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Disruptions in Serotonergic Regulation of Cortical Glutamate Release in Primate Insular Cortex in Response to Chronic Ethanol and Nursery Rearing

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Abstract

Early-life stress has been shown to increase susceptibility to anxiety and substance abuse. Disrupted activity within the anterior insular cortex (AIC) has been shown to play a role in both of these disorders. Altered serotonergic processing is implicated in controlling the activity levels of the associated cognitive networks. We therefore investigated changes in both serotonin receptor expression and glutamatergic synaptic activity in the AIC of alcohol-drinking rhesus monkeys. We studied tissues from male rhesus monkeys raised under two conditions: Male rhesus monkeys 1) “Mother reared” (MR) by adult females (n=9), or; 2) “Nursery reared” (NR), i.e., separated from their mothers and reared as a separate group under surrogate/peer-reared conditions (n=9). The NR condition represents a long-standing and well-validated nonhuman primate model of early life stress. All monkeys were trained to self-administer ethanol (4% w/v) or an isocaloric maltose-dextrin control solution. Subsets from each rearing condition were then given daily access to either ethanol, water or maltose dextrin for 12 months. Tissues were collected at necropsy and were further analyzed. Using real time RT-PCR we found that ethanol-naïve, NR monkeys had lower AIC levels of 5-HT_{1A} and 5-HT_{2A} receptor mRNA compared to ethanol-naïve, MR animals. While NR monkeys consumed more ethanol over the 12-month period compared to MR animals, both MR and NR animals expressed greater 5-HT_{1A} and 5-HT_{2A} receptor mRNA levels following chronic alcohol self-administration. The interaction between nursery-rearing conditions and alcohol consumption resulted in a significant enhancement of both 5-HT_{1A} and 5-HT_{2A} receptor mRNA levels such that lower expression levels observed in nursery rearing conditions were not found in the alcohol self-administration group. Using voltage clamp recordings in the whole cell configuration we recorded excitatory postsynaptic currents in both ethanol-naïve and chronic self administration groups of NR and MR monkeys. Both groups that self-administered ethanol showed greater glutamatergic activity within the AIC. This AIC hyperactivity in MR, alcohol-consuming monkeys was accompanied by an increased sensitivity to regulation by presynaptic 5-HT_{1A} receptors that was not apparent in the ethanol-naïve, MR group. Our data indicate that chronic alcohol consumption leads to greater AIC activity, and may indicate a compensatory upregulation of presynaptic 5-HT_{1A} receptors. Our results also indicate that AIC activity may be less effectively regulated by 5-HT in ethanol-naïve NR animals than in NR monkeys in response to chronic ethanol self-administration. These data suggest possible mechanisms for increased alcohol seeking and possible addiction potential among young adults who had previously experienced early-life stress that include disruptions in both AIC activity and serotonin system dynamics.

Keywords

anterior insula; ethanol; serotonin; stress; 5-HT_{1A}

Introduction

The anterior insular cortex (AIC) has recently become an area of interest in both anxiety (Paulus and Stein, 2006) and addiction (Goldstein et al., 2009; Naqvi and Bechara, 2009)

because of its unique role as both an integrator and evaluator of internal, homeostatic information from the external environment (Craig 2002; Goldstein et al., 2009; Paulus and Stein, 2006). Insular hyperactivity has been shown in anxiety-prone individuals, patients with general social phobias (Lorberbaum et al., 2004; Simmons et al., 2006; Stein et al., 2007) and in anticipatory anxiety (Chua et al., 1999; Nitschke et al., 2006). Smokers with brain damage involving the insula are reportedly more likely to quit smoking (Naqvi et al., 2007), and neuroimaging studies have shown insular activation in response to cravings for cigarettes, food and cocaine (Bonson et al., 2002; Pelchat et al., 2004; Wang et al., 2007). Additionally, a study by Contreras et al. (2007) suggested that drug-craving in rats was eliminated by focal inactivation of the insula as assessed by condition place preference paradigm. These findings provide evidence for the important role of the AIC in networks determining both anxiety and addiction potential.

