived from UPC rats either 1 or 24 h after the completion of the UPC paradigm. Similarly, within a brain region and subcellular fraction, no significant differences were found between the measures of UH rat PLC-β1a protein level and PLC-β1a protein level in UPC rats either 1 or 24 h after delivery of the foot shock. That is, compared to measures recorded in UH rats, the UPC paradigm did not significantly alter subcellular PLC-β1a enzyme activity or PLC-β1a protein level.

One-way ANOVA revealed that in only a single case did one-trial fear conditioning

FIG. 2. PLC-β1a enzyme activity (A–C) and PLC-β1a protein level (D–F) in membrane, cytosolic, and nuclear subcellular fractions prepared from the hippocampal formation of naïve unhandled (UH) control rats (shaded bars), rats subjected to the unpaired control (UPC) behavioral paradigm (open bars), and rats having undergone the one-trial fear conditioning (tone-shock) paradigm (filled bars). UPC and one-trial fear conditioning rats were sacrificed either 1 or 24 h following delivery of the foot shock. Data bars represent the mean ± the SEM from four rats under each condition. *, ** The data for the one-trial fear conditioning rats are significantly ($p < .05$ and $p < .01$, respectively) different than the UPC and UH data, as determined by one-way ANOVA followed by Tukey's multiple comparison post hoc test. The PLC-β1a protein level was significantly decreased in the membrane fraction 1 h after one-trial fear conditioning [$F(2, 11) = 8.97$, $p < .01$], whereas, in the cytosolic fraction, the PLC-β1a protein level was significantly elevated 1 h after one-trial fear conditioning [$F(2, 11) = 6.32$, $p < .05$] but reduced 24 h after one-trial fear conditioning [$F(2, 11) = 16.5$, $p < .01$].


HIPPOCAMPAL FORMATION
UH
UPC
TONE-SHOCK
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
PLC-β1a enzyme activity (nmol / min / mg total protein)
1 hour
24 hours
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
PLC-β1a protein level (unit / μg total protein)
*
**


MEDIAL FRONTAL CORTEX
UH
UPC
TONE-SHOCK
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
PLC-β1a enzyme activity (nmol / min / mg total protein)
1 hour
24 hours
7.5
5.0
2.5
0.0
*
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
PLC-β1a protein level (unit / μg total protein)
PLC-β1a protein level (unit / μg protein)
1.5
1.0
0.5
0.0
**

significantly alter PLC-β1a enzyme activity: in the medial frontal cortex cytosolic fraction 24 h after fear conditioning (Fig. 3B). In contrast, fear conditioning was associated with a significant change in PLC-β1a protein level in several instances. In the hippocampal membrane (Fig. 2D), the medial frontal cortical membrane (Fig. 3D) and the medial frontal cortical cytosolic (Fig. 3E) fractions, the PLC-β1a protein level was significantly reduced 1 h following fear conditioning, whereas in the hippocampal cytosolic fraction (Fig. 2E), the PLC-β1a protein level was elevated 1 h after fear conditioning. Twenty-four hours after fear conditioning, the PLC-β1a protein level in the hippocampal cytosolic fraction (Fig. 2E) was significantly reduced and the PLC-β1a protein level in the nuclear fraction derived from the medial frontal cortex (Fig. 3F) was significantly elevated.

Prenatal Ethanol Exposure Is Associated with Reduced Contextual Fear Conditioning

Adult rats, which had been exposed in utero to moderate levels of ethanol, or were reared from dams fed one of two control diets, were subjected to the one-trial fear conditioning paradigm and then 24 h later assessed for their level of freezing to the context and to the tone (see Methods). First, it should be noted that the procedure produced a high degree of associative learning, as evidenced by the fact that all three groups of rats showed considerable freezing to the context, when returned to the conditioning chamber 24 h posttraining, and to the tone, when presented in a novel context (Fig. 4). Compared to both of the diet control groups, rats that had been exposed prenatally to ethanol displayed significantly lower freezing scores in the contextual conditioning, but not the auditory cue, test (Fig. 4).

Prenatal Ethanol Exposure Alters PLC-β1a Enzyme Activity and PLC-β1a Protein Level in UPC Rats

Prior to assessing the effects of prenatal ethanol exposure on one-trial fear conditioning-induced changes in PLC-β1a signaling, it is important to note the effects that fetal ethanol exposure exerted on PLC-β1a enzyme activity and PLC-β1a protein level in behavioral control, UPC, rats. Offspring from each of the three diet groups were sacrificed 1 or 24 h after completion of the UPC paradigm. Two-way ANOVAs (using time after receipt of the shock and diet as the variables) performed within a brain region and subcellular fraction revealed that in no case was there a significant effect of time on either PLC-β1a

FIG. 3. PLC-β1a enzyme activity (A–C) and PLC-β1a protein level (D–F) in membrane, cytosolic, and nuclear subcellular fractions prepared from the medial frontal cortex of naïve unhandled (UH) control rats (shaded bars), rats subjected to the unpaired control (UPC) behavioral paradigm (open bars), and rats having undergone the one-trial fear conditioning (tone-shock) paradigm (filled bars). UPC and one-trial fear conditioning rats were sacrificed either 1 or 24 h following delivery of the foot shock. Data bars represent the mean $\pm$ the SEM from four rats under each condition. *, ** The data for the one-trial fear conditioning rats are significantly ($p < .05$ and $p < .01$, respectively) different than the UPC and UH data, as determined by one-way ANOVA followed by Tukey's multiple comparison post hoc test. PLC-β1a enzyme activity was significantly increased in the cytosolic fraction 24 h after one-trial fear conditioning [$F(2, 11) = 5.05$, $p < .05$]. The PLC-β1a protein level was significantly decreased 1 h after one-trial fear conditioning in both the membrane fraction [$F(2, 11) = 5.70$, $p < .05$] and the cytosolic fraction [$F(2, 11) = 4.63$, $p < .05$]. The PLC-β1a protein level in the nuclear fraction was significantly elevated 24 h after one-trial fear conditioning [$F(2, 11) = 9.61$, $p < .01$].

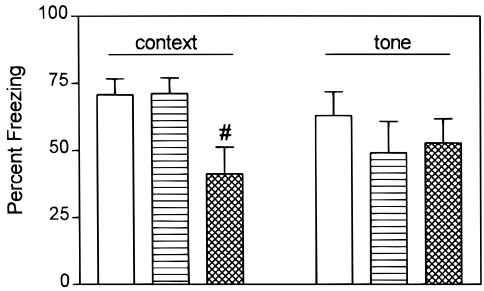
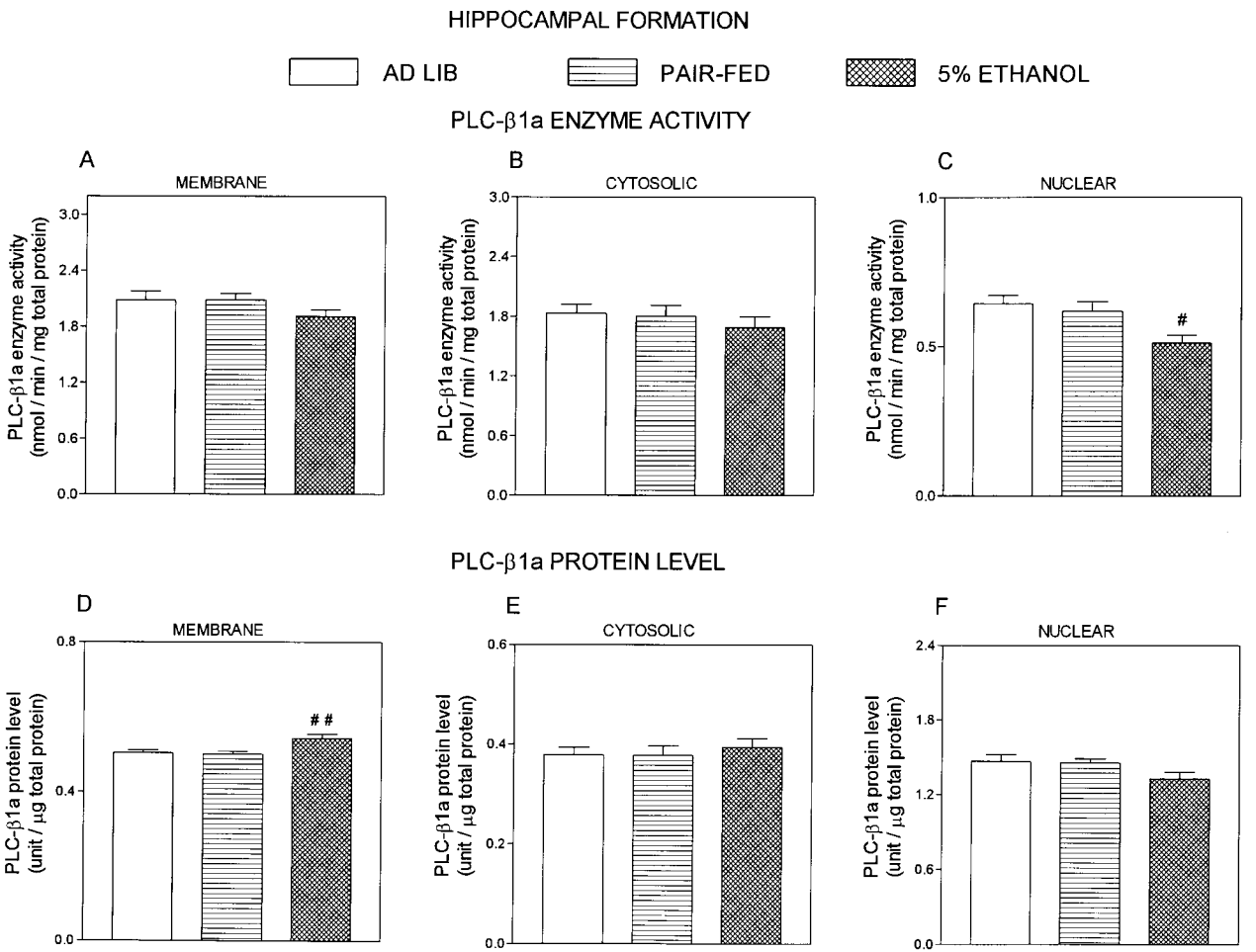


FIG. 4. Effect of prenatal ethanol exposure on contextual and tone fear conditioning. Rats were exposed prenatally to 5 % (v/v) ethanol (cross-hatched bars) or not (*ad lib*, open bars, and pair-fed, horizontally-filled bars, control) (see Methods). Rats from all three diet groups underwent one-trial fear conditioning as described under Methods. Twenty-four hours later, contextual and tone fear conditioning were determined as described under Methods. The data as expressed as percentage freezing (see Methods). Data bars represent the mean ± the SEM of six rats in each diet treatment group. # Data significantly ($p < .05$) different than both the *ad lib* and the pair-fed control groups based on a one-way ANOVA followed by Tukey's multiple comparison post hoc test. Rats exposed prenatally to ethanol exhibited a significant reduction in contextual fear conditioning [$F(2, 17) = 5.1$, $p < .05$] but did not differ significantly from control rats in freezing to the tone.

enzyme activity or PLC-β1a protein level. That is, within a subcellular fraction and diet group, PLC-β1a enzyme activity and PLC-β1a protein level did not differ between tissue isolated 1 and 24 h after completion of the UPC paradigm. Thus, within a diet group and brain region subcellular fraction, the 1- and 24-h data were pooled and then analyzed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. The results are graphed in Figs. 5 and 6. It should be noted that, unlike Figs. 2 and 3, in which the upper limit of the graph ordinate was held constant to facilitate comparisons of the relative PLC-β1a enzyme activity and PLC-β1a protein level across the subcellular fractions, in Figs. 5 and 6 the upper limit of the graph ordinate was varied so that differences between the diet groups within a subcellular fraction would be emphasized.

No significant differences were found between the measures of PLC-β1a enzyme activity in the pair-fed control and the *ad lib* control diet groups within a subcellular fraction (Figs. 5A–5C and Figs. 6A–6C). Similarly, no significant differences were recorded in PLC-β1a protein level within a subcellular fraction for these two groups of rats (Figs. 5D–5F and Figs. 6D–6F). In contrast, compared to pair-fed and *ad lib* diet rats, fetal ethanol-exposed rats exhibited significant reductions in PLC-β1a enzyme activity in the hippocampal nuclear fraction (Fig. 5C), the medial frontal cortical membrane fraction (Fig. 6A), and the medial frontal cortical cytosolic fraction (Fig. 6B). In addition, prenatal ethanol exposure was associated with significant elevations of the PLC-β1a protein level

FIG. 5. Effect of prenatal ethanol exposure on PLC-β1a enzyme activity (A–C) and PLC-β1a protein level (D–F) in subcellular fractions prepared from the hippocampal formation of behavioral control (UPC) rats. Rats were exposed prenatally to 5% (v/v) ethanol (cross-hatched bars) or not (pair-fed, horizontally-filled bars, and *ad lib*, open bars, control) (see Methods). Data bars represent the mean ± the SEM of eight rats in each diet treatment group. #, ## Data significantly ($p < .05$ and $p < .01$, respectively) different than both the *ad lib* and pair-fed control groups based on a one-way ANOVA followed by a Tukey's multiple comparison post hoc test. Rats exposed prenatally to ethanol exhibited significant reductions in PLC-β1a enzyme activity in the nuclear fraction [$F(2, 23) = 5.65$, $p < .05$] and significant elevations of PLC-β1a protein level in the membrane fraction [$F(2, 23) = 7.89$, $p < .01$].


HIPPOCAMPAL FORMATION
AD LIB
PAIR-FED
5% ETHANOL
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
PLC-β1a enzyme activity (nmol / min / mg total protein)
3.0
2.4
1.8
1.2
0.6
0.0
1.0
0.5
#
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
PLC-β1a protein level (unit / μg total protein)
0.8
0.4
0.2
0.6
2.4
##

in the hippocampal membrane fraction (Fig. 5D) and the medial frontal cortical cytosolic fraction (Fig. 6E).

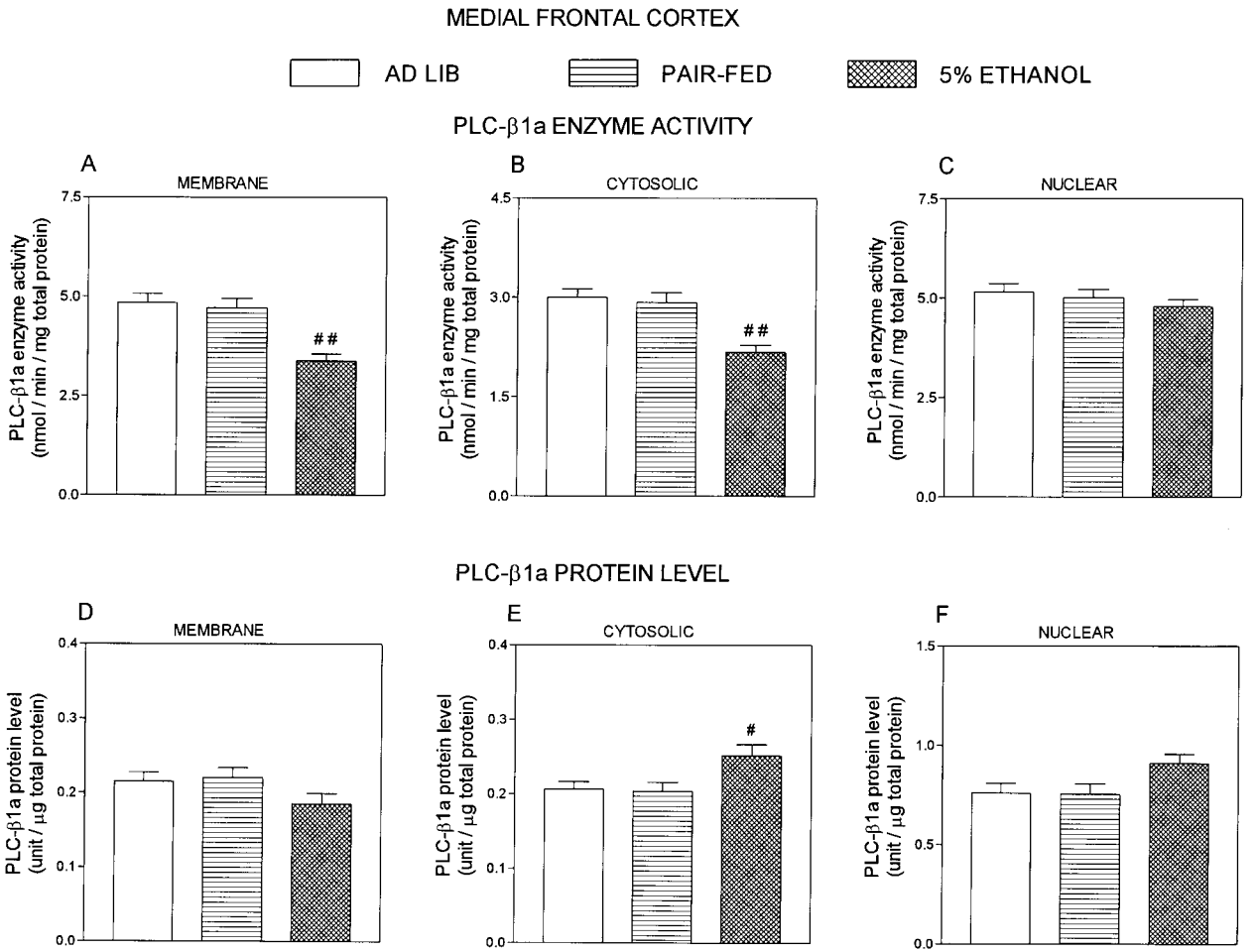
Prenatal Ethanol Exposure Alters the Pattern of One-Trial Fear Conditioning-Induced Changes in PLC-β1a Enzyme Activity and PLC-β1a Protein Level

The effects of one-trial fear conditioning on PLC-β1a enzyme activity and PLC-β1a protein level in hippocampal (Fig. 7) and medial frontal cortical (Fig. 8) subcellular fractions were assessed in offspring from dams fed each of the three diets. Tissues were isolated either 1 or 24 h after completion of the one-trial fear conditioning or UPC paradigm. In order to highlight the differences in the responses of the fetal ethanol-exposed and control rats to one-trial fear conditioning, the data are graphed as the change relative to the mean value obtained for the UPC group (within brain region, fraction, diet and time). The transformed data were analyzed by MANOVA to identify significant main effects and interactions among behavioral treatment (i.e., UPC or one-trial fear conditioning), diet, subcellular fraction, and time. Higher order interactions were subsequently probed using two types of post hoc analyses. First, the effect of one-trial fear conditioning was assessed within a diet group, fraction, and time using one sample *t* tests. In order to simply the figures, significant differences at the $p < .05$ and $p < .01$ level are not differentiated. Second, the effect of diet on the response to one-trial fear conditioning was assessed using Tukey's multiple comparison test, performed within a fraction and within time across the three diet groups; again, significant differences at both the $p < .05$ and $p < .01$ level are not distinguished.

MANOVA of the measurements of PLC-β1a enzyme activity in the hippocampal formation (Fig. 7A–7C) found a main effect of fraction and an interaction of behavioral treatment and fraction. Thus, we determined in which cases there was a significant effect of one-trial fear conditioning. One-trial fear conditioning significantly altered PLC-β1a enzyme activity in the cytosolic fraction derived from fetal ethanol-exposed rats 1 h after conditioning (Fig. 7B) and in the nuclear fraction obtained from pair-fed and prenatal ethanol-exposed rats 24 h after one-trial fear conditioning (Fig. 7C).