Disruptions of the serotonin system have also been implicated in both anxiety and addiction disorders. For example, studies have demonstrated reduced 5-HT_{1A} receptor binding in the insula of patients with social anxiety disorder (Lanzenberger et al., 2007) and depression (Bhagwagar et al. 2004; Sargent et al. 2000). Furthermore, rhesus monkeys exposed to early-life stress have been reported to have reduced 5-HT_{1A} receptor binding throughout the majority of the brain (Spinelli et al., 2010), and monkeys exposed to this rearing condition exhibit behavioral and chemical indications of high anxiety, including increased frequency of serious aggression, decreased complexity of social behavior, lower social rank achieved, increased alcohol consumption and increased adrenocorticotropin releasing hormone (ACTH) and cortisol levels in response to acute stress (Barr et al., 2009; Higley et al., 1991, 1996a,b, Suomi et al., 1997). Similarly, decreased 5-HT_{2A} receptor binding in several cortical structures, including the insula, has been shown in patients with obsessive-compulsive disorder (OCD) (Perani et al. 2008). Based on the involvement of the 5-HT system in behavioral abnormalities observed in animals exposed to early life stress and the involvement in psychopathology, as evidenced by specific serotonin reuptake inhibitors (SSRIs) and other 5-HT-acting agents being the most widely prescribed psychotropic medications (Golden, 2004), we chose to investigate the 5-HT system in our primate model. We investigated 5-HT_{1A}, 2A, 2B and 2C in cortical brain tissue. Of these receptors, 5-HT_{1A} and 5-HT_{2A} are most heavily expressed in cortex. The 5-HT_{1A} receptor is expressed on presynaptic terminals where it regulates glutamate and 5-HT release and is also active postsynaptically, whereby it hyperpolarizes pyramidal neurons. By contrast, 5-HT_{2B} receptors induce a net depolarization of pyramidal neurons (for review, see Andrade, 2011).

It has also been suggested that disruptions of the serotonergic system may be involved in the development of alcoholism (Johnson, 2004; Verheul et al., 1999). Serotonin receptor expression has been shown to be altered in animals and humans exposed to chronic ethanol, as well as in humans with mood disorders (Heinz et al., 2004; Lopez-Figera et al., 2004). Additionally, previous data from our group has demonstrated ethanol-induced alterations in 5-HT_{1A} receptor expression in specific regions of the hippocampus in both cynomolgus and rhesus macaques (Davenport et al., 2005, 2007). Indeed, SSRIs have been shown to decrease spontaneous ethanol consumption in rodents (Kelai et al., 2003; Maurel et al. 1999). More specifically, stimulation of 5-HT_{1A} receptors decreases ethanol intake (McKenzie-Quirk and Miczek, 2003; Kelai et al., 2006), and chronic ethanol consumption has been shown to alter

5-HT_{1A} receptor expression in the brain as well as increase receptor sensitization (Kelai et al., 2008; Nevo et al., 1995; Storvik et al., 2009). These findings suggest that altered serotonin function may be a common factor contributing to both mood disorders and alcohol addiction potential (Lesch, 2005). One mechanism by which serotonin regulates cortical function is through modulation of glutamatergic signaling, suggesting that serotonergic regulation of cortical hyperexcitability could be involved in mechanisms of mood disorders and alcohol addiction potential. We therefore focused on AIC as an important region to examine the regulation of neural excitability by serotonin and how infant rearing conditions and chronic drinking may affect serotonergic function in insular cortex.