MANOVA of the measurements of PLC-β1a protein level in the hippocampal formation (Figs. 7D–7F) revealed main effects of fraction and of time. Interactions were found between (1) behavioral treatment and fraction, (2) behavioral treatment and time, (3) diet and fraction, (4) fraction and time, (5) behavioral treatment, diet, and fraction, and (6) behavioral treatment, fraction, and time. The interaction of behavioral treatment, fraction, and time is observed as decreased (relative to UPC) PLC-β1a protein level in the membrane (Fig. 7D) fraction and increased PLC-β1a protein level in the cytosolic (Fig. 7E) and

FIG. 6. Effect of prenatal ethanol exposure on PLC-β1a enzyme activity (A–C) and PLC-β1a protein level (D–F) in subcellular fractions prepared from the medial frontal cortex of behavioral control (UPC) rats. Rats were exposed prenatally to 5% (v/v) ethanol (cross-hatched bars) or not (pair-fed, horizontally filled bars, and *ad lib*, open bars, control) (see Methods). Data bars represent the mean ± the SEM of eight rats in each diet treatment group. #, ## Data significantly ($p < .05$ and $p < .01$, respectively) different than both the *ad lib* and pair-fed control groups based on a one-way ANOVA followed by Tukey's multiple comparison post hoc test. Fetal ethanol-exposed rats exhibited significant reductions in PLC-β1a enzyme activity in the membrane [$F(2, 23) = 14.33$, $p < .01$] and cytosolic [$F(2, 23) = 13.03$, $p < .01$] fractions and significant elevations of PLC-β1a protein level in the cytosolic fraction [$F(2, 23) = 4.55$, $p < .05$].

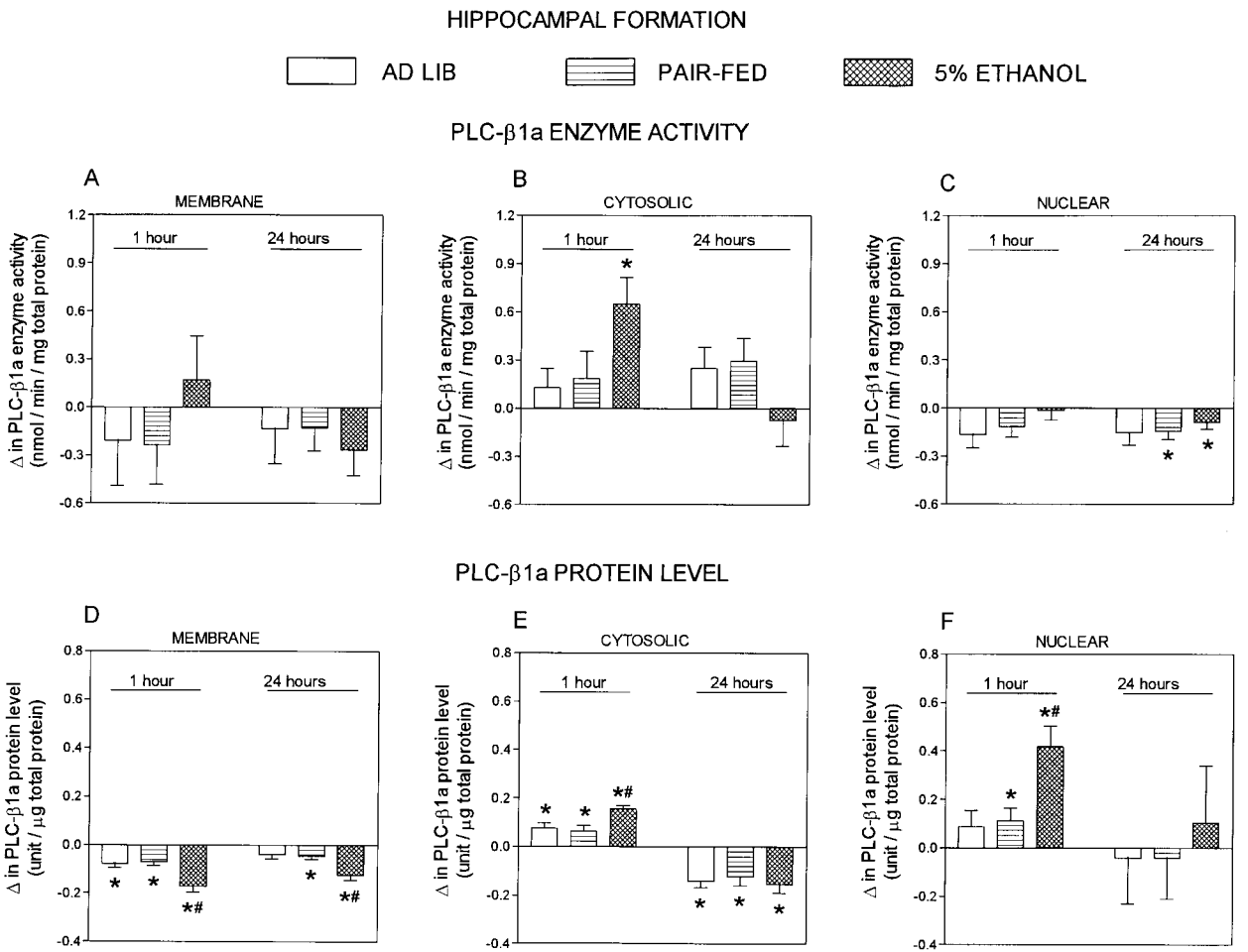

MEDIAL FRONTAL CORTEX
AD LIB
PAIR-FED
5% ETHANOL
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
PLC-β1a enzyme activity (nmol / min / mg total protein)
7.5
5.0
2.5
0.0
4.5
3.0
1.5
##
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
PLC-β1a protein level (unit / μg total protein)
0.4
0.3
0.2
0.1
1.5
1.0
0.5
#

nuclear (Fig. 7F) fractions in all diet groups 1 h after one-trial fear conditioning. Twenty-four hours after conditioning, membrane PLC-β1a protein level remained decreased, while cytosolic PLC-β1a protein level also was reduced and nuclear PLC-β1a protein level was not different from UPC levels. The behavioral treatment, diet, and fraction interaction is seen as a significant increase in the magnitude of the response in rats exposed prenatally to ethanol in all fractions 1 h after one-trial fear conditioning but only in the membrane fraction 24 h after conditioning. Post hoc analyses revealed that, in most cases, one-trial fear conditioning significantly altered PLC-β1a protein level and that diet affected the response in all three fractions 1 h after one-trial fear conditioning and in the membrane fraction 24 h after one-trial fear conditioning.

Several significant effects were revealed by MANOVA of the medial frontal cortical PLC-β1a enzyme activity data (Fig. 8A–8C): (1) main effect of diet, (2) interaction of behavioral treatment and diet, (3) interaction of diet and fraction, (4) interaction of fraction and time, (5) interaction of behavioral treatment, diet, and fraction, and (6) interaction of behavioral treatment, fraction, and time. The interactions of behavioral treatment, fraction, and time and of behavioral treatment, diet, and fraction are the result, primarily, of the difference in the pattern of the responses in the cytosolic fraction compared to that observed in the membrane and nuclear fractions. Twenty-four hours after one-trial fear conditioning, PLC-β1a enzyme activity was increased (relative to UPC) in all diet groups in the cytosolic fraction (Fig. 8B), whereas at 1 h in the cytosolic fraction and at both 1 and 24 h in the membrane (Fig. 8A) and nuclear (Fig. 8C) fractions, prenatal ethanol exposure altered the direction (increase, rather than decrease) of the response. This reversal was significant only at 1 h in the membrane and cytosolic fractions. Post hoc analyses revealed four cases of significant effects of one-trial fear conditioning.

MANOVA of the medial frontal cortical PLC-β1a protein level data (Figs. 8D–8F) revealed a main effect of fraction and of time. Significant interactions were found between (1) behavioral treatment and fraction, (2) behavioral treatment and time, (3) fraction and time, and (4) behavioral treatment, fraction, and time. The primary source of this latter

FIG. 7. Effect of prenatal alcohol exposure on the response to one-trial fear conditioning in hippocampal subcellular fractions. Rats were exposed prenatally to 5% (v/v) ethanol (cross-hatched bars) or not (pair-fed, horizontally filled bars, and *ad lib*, open bars, control) (see Methods). One or 24 h following delivery of the shock in the one-trial fear conditioning or UPC paradigm, rats were sacrificed, the hippocampal formation was isolated, and subcellular fractions were prepared as described under Methods. The data are graphed as the change in PLC-β1a enzyme activity (A–C) or PLC-β1a protein level (D–F) and were calculated as the difference between the individual values and the mean of the UPC values (see text). * Data that are significantly different (either $p < .05$ or $p < .01$) from the UPC mean (within diet group, subcellular fraction, and time), as determined by a one-sample *t* test. # Data significantly different (either $p < .05$ or $p < .01$) than both the *ad lib* and pair-fed control groups (within fraction and time) based on a one-way ANOVA followed by a Tukey's multiple comparison post hoc test. MANOVA of the measurements of PLC-β1a enzyme activity revealed a main effect of fraction [$F(2, 36) = 9.95$, $p < .01$] and an interaction of behavioral treatment and fraction [$F(2, 36) = 10.0$, $p < .01$]. MANOVA of the measurements of PLC-β1a protein level revealed a main effect of fraction [$F(2, 36) = 9.84$, $p < .01$], a main effect of time [$F(1, 18) = 7.17$, $p < .01$], an interaction of behavioral treatment and fraction [$F(2, 36) = 9.57$, $p < .01$], an interaction of behavioral treatment and time [$F(1, 18) = 7.53$, $p < .01$], an interaction of diet and fraction [$F(4, 36) = 3.27$, $p < .05$], an interaction of fraction and time [$F(2, 36) = 4.13$, $p < .05$], an interaction of behavioral treatment, diet, and fraction [$F(4, 36) = 3.35$, $p < .05$] and an interaction of behavioral treatment, fraction, and time [$F(2, 36) = 4.21$, $p < .05$].


HIPPOCAMPAL FORMATION
AD LIB
PAIR-FED
5% ETHANOL
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
1 hour
24 hours
Δ in PLC-β1a enzyme activity (nmol / min / mg total protein)
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
Δ in PLC-β1a protein level (unit / μg total protein)

interaction is the significant increase in PLC-β1a protein level in all diet groups in the nuclear fraction 24 h after one-trial fear conditioning (Fig. 8F), which contrasts with the significant decreases in all diet groups in all three fractions 1 h after conditioning. Post hoc analyses revealed significant effects of one-trial fear conditioning in several cases.

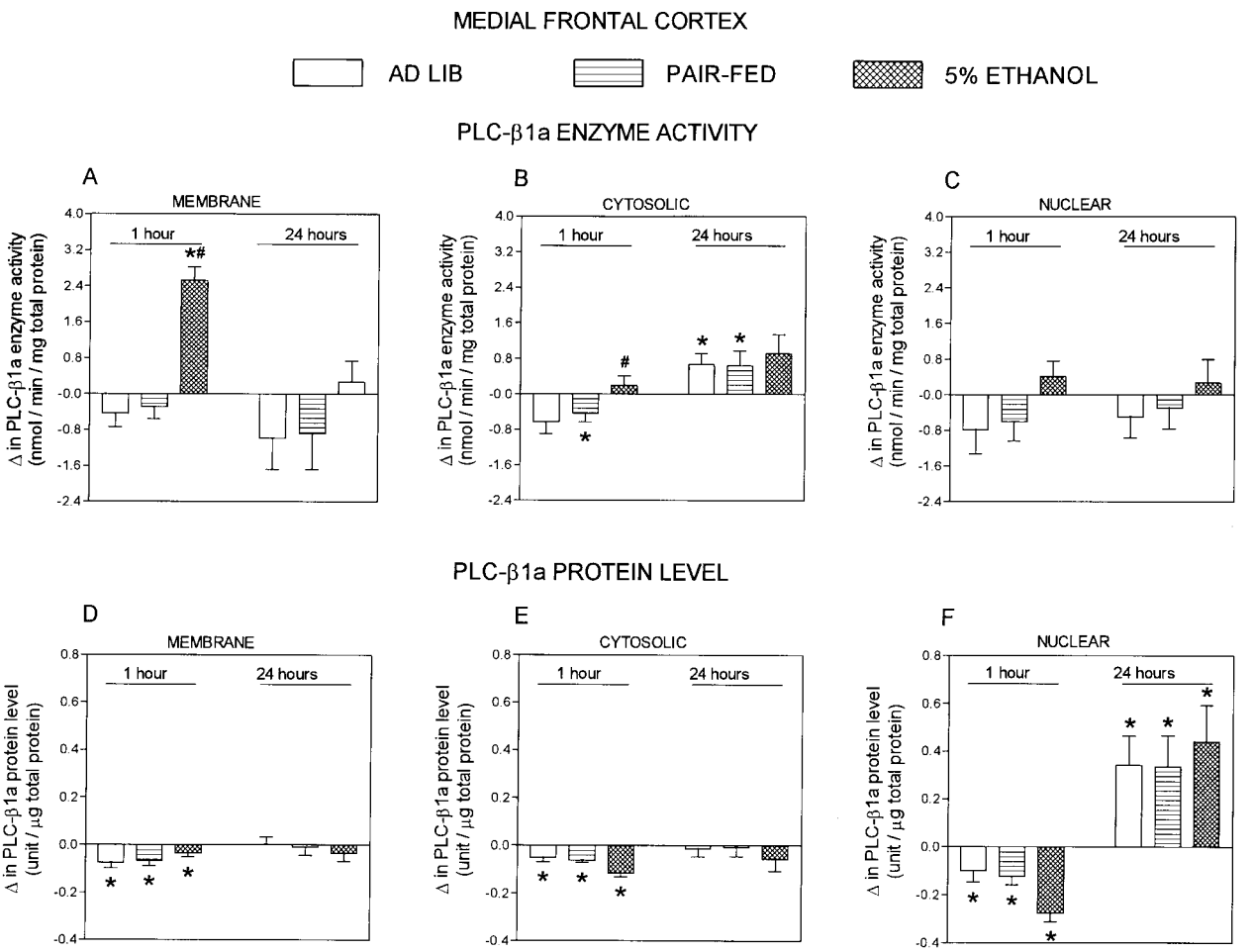
DISCUSSION

One-Trial Fear Conditioning Is Associated with Altered Subcellular PLC-β1a Enzyme Activity and PLC-β1a Protein Level

One-trial fear conditioning was associated with several changes in PLC-β1a enzyme activity and PLC-β1a protein level in hippocampal formation and medial frontal cortical subcellular fractions. In both the hippocampal formation (Figs. 2 and 7) and the medial frontal cortex (Figs. 3 and 8) isolated from *ad lib* diet control rats, fear conditioning was associated with altered PLC-β1a protein level in the membrane and cytosolic subcellular fractions. In addition, in the medial frontal cortex, fear conditioning was associated with altered PLC-β1a protein level in the nuclear fraction and changes in PLC-β1a enzyme activity in the cytosolic fraction. Because none of these changes were detected in the behavioral control (UPC) rats, we conclude that they reflect processes underlying the formation of associations among the elements of the learning episode, rather than unconditioned responses to the stimuli that comprise the fear conditioning paradigm. Thus, the present studies support our hypothesis that changes in PLC-β1a-dependent signal transduction play a role in fear learning and memory.

In diet control (*ad lib* and pair-fed) rats, the effects of fear conditioning on PLC-β1a enzyme activity in hippocampal subcellular fractions (Figs. 2A–2C and 7A–7C) paralleled, in general, those in medial frontal cortical subcellular fractions (Figs. 3A–3C and 8A–8C), both 1 and 24 h after conditioning. The exception to this statement is the difference in the measures of enzyme activity in the cytosolic fraction 1 h after fear conditioning,

FIG. 8. Effect of prenatal alcohol exposure on the response to one-trial fear conditioning in medial frontal cortical subcellular fractions. Rats were exposed prenatally to 5% (v/v) ethanol (cross-hatched bars) or not (pair-fed, horizontally filled bars, and *ad lib*, open bars, control) (see Methods). One or 24 h following delivery of the shock, the rats were sacrificed, the medial frontal cortex was isolated, and subcellular fractions were prepared as described under Methods. The data are graphed as the change in PLC-β1a enzyme activity (A–C) or PLC-β1a protein level (D–F) and were calculated as the difference between the individual values and the mean of the UPC values (see text). * Data that are significantly different (either $p < .05$ or $p < .01$) from the UPC mean (within diet group, subcellular fraction, and time), as determined by a one-sample *t* test. # Data significantly different (either $p < .05$ or $p < .01$) than both the *ad lib* and pair-fed control groups (within fraction and time) based on a one-way ANOVA followed by Tukey's multiple comparison post hoc test. MANOVA of the PLC-β1a enzyme activity data revealed a main effect of diet [$F(2, 18) = 9.44$, $p < .01$], an interaction of behavioral treatment and diet [$F(2, 18) = 9.46$, $p < .01$], an interaction of diet and fraction [$F(4, 36) = 2.84$, $p < .05$], an interaction of fraction and time [$F(2, 36) = 9.09$, $p < .01$], an interaction of behavioral treatment, diet, and fraction [$F(4, 36) = 2.86$, $p < .05$], and an interaction of behavioral treatment, fraction, and time [$F(2, 36) = 9.24$, $p < .01$]. MANOVA of the PLC-β1a protein level data revealed a main effect of fraction [$F(2, 36) = 9.45$, $p < .01$], a main effect of time [$F(1, 18) = 46.24$, $p < .01$], an interaction of behavioral treatment and fraction [$F(2, 36) = 9.46$, $p < .01$], an interaction of behavioral treatment and time [$F(1, 18) = 46.3$, $p < .01$], an interaction of fraction and time [$F(2, 36) = 35.6$, $p < .01$], and an interaction of behavioral treatment, fraction and time [$F(2, 36) = 35.7$, $p < .01$].


MEDIAL FRONTAL CORTEX
AD LIB
PAIR-FED
5% ETHANOL
PLC-β1a ENZYME ACTIVITY
A
MEMBRANE
B
CYTOSOLIC
C
NUCLEAR
1 hour
24 hours
Δ in PLC-β1a enzyme activity (nmol / min / mg total protein)
PLC-β1a PROTEIN LEVEL
D
MEMBRANE
E
CYTOSOLIC
F
NUCLEAR
Δ in PLC-β1a protein level (unit / μg total protein)

when enzyme activity was decreased in the medial frontal cortex and increased in the hippocampal formation. There was not an apparent similar parallel in the effects of fear conditioning on PLC-β1a protein level in subcellular fractions derived from the hippocampal formation (Figs. 2A–2C and 7D–7F) and the medial frontal cortex (Figs. 3A–3C and 8D–8F) of diet control rats. Consequently, there are differences in the effects of fear conditioning on PLC-β1a specific activity (catalytic activity/protein level) across brain regions and subcellular fractions. These results indicate that there is differential regulation of PLC-β1a catalytic activity in response to one-trial fear conditioning, depending on the brain region and subcellular fraction in which the enzyme resides.