We used nursery-reared nonhuman primates as a model to determine possible mechanisms by which early-life stress might lead to later alcohol drinking behavior. Nursery-rearing in nonhuman primates has been shown to result in behavioral and neurochemical changes later in life consistent with affective disorders, and these animals have been reported to consume greater quantities of ethanol relative to MR animals (Harlow et al., 1959; Harlow et al., 1965; Harlow & Suomi, 1974; Seay & Harlow, 1965; Suomi, 1997; Shannon et al., 1998; Sanchez et al., 2001; Machado & Bachevalier, 2003; Cirulli et al., 2009; Higley et al., 1991). Consistent with this, a parallel study using the mother-reared (MR) and nursery-reared (NR) ethanol-naïve monkeys that were also used in the current study reported altered socioaffective behavior in NR monkeys compared to MR monkeys (Corcoran et al., 2011). This model thus provides a unique resource to understand neurobiological changes similar to those that may occur in human children exposed to early-life stress and how these changes may contribute to the development of addictive behaviors later in life.

Using this model, we hypothesized that chronic ethanol exposure and nursery-rearing may disrupt serotonin receptor expression and function in AIC. To test this hypothesis, we assessed serotonin receptor gene expression as well as GAPDH and actin gene expression by measuring mRNA levels in AIC tissue isolated from control and chronic ethanol consuming MR and NR animals. In addition, we hypothesized that chronic self-administration of ethanol would disrupt AIC network activity and that serotonin regulation of excitatory signaling would be significantly altered. We further tested the hypothesis that nursery-rearing or chronic ethanol consumption in adulthood would produce a hyperexcitable state in the prefrontal cortical network in response to reduced serotonergic regulation. We found an interaction between nursery-rearing and chronic alcohol consumption that resulted in a synergistic enhancement of serotonin receptor expression and altered AIC excitability.

Materials and Methods

Experimental subjects

Eighteen male rhesus monkeys housed at the Wake Forest University Primate Center were used in the study. All animals were born at the Laboratory of Comparative Ethology at the National Institute of Child Health and Human Development and transferred to Wake Forest University School of Medicine in two cohorts as adolescents. For testing, the animals were initially acclimated to individual housing (76 × 60 × 70 cm³) for three months before undergoing training for self-administration. Throughout the study period, monkeys were

maintained at a free-feeding weight on a diet of Primate Food pellets (Research Diets Inc., New Brunswick, N.J.) supplemented with fresh produce.

Manipulation of Early Rearing Environment

Each subject experienced one of two early rearing environments: 1) rearing by an adult female in social groups comprised of multiple mother-infant dyads and two adult males (MR group; n=9). These subjects lived from birth to approximately seven months-of-age in social groups housed in indoor-outdoor cages. Over the course of the first seven months the diet of the MR subjects consisted of breast milk and weaning to chow and water. 2) Nursery-reared animals (NR group; n=9) that were separated from their mothers within twenty-four hours of birth, moved to a neonatal nursery, and reared under surrogate-peer-reared conditions using procedures based on those developed at the University of Wisconsin Harlow Primate Laboratory (see Novak & Sackett, 2006 for detailed description and comparison of nursery-rearing conditions; Shannon et al., 1998). Briefly, infants were housed in an incubator containing an inanimate surrogate equipped with a spring to allow rocking motion, covered with a heating pad, and encased in thick fleece. Infants were fed formula (50:50 Similac:Primilac) and weaned to solid foods and water over the course of 6 months. Beginning at 14 days, infants were moved to one quadrant of a cage within a room of the nursery and allowing for visual, auditory, and olfactory contact with other monkeys. At approximately 37 days of age, daily two-hour socialization periods were given in which four infants were united in a play cage. Animals from both rearing groups experienced identical environments following weaning at 6–7 months of age.