Prenatal Exposure to Moderate Levels of Ethanol Is Associated with Reduced Contextual Fear Conditioning

In utero exposure to ethanol has been shown to be associated with deficits in learning and memory in a variety of tests (Berman, & Hannigan, 2000; Furuya et al., 1996; Gianoulakis, 1990; Reyes et al., 1989; Westergren et al., 1996; Zimmerberg et al., 1989). We found that prenatal exposure of rats to moderate levels of ethanol was associated with reduced contextual, but not auditory, fear conditioning measured 24 h posttraining (Figure 4). This deficit could be the result of a deficit in one, or a combination, of the following: the ability to learn (i.e., to acquire/process information), the ability to consolidate information into memory, the ability to retain and/or retrieve memory, or the ability to express a fear response. Preliminary results from ongoing studies by one of us (R.J.S.) demonstrate that both 75 min and 1 week following completion of the one-trial fear conditioning paradigm, rats exposed prenatally to ethanol display deficits in contextual, but not auditory, conditioning similar to that reported 24 h posttraining in the present study. These results indicate that fetal ethanol exposure selectively alters "early" (occurring at times $<$75 min posttraining), rather than "late", processes involved in contextual fear conditioning. Our identification of effects of prenatal ethanol exposure on fear conditioning-induced changes in PLC-β1a protein level and enzyme activity measured 60 min following conditioning identify one possible biochemical mechanism that could underlie these deficits in contextual fear conditioning.

Fetal Ethanol Exposure Alters Basal PLC-β1a Enzyme Activity and PLC-β1a Protein Level in Behavioral Control Rats

Previously, we reported that prenatal exposure to ethanol significantly reduced PLC-β1a basal enzyme activity measured in hippocampal formation and medial frontal cortical crude soluble (S1) fractions, which contain both cytosolic and membrane components (Allan et al., 1997). In order to characterize these deficits further, we determined whether they were confined to either the cytosolic or the membrane compartment, or whether they were present in both compartments. Further, we determined whether they were correlated with deficits in the amount of PLC-β1a protein present in the compartment. Finally, we also assessed whether prenatal ethanol exposure was associated with alterations in PLC-β1a enzyme activity or protein level in hippocampal formation and medial frontal cortical nuclear fractions.

Fetal ethanol exposure was associated with significant reductions in PLC-β1a basal

enzyme activity in both the cytosolic and the membrane fractions isolated from the medial frontal cortex (Figs. 6A–6B). Similarly, PLC-β1a basal enzyme activity was decreased in hippocampal cytosolic and membrane fractions, however, these decreases did not achieve statistical significance (Figs. 5A and 6B). It is possible that the reduction in the magnitude of the effect of prenatal ethanol exposure on hippocampal formation PLC-β1a enzyme activity in the present studies compared to our previous studies is the result of the contamination of the S1 preparations employed in our previous studies with the nuclear fraction, in which there is a significant decrease in PLC-β1a enzyme activity in fetal ethanol-exposed rats (see Fig. 5C).

The reductions in PLC-β1a basal enzyme activity in the medial frontal cortical membrane and hippocampal nuclear fractions derived from fetal ethanol-exposed animals appear to be, at least in part, the result of decreases in the PLC-β1a protein level in these fractions. In contrast, the deficit in medial frontal cortical cytosolic PLC-β1a basal enzyme activity cannot be explained by a reduction in PLC-β1a protein level, as the PLC-β1a level was significantly increased, rather than decreased, in this fraction. Similarly, the increase in PLC-β1a protein level in the hippocampal membrane fraction was associated with decreased, rather than increased, basal enzyme activity. These results indicate that, in the medial frontal cortical cytosolic and hippocampal membrane fractions, prenatal exposure to moderate levels of ethanol reduces PLC-β1a basal enzyme activity as the result of changes in enzyme regulatory and/or catalytic processes, rather than decreases in the amount of enzyme present in these subcellular fractions. The mechanism(s) underlying these effects of prenatal ethanol exposure on PLC-β1a protein level and enzyme regulatory/catalytic activity are uncertain.

Effects of Fetal Ethanol Exposure on One-Trial Fear Conditioning-Induced Changes in PLC-β1a Enzyme Activity and PLC-β1a Protein Level

We observed three types of relationships between the effects of one-trial fear conditioning on PLC-β1a in fetal ethanol-exposed rats and control rats (Figs. 7 and 8). First, cases where the direction (i.e., increase or decrease relative to UPC) and magnitude of the change in fetal ethanol-exposed rats were not significantly different from those of control rats. Second, cases where the direction of change in response to one-trial fear conditioning in rats exposed prenatally to ethanol was the same as that measured in control rats; however, the magnitude of the change was greater (i.e., an exaggerated response). Third, cases where the direction of the change in prenatal ethanol-exposed rats was opposite to that measured in control rats. In these latter cases, the magnitudes of the responses in fetal ethanol-exposed rats ranged from less than to greater than those measured in control rats. These relationships indicate that, in some instances, mechanisms underlying the regulation of PLC-β1a catalytic activity and subcellular distribution may be altered by prenatal exposure to ethanol.

The majority of the significant effects of fetal ethanol exposure on the response to fear conditioning were recorded in subcellular fractions derived from rats sacrificed 1 h, rather than 24 h, after one-trial fear conditioning. These results indicate that, in the case of PLC-β1a, prenatal exposure to ethanol alters the initial biochemical responses to fear conditioning, without profoundly altering the later responses. Interestingly, this mirrors the effects of prenatal ethanol on behavioral responses to fear conditioning noted above.

If PLC-β1a signaling plays an important role in the deficits in contextual fear conditioning that occur in animals exposed prenatally to ethanol (Fig. 4), then, cases where the response of fetal ethanol-exposed rats differed significantly from the response of diet control rats identify a subset of fear conditioning-induced changes in PLC-β1a signaling that are critical for the acquisition and retention of contextual fear.

Potential Amplification of the Effects of One-Trial Fear Conditioning and Prenatal Ethanol Exposure

It is important to recognize that PLC-β1a is positioned at a critical step in the transduction of input from the environment into a cellular response. Hoek and Kholodenko (1998) have developed models to describe the relationships between signal strength at individual steps in a signal transduction pathway and the response of the end target of the pathway. They demonstrate that, even though ethanol may elicit a relatively small (30%) decrease in the functioning of one of the steps (e.g., at the level of a receptor or an effector) of a signaling network, this decrease may markedly reduce the sigmoidicity of the relationship between the signal strength and a cellular response, such that more than a 3-fold increase in signal strength is needed to elicit a maximal cellular response. Thus, the 20–40% changes in effector (PLC-β1a) activity and protein level that we observed in several instances following one-trial fear conditioning, as well as in association with fetal ethanol exposure, may grossly after cellular functioning and could constitute a principle mechanism underlying altered synaptic plasticity and, ultimately, learning and memory.

Proposed Roles for the Observed Changes in PLC-β1a Protein Level and Catalytic Activity in Fear Conditioning and Prenatal Ethanol-Induced Contextual Fear Deficits

The present studies do not define whether fear conditioning-induced changes in PLC-β1a enzyme activity and protein level are associated with contextual or CS fear. However, we propose that that they are associated with contextual, rather than CS, fear conditioning given that conditioning to an elemental CS, such as a tone, can occur in the absence of a functional hippocampal formation (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Sacchetti et al., 1999) or medial frontal cortex (Morgan, Romanski, & LeDoux, 1993; Morgan & LeDoux, 1995), whereas contextual conditioning is dependent upon hippocampal function (Chen et al., 1996; Kim et al., 1993; Maren, Anagnostaras, & Fanselow, 1998; Phillips & LeDoux, 1992; Sacchetti et al., 1999) and may be modulated by the medial frontal cortex (Morgan & LeDoux, 1995; Morrow et al., 1999). This conclusion predicts that fear conditioning differentially affects PLC-β1a enzyme activity and/or PLC-β1a protein level in the hippocampal formation, the medial frontal cortex, or both, of animals that differ in contextual conditioning. Indeed, this is what we observed in studies employing rats that had been exposed prenatally to ethanol. These animals, which displayed reduced contextual, but not auditory, fear compared to controls, exhibited several differences in the effects of fear conditioning on PLC-β1a enzyme activity and protein level. Thus, the altered effects of fear conditioning on hippocampal formation and medial frontal cortical PLC-β1a enzyme activity and PLC-β1 protein level in fetal ethanol-exposed rats could underlie their deficits in contextual conditioning.

Although the results of the present studies provide the basis for hypothesizing that hippocampal formation and medial frontal cortical PLC-β1a play a role in contextual fear conditioning, as well as in the learning deficits associated with prenatal ethanol exposure, we cannot, at present, develop a simple model that defines the role(s) of PLC-β1a in fear conditioning and prenatal ethanol-induced cognitive dysfunctions. This is, in part, due to the fact that the complicated pattern of the changes in PLC-β1a protein level and catalytic activity that we found associated with fear conditioning and fetal ethanol exposure do not render themselves to simplistic interpretation. In addition, it is the result of the current lack of knowledge concerning the function(s) of PLC-β1a in distinct subcellular compartments.

The physiologic functions of membrane-associated, cytoplasmic, and nuclear-associated PLC-β1a are, at present, uncertain. As noted in the introduction, plasma membrane-associated PLC-β1a is thought to play a role in transducing signals from G-protein-coupled receptors into intracellular signals. Thus, changes in plasma membrane-associated PLC-β1a signaling that are induced by fear conditioning (or by prenatal ethanol exposure) are predicted to alter intracellular Ins(1,4,5)P_3 and DAG levels and/or turnover rates and, as a consequence, to alter Ca^{2+}- and PKC-dependent downstream responses. In this light, plasma membrane-associated PLC-β1a could play a role in the induction of fear conditioning, which is likely to depend on the alteration of existing synapses through the modification (e.g., phosphorylation) of preexisting cellular proteins (Atkins et al., 1998; Bourtchouladze et al., 1998; Young et al., 2000). It is more difficult to evaluate the physiologic significance of changes in cytosolic PLC-β1a activity and protein level, since roles for PLC-β1a in this compartment have not been elucidated. Cockcroft and Thomas (1992) proposed that cytosolic PLC isozymes might be in equilibrium with subcellular membranes. In agreement with this, we believe that cytosolic PLC-β1a may serve as a reservoir of enzyme to ensure immediate availability to the nucleus and plasma membrane, as well as other subcellular compartments. Similarly, the role of PLC-β1a in the nucleus is unclear. Immunohistochemical studies have identified PLC-β1a in the nucleus of a variety of cell lines (Cocco, Capitani, Maraldi, Mazzotti, Barnabei, Rizzoli, Gilmour, Wirtz, Rhee, & Manzoli, 1998), as well as rat liver (Divecha, Rhee, Letcher, & Irvine, 1993) and maturing germ cells (Caramelli, Matteucci, Zini, Carini, Guidotti, Ricci, Maraldi, & Capitani, 1996). Within the nucleus, PLC-β1a is localized to the interheterochromatin border (Marmiroli et al., 1996; Zini, Mazzoni, Neri, Bavelloni, Marmiroli, Capitani, & Maraldi, 1994; Zini et al., 1996), indicating that it may be involved in the control of gene transcription. Therefore, nuclear PLC-β1a could play a role in the acquisition of fear conditioning and in the consolidation and maintenance of fear-conditioned memory, all of which require gene transcription and the synthesis of new proteins (Bailey, Kim, Sun, Thompson, & Helmstetter, 1999; Bourtchouladze et al., 1998; Impey, Smith, Obrietan, Donahue, Wade, & Strom, 1998; Morrow et al., 1999; Radulovic, Kammermeier, & Spiess, 1998; Schafe et al., 1999; Stiedl, Palve, Radulovic, Birkenfeld, & Spiess, 1999).

Proposed Mechanisms Underlying the Effects of One-Trial Fear Conditioning and Prenatal Ethanol Exposure

The molecular bases that underlie the observed changes in PLC-β1a enzyme activity and PLC-β1a protein level following fear conditioning and prenatal ethanol exposure are

unknown. Changes in enzyme activity that cannot be accounted for by corresponding changes in enzyme protein level, such as was noted in the medial frontal cortical membrane fraction isolated from fetal ethanol-exposed rats, indicate the existence of underlying regulatory mechanisms. Phosphorylation is a candidate regulatory mechanism. Litosch (1996) has reported that the PKC-dependent phosphorylation of PLC-β1 alters the Ca^{2+}-dependency of Ptdlns(4,5)P_2 hydrolysis. Alternatively, changes in PLC-β1a enzyme activity may result from changes in the association of the enzyme with a regulatory cofactor (e.g., G-protein subunit) that coimmunoprecipitates with the enzyme. In support of this latter mechanism, Piiper, Stryjek-Kaminska, Klengel, and Zeuzem (1997) have reported that both $G_{i\alpha 1}$ and $G_{i\alpha 2}$ subunits can be coimmunoprecipitated with PLC-β1.

The PLC-β1a protein level in a particular subcellular compartment is controlled by multiple processes, including the relative rates of protein synthesis and degradation in the compartment and factors governing the movement of the enzyme into and out of the compartment. Thus, the changes in cytosolic PLC-β1a protein levels that we observed following fear conditioning reflect alterations in the turnover rate of the protein or translocation of the protein to a different subcellular location. More detailed kinetic studies will be needed to differentiate between these mechanisms. The decreases in hippocampal formation and medial frontal cortical membrane PLC-β1a protein levels observed following fear conditioning could reflect reductions in the amount of PLC-β1a associated with the plasma membrane. Plasma membrane binding of PLC-β1 is believed to involve the cooperative binding of G-protein $\beta\gamma$-subunits and phosphatidylinositol 3-phosphate to the pleckstrin homology domain of the enzyme (Razzini, Brancaccio, Lemmon, Guarnieri, & Falasca, 2000). Thus, the observed decreases in membrane PLC-β1a protein levels could result from decreased plasma membrane content of phosphatidylinositol 3-phosphate following fear conditioning. The observed increases in the amount of PLC-β1a present in the hippocampal nuclear fraction 1 h following fear conditioning may reflect increased localization of the enzyme to the nucleus, which is mediated by basic residues in the carboxyl-terminus of the protein (Kim, Park, & Rhee, 1996), while the large increases in the PLC-β1a protein level observed in the medial frontal cortical nuclear fraction may be attributable to altered protein turnover rates. Studies aimed at addressing these issue are currently underway in our laboratories.

Conclusions

In conclusion, we found that one-trial fear conditioning altered PLC-β1a enzyme activity and PLC-β1a protein level in rat hippocampal formation and medial frontal cortex subcellular fractions and that the patterns of changes induced by fear conditioning were, in some instances, different in rats that were exposed prenatally to ethanol. Thus, the present data support the hypothesis that activity-dependent changes in PLC-β1a signal transduction play an important role in fear conditioning. In addition, they support the hypothesis that prenatal ethanol exposure alters PLC-β1a signaling, which, in turn, may contribute to the neurophysiologic and the neurobehavioral deficits observed in fetal ethanol-exposed animals.

REFERENCES

Aiba, A., Chen, C., Herrup, K., Rosenmund, C., Stevens, C. F., & Tonegawa, S. (1994). Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell,* **79,** 365–375.

Alkon, D. L., Nelson, T. J., Zhao, W., & Cavallaro, S. (1998). Time domains of neuronal Ca^{2+} signaling and associative memory: Steps through a calexcitin, ryanodine receptor, K^+ channel cascade. *Trends in Neurosciences,* **21,** 529–537.

Allan, A. M., Weeber, E. J., Savage, D. D., & Caldwell, K. K. (1997). Effects of prenatal ethanol exposure on phospholipase C-β1 and phospholipase A_2 in hippocampus and medial frontal cortex of adult rat offspring. *Alcoholism: Clinical and Experimental Research,* **21,** 1534–1541.

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: Within-subjects examination. *The Journal of Neuroscience,* **19,** 1106–1114.

Atkins, C. M., Selcher, J. C., Petraitis, J. J., Trzaskos, J. M., & Sweatt, J. D. (1998). The MAPK cascade is required for mammalian associative learning. *Nature Neuroscience,* **1,** 602–609.

Ayyagari, P. V., Gerber, M., Joseph, J. A., & Crews, F. T. (1998). Uncoupling of muscarinic cholinergic phosphoinositide signals in senescent cerebral cortical and hippocampal membranes. *Neurochemistry International,* **32,** 107–115.

Bahk, Y. Y., Lee, Y. H., Lee, T. G., Seo, J., Ryu, S. H., & Suh, P.-G. (1994). Two forms of phospholipase C-β1 generated by alternative splicing. *The Journal of Biological Chemistry,* **269,** 8240–8245.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mRNA in the amygdala. *Behavioral Neuroscience,* **113,** 276–282.

Berman, R. E., & Hannigan, J. H. (2000). Effects of prenatal alcohol exposure on the hippocampus: Spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus,* **10,** 94–110.

Berridge, M. J. (1993). Inositol trisphosphate and calcium signalling. *Nature,* **361,** 315–325.

Bevins, R. A., & Ayres, J. J. B. (1994). A deficit in one-trial context fear conditioning is not due to opioid analgesia. *Pharmacology Biochemistry and Behavior,* **49,** 183–186.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., & Kandel, E. R. (1998). Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. *Learning & Memory,* **5,** 365–374.

Bouton, M. E., & Bolles, R. C. (1980). Conditioned fear assessed by freezing and by the suppression of three different baselines. *Animal Learning & Behavior,* **8,** 429–434.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* **72,** 248–254.

Caramelli, E., Matteucci, A., Zini, N., Carini, C., Guidotti, L., Ricci, D., Maraldi, N. M., & Capitani, S. (1996). Nuclear phosphoinositide-specific phospholipase C, phosphatidylinositol 4,5-bisphosphate and protein kinase C during rat spermatogenesis. *European Journal of Cell Biology,* **71,** 154–164.

Chen, C., Kim, J. J., Thompson, R. F., & Tonegawa, S. (1996). Hippocampal lesions impair contextual fear conditioning in two strains of mice. *Behavioral Neuroscience,* **110,** 1177–1180.

Chouinard, M. L., Gallagher, M., Yasuda, R. P., Wolfe, B. B., & McKinney, M. (1995). Hippocampal muscarinic receptor function in spatial learning-impaired aged rats. *Neurobiology of Aging,* **16,** 955–963.

Christoffersen, G. R. J., Christensen, L. H., Harrington, N. R., Macphail, E. M., & Riedel, G. (1999). Task-specific enhancement of short-term, but not long-term, memory by class I metabotropic glutamate receptor antagonist 1-aminoindan-1,5-dicarboxylic acid in rats. *Behavioural Brain Research,* **101,** 215–226.

Cocco, L., Capitani, S., Maraldi, N. M., Mazzotti, G., Barnabei, O., Rizzoli, R., Gilmour, R. S., Wirtz, K. W. A., Rhee, S.-G., & Manzoli, F. A. (1998). Inositides in the nucleus: Taking stock of PLCβ_1. *Advances in Enzyme Regulation,* **38,** 351–363.

Cockcroft, S., & Thomas, G. M. H. (1992). Inositol-lipid-specific phospholipase C isoezymes and their differential regulation by receptors. *Biochemical Journal,* **288,** 1–14.

Colombo, P. J., Wetsel, W. C., & Gallagher, M. (1997). Spatial memory is related to hippocampal subcellular concentrations of calcium-dependent protein kinase C isoforms in young and aged rats. *Proceedings of the National Academy of Sciences USA,* **94,** 14195–14199.

Cordero, M. I., & Sandi, C. (1998). A role for brain glucocorticoid receptors in contextual fear conditioning: Dependence upon training intensity. *Brain Research,* **786,** 11–17.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience,* **112,** 1092–1103.