Ethanol self-administration

Monkeys were divided into four experimental groups by separating both the MR and NR monkeys into control and ethanol groups. Thus, the four experimental groups included NR controls (n=5), MR controls (n=5), NR ethanol (n=4), and MR ethanol (n=4). All monkeys were trained to operate a drinking panel and consume water under scheduled pellet deliveries (i.e., schedule-induced polydipsia; Falk, 1971) followed by 4% w/v ethanol (ethanol group), as previously described (Vivian et al., 2001; Grant et al., 2008). The daily amount of ethanol increased in a stepwise fashion over three 30-day epochs from 0.5 g/kg to 1.5 g/kg. During this same induction period, the control group self-administered maltose-dextrin under identical conditions. At the conclusion of this induction period the animals were then allowed free access to 4% ethanol (w/v) and water (ethanol group) or isocaloric maltose dextrin and water (control group) for 22 h each day for 12 consecutive months. All of the animals in the first cohort (n=8) had access to ethanol and water, while the second cohort (n=10) had access to a maltose dextrin solution and water. During the 22 h self-administration period, animals were allowed to self-administer a banana pellet diet rationed into three meals per day supplemented with daily fresh produce. The study design was such that all animals began the 22 hr self-administration portion of the paradigm between five and six years of age, which corresponds to young adulthood in rhesus macaques (Lewis, 1997; Watts & Gavan, 1982). Blood samples were obtained for analysis of blood ethanol concentration (BEC) via awake venipuncture of the femoral or saphenous vein every five days throughout the 22-h self-administration portion of the drinking paradigm. Blood samples were collected just before lights out at 6:00PM, approximately six hours following

the onset of the daily session. Samples were stored at -20°C until the end of the study period at which point they were assayed simultaneously. BEC was determined by gas chromatograph (Agilent GC 7890A, Agilent Technologies, Santa Clara, CA).

Both cohorts exhibited a progressive increase in body weight over the entire study period. No significant difference was observed in body weights at the end of the study period between cohorts (data not shown). Monkeys were necropsied immediately following the final 22 h access period. During necropsy, monkeys were perfused through the heart with ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 2 MgSO_4 , 2 CaCl_2 , 23 NaHCO_3 , 3 NaH_2PO_4 , 10 glucose (pH 7.4, osmolarity 290–300 mOsm) oxygenated with 95% O_2 :5% CO_2 . A tissue block containing the AIC was isolated and immediately immersed in oxygenated (95% O_2 :5% CO_2), ice cold sucrose-substituted ACSF containing (in mM): 220 sucrose, 12 MgSO_4 , 10 glucose, 2 KCl, 1.5 NaH_2PO_4 , 26 NaHCO_3 , 0.2 CaCl_2 , (pH 7.4, Osmolarity 290–300 mOsm). Vibratome sections for in vitro electrophysiology were made from the tissue block. In addition, a portion of the AIC tissue block was snap frozen in liquid nitrogen and stored at -80°C until tissue was collected from each animal and gene expression analyses could be performed on all samples simultaneously.

Real-Time RT-PCR

After removal from storage, tissue was immediately homogenized in TriREAGENT (Molecular Research Center, Inc. Cincinnati OH) using a PowerGen 125 Tissue Homogenizer (Fisher Scientific, Hampton, NH). Total RNA was isolated as described in the manufacturer's protocols. Absence of DNA contamination was verified in the purified RNA preparation by performing cDNA synthesis in the absence of reverse transcriptase (–RT control) followed by RT-PCR (see below). As an added precaution, RNA was further purified using Qiagen RNeasy spin columns (Qiagen, Valencia, CA) to remove possible contaminants including genomic DNA. RNA concentration was measured using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE), and quality was assessed by electrophoresis in 1% agarose formaldehyde gels. cDNA was synthesized using the SuperScript III First Strand Synthesis System (Invitrogen, Carlsbad, CA) for real-time PCR with random hexamer primers as per manufacturer's instructions. Real-time PCR was performed using the 5'-exonuclease method (Taqman, Applied Biosystems, Foster City, CA). Real-time PCR was performed on an ABI 7300 thermal cycler (Applied Biosystems) using 2 ng of amplified sample cDNA with Taqman specific gene assays, as described by Taqman Universal PCR Master Mix (Applied Biosystems) protocols. The cycling parameters were as follows: 50°C for 4 minutes followed by a DNA polymerase activation step at 95°C for 10 minutes and a two-temperature PCR of 40 cycles at 95°C for 15 seconds (denaturing step) followed by 55°C for 1 minute (annealing step). Relative expression levels for the 5-HT receptors, GAPDH and actin were quantified according to the C_t method (Livak and Schmittgen, 2001). Each gene/animal was run in triplicate and normalized to actin gene expression. Data are represented as the group mean $\pm$ the SEM. One tissue sample from the MR ethanol group was excluded from analysis due to contamination during the mRNA isolation process.