Davis, M. (1997). Neurobiology of fear responses: The role of the amygdala. *The Journal of Neuropsychiatry and Clinical Neuroscience,* **9,** 382–402.

Davis, M., Falls, W. A., Campeau, S., & Kim, M. (1993). Fear-potentiated startle: A neural and pharmacological analysis. *Behavioural Brain Research,* **58,** 175–198.

de Bruin, J. P. C., Facchinetti, F., Tóth, E., Balázs, R., & Joosten, R. N. J. M. A. (1996). Differential effects of learning and recall of a spatial task on phosphoinositide hydrolysis induced by the metabotropic glutamate receptor agonist $1_S,3_R$-ACPD ($1_S,3_R$-1-amino-cyclopentane-1,3-dicarboxylic acid) in the hippocampus and the prefrontal cortex of rats. *Neuroscience Letters,* **204,** 57–60.

Desmedt, A., Garcia, R., & Jaffard, R. (1998). Differential modulation of changes in hippocampal-septal synaptic excitability by the amygdala as a function of either elemental or contextual fear conditioning in mice. *The Journal of Neuroscience,* **18,** 480–487.

Divecha, N., Rhee, S.-G., Letcher, A. J., & Irvine, R. F. (1993). Phosphoinositide signalling enzymes in rat liver nuclei: Phosphoinositidase C isoform $\beta 1$ is specifically, but not predominantly, located in the nucleus. *Biochemical Journal,* **289,** 617–620.

Doyère, V., Burette, F., Rédini-Del Negro, C., & Laroche, S. (1993). Long-term potentiation of hippocampal afferents and efferents to prefrontal cortex: Implications for associative learning. *Neuropsychologia,* **31,** 1031–1053.

Exton, J. H. (1996). Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annual Review of Pharmacology and Toxicology,* **36,** 481–509.

Fanselow, M. S. (1980). Conditional and unconditional components of post-shock freezing. *Pavlovian Journal of Biological Sciences,* **15,** 177–182.

Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. *Journal of Comparative and Physiological Psychology,* **93,** 736–744.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behavioral Neuroscience,* **108,** 210–212.

Fanselow, M. S., & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron,* **23,** 229–232.

Feliciello, A., Gallo, A., Mele, E., Porcellini, A., Troncone, G., Garbi, C., Gottesman, M. E., & Avvedimento, E. V. (2000). The localization and activity of cAMP-dependent protein kinase affect cell cycle progression in thyroid cells. *The Journal of Biological Chemistry,* **275,** 303–311.

Fordyce, D. E., Clark, V. J., Paylor, R., & Wehner, J. M. (1995). Enhancement of hippocampally-mediated learning and protein kinase C activity by oxiracetam in learning-impaired DBA/2 mice. *Brain Research,* **672,** 170–176.

Frohardt, R. J., Guarraci, F. A., & Young, S. L. (1999). Intrahippocampal infusions of a metabotropic glutamate receptor antagonist block the memory of context-specific but not tone-specific conditioned fear. *Behavioral Neuroscience,* **113,** 222–227.

Frysztak, R. J., & Neafsey, E. J. (1991). The effect of medial frontal cortex lesions on respiration, "freezing", and ultrasonic vocalizations during conditioned emotional responses in rats. *Cerebral Cortex,* **1,** 418–425.

Furuya, H., Aikawa, H., Yoshida, T., & Okazaki, I. (1996). Effects of ethyl alcohol administration to THA rat dams during their gestation period on learning behavior and on levels of monoamines and metabolites in the brain of pups after birth. *Alcoholism: Clinical and Experimental Research,* **20,** 305A–310A.

Gerfen, C. R., Choi, W. C., Suh, P. G., & Rhee, S. G. (1988). Phospholipase C I and II brain isozymes: Immunohistochemical localization in neuronal systems in rat brain. *Proceedings of the National Academy of Sciences USA,* **85,** 3208–3212.

Gianoulakis, C. (1990). Rats exposed prenatally to alcohol exhibit impairment in spatial navigation test. *Behavioural Brain Research,* **36,** 217–228.

Graham, J. M. (1993). The isolation of nuclei and nuclear membranes from rat liver. *In* J. M. Graham & J. A. Higgins (Eds.). *Methods in molecular biology, Vol. 19: Biomembrane protocols: I. Isolation and analysis* (pp. 19–28). Totowa, NJ: Humana Press.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience,* **108,** 1005–1009.

Hodgkin, M. N., Pettitt, T. R., Martin, A., Michell, R. H., Pemberton, A. J., & Wakelam, M. J. O. (1998). Diacyglycerols and phosphatidates: Which molecular species are intracellular messengers? *Trends in Biochemical Sciences,* **23,** 200–204.

Hoek, J. B., & Kholodenko, B. N. (1998). The Intracellular signaling network as a target for ethanol. *Alcoholism: Clinical and Experimental Research,* **22,** 224S–230S.

Hwang, P. M., Bredt, D. S., & Snyder, S. H. (1990). Autoradiographic imaging of phosphoinositide turnover in the brain. *Science,* **249,** 802–804.

Impey, S., Smith, D. M., Obrietan, K., Donahue, R., Wade, C., & Storm, D. R. (1998). Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nature Neuroscience,* **1,** 595–601.

Kim, C. G., Park, D., & Rhee, S.-G. (1996). The role of carboxyl-terminal basic amino acids in $G_q\alpha$-dependent activation, particulate association, and nuclear localization of phospholipase C-β1. *The Journal of Biological Chemistry,* **271,** 21187–21192.

Kim, C. K., Kalynchuk, L. E., Kornecook, T. J., Mumby, D. G., Dadgar, N. A., Pinel, J. P. J., & Weinberg, J. (1997). Object-recognition and spatial learning and memory in rats prenatally exposed to ethanol. *Behavioral Neuroscience,* **111,** 985–995.

Kim, J. J., DeCola, J. P., Landeira-Fernandez, J., and Fanselow, M. S. (1991). *N*-Methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. *Behavioral Neuroscience,* **105,** 126–133.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science,* **256,** 675–677.

Kim, J. J., Fanselow, M. S., DeCola, J. P., & Landeira-Fernandez, J. (1992). Selective impairment of long-term but not short-term conditional fear by the *N*-methyl-D-aspartate antagonist APV. *Behavioral Neuroscience,* **106,** 591–596.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience,* **107,** 1093–1098.

Kim, J. J., Shih, J. C., Chen, K., Chen, L., Bao, S., Maren, S., Anagnostaras, S. G., Fanselow, M. S., De Maeyer, E., Seif, I., & Thompson, R. F. (1997). Selective enhancement of emotional, but not motor, learning in monoamine oxidase A-deficient mice. *Proceedings of the National Academy of Sciences USA,* **94,** 5929–5933.

Komatsu, Y. (1996). $GABA_B$ receptors, monoamine receptors, and postsynaptic inositol trisphosphate-induced Ca^{2+} release are involved in the induction of long-term potentiation at visual cortical inhibitory synapses. *Journal of Neuroscience,* **16,** 6342–6352.

Kusuki, T., Imahori, Y., Fujii, R., Inokuchi, K., Kimura, M., & Ueda, S. (1998). Potentiation of phosphoinositide-derived signals during LTP in intact rat brain. *NeuroReport,* **9,** 2085–2088.

Laroche, S., Redini-Del Negro, C., Clements, M. P., & Lynch, M. A. (1990). Long-term activation of phosphoinositide turnover associated with increased release of amino acids in the dentate gyrus and hippocampus following classical conditioning in the rat. *European Journal of Neuroscience,* **2,** 534–543.

Leahey, T. H., & Harris, R. J. (1980). *Learning and cognition.* Upper Saddle River, NJ: Prentice-Hall.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Reviews of Neuroscience,* **23,** 155–184.

Lee, H., & Kim, J. J. (1998). Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. *Journal of Neuroscience,* **18,** 8444–8454.

Litosch, I. (1996). Protein kinase C inhibits the Ca^{2+}-dependent stimulation of phospholipase C-β_1 in vitro. *Receptors and Signal Transduction,* **6,** 87–98.

Lu, Y.-M., Jia, Z., Janus, C., Henderson, J. T., Gerlai, R., Wojtowicz, J. M., & Roder, J. C. (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *Journal of Neuroscience,* **17,** 5196–5205.

Lu, Y., & Wehner, J. M. (1997). Enhancement of contextual fear conditioning by putative (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor modulators and *N*-methyl-D-aspartate (NMDA) receptor antagonists in DBA/2J mice. *Brain Research,* **768,** 197–207.

Lynch, M. A., Clements, M. P., Errington, M. L., & Bliss, T. V. P. (1988). Increased hydrolysis of phosphatidylinositol-4,5-bisphosphate in long-term potentiation. *Neuroscience Letters,* **84,** 291–296.

Lynch, M. A., Voss, K. L., & Gower, A. J. (1994). Impaired spatial memory in aged rats is associated with alterations in inositol phospholipid metabolism. *NeuroReport,* **5,** 1493–1497.

Mackintosh, N. J. (1983). *Conditioning and associative learning*. Oxford Psychology Series No. 3. New York: Oxford Univ. Press.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditioned fear in rats. *Journal of Neuroscience,* **19,** 8696–8703.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural Brain Research,* **88,** 261–274.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996). *N*-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience,* **110,** 1365–1374.

Maren, S., Anagnostaras, S. G., & Fanselow, M. S. (1998). The startled seahorse: Is the hippocampus necessary for contextual fear conditioning? *Trends in Cognitive Sciences,* **2,** 39–42.

Marmiroli, S., Zini, N., Bavelloni, A., Faenza, I., Ognibene, A., & Maraldi, N. M. (1996). Transfected Saos-2 cells overexpressing phosphoinositidase C β_1 isoform accumulate it within the nucleus. *Biology of the Cell,* **86,** 121–126.

Matteucci, A., Faenza, I., Gilmour, R. S., Manzoli, L., Billi, A. M., Peruzzi, D., Bavelloni, A., Rhee, S.-G., & Cocco, L. (1998). Nuclear but not cytoplasmic phospholipase C β_1 inhibits differentiation of erythroleukemia cells. *Cancer Research,* **58,** 5057–5060.

McEchron, M. D., Bouwmeester, H., Tseng, W., Weiss, C., & Disterhoft, J. F. (1998). Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus,* **8,** 638–646.

McGahon, B., & Lynch, M. A. (1998). Analysis of the interaction between arachidonic acid and metabotropic glutamate receptor activation reveals that phospholipase C acts as a coincidence detector in the expression of long-term potentiation in the rat dentate gyrus. *Hippocampus,* **8,** 48–56.

McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in-vitro. *Nature,* **390,** 607–611.

McNish, K. A., Gewirtz, J. C., & Davis, M. (1997). Evidence of contextual fear after lesions of the hippocampus: A disruption of freezing but not fear-potentiated startle. *The Journal of Neuroscience,* **17,** 9353–9360.

McNish, K. A., Gewirtz, J. C., & Davis, M. (2000). Disruption of contextual freezing, but not contextual blocking of fear-potentiated startle, after lesions of the dorsal hippocampus. *Behavioral Neuroscience,* **114,** 64–76.

Melia, K. R., Falls, W. A., & Davis, M. (1992). Involvement of pertussis toxin sensitive G-proteins in conditioned fear-potentiated startle: Possible involvement of the amygdala. *Brain Research,* **584,** 141–148.

Mochly-Rosen, D. (1995). Localization of protein kinases by anchoring proteins: A theme in signal transduction. *Science,* **268,** 247–251.

Morgan, M. A., & LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral Neuroscience,* **109,** 681–688.

Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial frontal cortex. *Neuroscience Letters,* **163,** 109–113.

Morrow, B. A., Elsworth, J. D., Inglis, F. M., & Roth, R. H. (1999). An antisense oligonucleotide reverses the footshock-induced expression of Fos in the rat medial prefrontal cortex and the subsequent expression of conditioned fear-induced immobility. *The Journal of Neuroscience,* **19,** 5666–5673.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience,* **111,** 683–691.

Nicolle, M. M., Colombo, P. J., Gallagher, M., & McKinney, M. (1999). Metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in spatial learning-impaired aged rats. *The Journal of Neuroscience,* **19,** 9604–9610.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience,* **106,** 274–285.

Piiper, A., Stryjek-Kaminska, D., Klengel, R., & Zeuzem, S. (1997). Epidermal growth factor inhibits bombesin-induced activation of phospholipase C-β_1 in rat pancreatic acinar cells. *Gastroenterology,* **113,** 1747–1755.

Pugh, C. R., Fleshner, M., & Rudy, J. W. (1997). Type II glucocorticoid receptor antagonists impair contextual but not auditory-cue fear conditioning in juvenile rats. *Neurobiology of Learning and Memory,* **67,** 75–79.

Pugh, C. R., Tremblay, D., Fleshner, M., & Rudy, J. W. (1997). A selective role for corticosterone in contextual-fear conditioning. *Behavioral Neuroscience,* **111,** 503–511.

Queen, S. A., Sanchez, C. F., Lopez, S. R., Paxton, L. L., & Savage, D. D. (1993). Dose- and age-dependent effects of prenatal ethanol exposure on hippocampal metabotropic-glutamate receptor-stimulated phosphoinositide hydrolysis. *Alcoholism: Clinical and Experimental Research,* **17,** 887–893.

Radulovic, J., Kammermeier, J., & Spiess, J. (1998). Relationship between fos production and classical fear conditioning: Effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *The Journal of Neuroscience,* **18,** 7452–7461.

Razzini, G., Brancaccio, A., Lemmon, M. A., Guarnieri, S., & Falasca, M. (2000). The role of the pleckstrin homology domain in membrane targeting and activation of phospholipase C β_1. *The Journal of Biological Chemistry,* **275,** 14873–14881.

Rescorla, R. A. (1988). Pavlovian conditioning: It's not what you think it is. *American Psychologist,* **43,** 151–160.

Reyes, E., Wolfe, J., & Savage, D. D. (1989). The effects of prenatal alcohol exposure on radial arm maze performance in adult rats. *Physiology & Behavior,* **46,** 45–48.

Rhee, S. G., & Bae, Y. S. (1997). Regulation of Phosphoinositide-specific Phospholipase C Isozymes. *The Journal of Biological Chemistry,* **272,** 15045–15048.

Richmond, M. A., Yee, B. K., Pouzet, B., Veenman, L., Rawlins, J. N., Feldon, J., & Bannerman, D. M. (1999). Dissociating context and space within the hippocampus: Effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. *Behavioral Neurosciences,* **113,** 1189–1203.

Rogan, M. T., Stäubli, U. V., & LeDoux, J. E. (1997). AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. *The Journal of Neuroscience,* **17,** 5928–5935.

Ross, C. A., MacCumber, M. W., Glatt, C. E., & Snyder, S. H. (1989). Brain phospholipase C isozymes: Differential mRNA localizations by in situ hybridization. *Proceedings of the National Academy of Sciences USA,* **86,** 2923–2927.

Sacchetti, B., Lorenzini, C. A., Baldi, E., Tassoni, G., & Bucherelli, C. (1999). Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *The Journal of Neuroscience,* **19,** 9570–9578.

Schafe, G. E., Nadel, N. V., Sullivan, G. M., Harris, A., & LeDoux, J. E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA and MAP kinase. *Learning & Memory,* **6,** 97–110.

Schoepp, D. D., Salhoff, C. R., Wright, R. A., Johnson, B. G., Burnett, J. P., Mayne, N. G., Belagaje, R., Wu, S., & Monn, J. A. (1996). The novel metabotropic receptor agonist 2*R*,4*R*-APDC potentiates stimulation of phosphoinositide hydrolysis in the rat hippocampus by 3,5-dihydroxyphenylglycine: Evidence for a synergistic interaction between group 1 and group 2 receptors. *Neuropharmacology,* **35,** 1661–1672.

Selcher, J. C., Atkins, C. M., Trzaskos, J. M., Paylor, R., & Sweatt, J. D. (1999). A necessity for MAP kinase activation in mammalian spatial learning. *Learning & Memory,* **6,** 478–490.

Silva, A. J., Rosahl, T. W., Chapman, P. F., Marowitz, Z., Friedman, E., Frankland, P. W., Cestari, V., Cioffi, D., Südhof, T. C., & Bourtchuladze, R. (1996). Impaired learning in mice with abnormal short-lived plasticity. *Current Biology,* **6,** 1509–1518.

Stiedl, O., Palve, M., Radulovic, J., Birkenfeld, K., & Spiess, J. (1999). Differential impairment of auditory and contextual fear conditioning by protein synthesis inhibition in C57BL/6N mice. *Behavioral Neuroscience,* **113,** 496–506.

Sutherland, R. J., McDonald, R. J., & Savage, D. D. (1997). Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. *Hippocampus,* **7,** 232–238.

Sutherland, R. J., & Rudy, J. W. (1989). Configural association theory: The role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology,* **17,** 129–144.

Tandon, P., Mundy, W. R., Ali, S. F., Nanry, K., Rogers, B. C., & Tilson, H. A. (1991). Age-dependent changes in receptor-stimulated phosphoinositide turnover in the rat hippocampus. *Pharmacology Biochemistry and Behavior,* **38,** 861–867.

Undie, A. S., Wang, H.-Y., & Friedman, E. (1995). Decreased phospholipase C-β immunoreactivity, phosphoinositide metabolism, and protein kinase C activation in senescent F-344 rat brain. *Neurobiology of Aging,* **16,** 19–28.

Watanabe, M., Nakamura, M., Sato, K., Kano, M., Simon, M. I., & Inoue, Y. (1998). Patterns of expression for the mRNA corresponding to the four isoforms of phospholipase Cβ in mouse brain. *European Journal of Neuroscience,* **10,** 2016–2025.

Westergren, S., Rydenhag, B., Bassen, M., Archer, T., & Conradi, N. G. (1996). Effects of prenatal alcohol exposure on activity and learning in Sprague-Dawley rats. *Pharmacology, Biochemistry and Behavior,* **55,** 515–520.

Woolf, N. J., Young, S. L., Johnson, G. V. W., & Fanselow, M. S. (1994). Pavlovian conditioning alters cortical microtubule-associated protein-2. *NeuroReport,* **5,** 1045–1048.

Yin, J. C.-P. (1999). Location, location, location: The many addresses of memory formation. *Proceedings of the National Academy of Sciences USA,* **96,** 9985–9986.

Young, E. A., Owen, E. H., Meiri, K. F., & Wehner, J. M. (2000). Alterations in hippocampal GAP-43 phosphorylation and protein level following contextual fear conditioning. *Brain Research,* **860,** 95–103.

Zimmerberg, B., Mattson, S., & Riley, E. P. (1989). Impaired alternation test performance in adult rats following prenatal alcohol exposure. *Pharmacology Biochemistry and Behavior,* **32,** 293–299.

Zini, N., Mazzoni, M., Neri, L. M., Bavelloni, A., Marmiroli, S., Capitani, S., & Maraldi, N. M. (1994). Immunocytochemical detection of the specific association of different PIC isoforms with cytoskeletal and nuclear matrix compartments in PC12 Cells. *European Journal of Cell Biology,* **65,** 206–213.

Zini N., Sabatelli P., Faenza I., Ognibene A., & Maraldi, N. M. (1996). Interleukin-1α induces variations of the intranuclear amount of phosphatidylinositol 4,5-bisphosphate and phospholipase C β_1 in human osteosarcoma Saos-2 cells. *Histochemical Journal,* **28,** 495–504.