Electrophysiology

The tissue block was sectioned on a vibratome (model OTS 4000, Electron Microscopy Sciences, Fort Washington, PA) at 400 μm , and slices containing the intermediate and lateral agranular insula areas (Iai and Ial, respectively) and precentral opercular cortex (PrCo) of AIC (Carmichael and Prince, 1994) were maintained in oxygenated, warm (34°C) ACSF of the same composition as the perfusion ACSF, for at least 1.5 hours before being transferred to another chamber for recordings (Harvard Apparatus, Holliston, MA). Slices were recorded using “blind” recording techniques from 400 μm slices, as previously described for recordings from primate tissue (Alexander et al., 2006). For whole cell patch recordings, slices were submerged in oxygenated (95% O_2 , 5% CO_2) ACSF at room temperature. Oxygenated ACSF was perfused at a flow rate of 2 ml per minute. Patch pipettes (5–10M Ω) were pulled from borosilicate glass (Sutter Instruments, Novato CA) with a PC-10 vertical puller (Narishige International USA, Inc., East Meadow, NY) and were filled with an internal solution containing the following (in mM): 100 Gluconic acid, 100 CsOH, 10 NaCl, 10 HEPES, 20 TEA-Cl, 1 EGTA, 4 ATP, (pH 7.3 with 2N CsOH, Osmolarity 270–290 mOsm). The calculated liquid junction potential was +7mV with this internal solution, and membrane potential measurements were corrected for the junction potential post hoc. Resting membrane potential measurements were made 1–2 min following establishment of whole-cell configuration. Cellular activity was acquired with an AxoClamp 2B amplifier (Axon Instruments, Union City, CA), digitized with a Digidata 1322 (Axon Instruments), and analyzed using pCLAMP 9.0 software (Axon Instruments). To acquire cells for voltage clamp, patch pipettes were advanced “blind” through tissue in bridge mode until encountering a cell, a >1 G Ω seal was formed, the membrane ruptured to allow whole cell access, and the amplifier was then switched to cSEVC mode. Pharmacological agents used in electrophysiology included picrotoxin (Sigma-RBI, St. Louis, MO, USA), DL-2-Amino-5-phosphonopentanoic acid (APV; Sigma-RBI), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; Tocris Bioscience, Ellisville, MO, USA), and ($\pm$)-8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT; Tocris Bioscience).

Statistical Analyses

A two-way ANOVA was used to assess differences in ethanol consumption behavior between MR and NR animals, with months of drinking and rearing group as factors. Unpaired *t* tests were used to assess differences in gene expression within each group, and a two-way ANOVA was used to test for interactions between rearing condition and ethanol exposure on gene expression levels. Unpaired *t* tests were used to compare physiological measures between groups of animals, and paired *t* tests or Wilcoxon rank tests were used to compare responses to agonist administration within groups. All consumption analyses were performed using SAS 9.1 (SAS Institute; Cary, NC). The remaining analyses were performed using Prism 5 (GraphPad Software; La Jolla, CA) Data were considered significant if $p < 0.05$.

Results

Alcohol self-administration

A two-way ANOVA revealed a main effect of rearing condition on ethanol intake. NR monkeys consumed significantly more ethanol than MR monkeys ($F(1,6)=54.51$, $p<0.01$; Fig. 1A). The average daily intake for MR monkeys was 2.2 ± 0.03 g/kg, and the average daily intake for NR monkeys was 2.9 ± 0.03 g/kg. These intake values surpass the NIAAA definition of a heavy drinker (>4 drinks/day or >14 drinks/week where 0.25 g/kg equals 1 drink) and thus, these animals should be considered an appropriate model of human heavy drinking. Further analyses also uncovered a significant effect of time ($F(365,2179)=5.47$, $p<0.0001$) and an interaction between rearing and time ($F(365,2179)=1.29$, $p<0.01$).

For BEC analyses, blood samples were collected just before lights out at 6:00PM to allow for as much day time drinking as possible with the aim being to obtain BEC values reflective of the maximum intake for a particular day. Whereas some animals drank the bulk of their daily intake during the day, as has been the case in previous cohorts, in this cohort, the two heaviest drinkers often consumed more than half their ethanol after 6:00 PM, drinking throughout the night and into the following morning.

That said, the BEC data showed that MR animals had significantly higher average BECs during the 22 hr self-administration period than NR animals ($p<0.001$) despite greater daily intake in the NR group. Using the data acquired from the 6:00 PM samples, MR monkeys had an average BEC of 69 mg/dL, ranging from 0–196 mg/dL while NR monkeys obtained an average BEC of 49 mg/dL, ranging from 0–170 mg/dL. Data acquired from a five-hour metabolism study in which all animals were given a standard dose of 1.0g/kg ethanol showed that both ethanol naïve and ethanol experienced MR and NR animals attained similar peak BECs (~ 100 mg/dL) but that the NR monkeys metabolized ethanol at a higher rate, thus clearing it more quickly regardless of ethanol exposure history ($p=0.02$). A manuscript describing this finding is in preparation. The higher rate of metabolism and large amount of drinking after 6:00 PM by the heaviest drinkers are both consistent with the lower BECs seen in the 6:00 PM samples. It is also worth noting that although the correlation between drinking amounts and BECs, while highly statistically significant ($p<0.0001$), revealed an r^2 of only 0.67 (Fig 1B), indicating that factors other than total daily intake *per se* influenced BECs taken at 6:00 PM.

For the remainder of this study, we examined cellular and molecular properties of AIC tissues isolated from these animals. However, for the gene expression studies, tissue from one MR monkey was not collected. The rearing effect on alcohol intake remained, however, despite the removal of this animal from the drinking data analysis ($n=3$ MR, $n=4$ NR) ($F(1,5)=31.83$, $p<0.01$; Fig. 1A).

Chronic ethanol self-administration alters serotonin receptor gene expression

We found that chronic ethanol self-administration and nursery-rearing differentially regulated mRNA levels of serotonin receptors and GAPDH in AIC tissues (Fig. 2). Because levels of GAPDH mRNA, which are typically used to normalize mRNA levels, differed significantly among groups we normalized serotonin receptor mRNA levels to that of actin,

an endogenous control that has been shown to be a stable reference gene in comparing human prefrontal brain tissues of chronic alcoholics and controls (Johansson et al., 2007). Actin mRNA expression levels showed no significant differences among the four groups tested. To probe for a main effect of ethanol exposure on gene expression, we compared relative mRNA expression levels between animals self-administering ethanol (n=3 MR monkeys and 4 NR monkeys) and ethanol-naïve controls (n=3 MR monkeys and 4 NR monkeys) with rearing conditions grouped. We found that chronic ethanol-consuming animals had significantly greater mRNA levels of 5-HT_{1A} ($p<0.05$, unpaired t test) and 5-HT_{2A} ($p<0.05$, unpaired t test). In contrast, mRNA levels of 5-HT_{1B} and 5-HT_{1D} did not differ between animals that chronically self-administered alcohol and controls not exposed to alcohol. Thus, ethanol exposure produced a main effect on serotonin receptors.

We then investigated whether or not rearing conditions affected gene expression in monkeys exposed to ethanol. To that end, we divided the monkeys into four groups based on ethanol-exposure and rearing condition and assessed relative change in gene expression for one variable at a time. We examined the effect of ethanol-exposure on each of the MR and NR groups (Fig. 2A), then we examined the effect of rearing for each of ethanol-naïve and chronic ethanol self-administration groups (Fig. 2B). The control groups for each of these comparisons were either ethanol-naïve or MR animals, so changes in gene expression were assessed relative to their appropriate control group for all comparisons. In comparing the effect of ethanol exposure, we found that MR animals chronically exposed to ethanol had significantly greater expression of GAPDH, 5-HT_{1A}, and 5-HT_{2A} mRNA (n=3, $p<0.05$, unpaired t test) (Fig. 2A). For the NR group, ethanol monkeys had significantly greater levels of GAPDH, 5-HT_{1A} and 5-HT_{2A} mRNA than ethanol-naïve animals (n=4, $p<0.05$, unpaired t test) (Fig. 2A). Furthermore, using raw C_t values we found a significant interaction between ethanol exposure and rearing condition, suggesting that chronic ethanol self-administration had a significantly greater impact on the transcriptional upregulation of 5-HT_{1A} ($F(1,10)=4.93$, $p<0.05$; two-way ANOVA) and 5-HT_{2A} ($F(1,10)=5.92$, $p<0.05$; two-way ANOVA) mRNA in the NR group. This enhancement in NR animals was not due to higher levels of ethanol consumption since no statistical correlation between gene expression and average daily ethanol intake was observed (data not shown).

Nursery-rearing alone alters serotonin receptor gene expression

In comparing the effect of rearing condition, we found that NR monkeys showed significantly lower expression levels of 5-HT_{1A} and 5-HT_{2A} mRNA among ethanol-naïve animals ($p<0.05$, unpaired t test) (Fig. 2B). However, among animals chronically self-administered ethanol, these differences between MR and NR monkeys were not apparent ($p>0.05$) (Fig. 2B).

Synaptic properties of AIC neurons

Altered activation of certain brain circuits is likely to occur following either chronic ethanol exposure or early-life stress. We hypothesized that the alterations in gene expression we observed may either contribute to, or be homeostatically regulated in response to, increased neuronal excitability in AIC neurons. To begin testing this hypothesis, we initially sought to characterize the synaptic and membrane properties of neurons in *Iai*, *Ial* and *PrCo* of AIC

(Carmichael and Price, 1994) because these properties of monkey AIC neurons are unknown. We therefore performed whole-cell recordings from deep layer neurons and placed bipolar stimulating electrodes in both the white matter adjacent to the recording electrode and more superficial mantle of the adjacent cortex.

In the presence of the GABA_A receptor blocker picrotoxin (100 μ M), we examined the composition of the white matter evoked excitatory postsynaptic currents (EPSCs). We found that bath application of the NMDA receptor blocker DL-APV (100 μ M) produced an EPSC with faster decay kinetics than the baseline EPSC (i.e., sharper), and subsequent bath application of the AMPA/kainate receptor inhibitor NBQX (1 μ M) abolished the remaining synaptic response. These findings indicate that the glutamatergic receptors that contribute to the EPSC within the monkey AIC include NMDA and AMPA/kainate receptors (Fig 3A). In a portion of the recordings (6 cells of 21 studied from MR control animals), in addition to the short-latency synaptic response, a second, polysynaptic response was elicited with increased stimulus intensity (>350 μ A). This response had a longer latency to onset with a peak amplitude approximately 100–150 msec following stimulation (Fig 3Bi). This longer-latency response likely arose from activation of intracortical circuitry because direct cortical stimulation elicited a polysynaptic response of similar profile but with a peak response amplitude occurring approximately 20 msec earlier than the peak response amplitude elicited by white matter stimulation (Fig 3Bii). Furthermore, synaptic stimulation sufficient to elicit the putative polysynaptic response was followed by an inward current that persisted for >1500 msec (Fig 3Bi). This response may be due to activation of metabotropic glutamate receptors because inward currents of a similar duration have been described in other brain regions in both monkeys and rodents (Anwyl, 1999), and mGluR1 has been shown to be expressed on dendritic spines of principal cells within monkey prefrontal cortex (Muly et al., 2003).

We then examined short term synaptic plasticity of intermediate layer cells upon stimulation of the white matter tract. We found that paired stimuli did not consistently result in paired-pulse depression or facilitation, yielding a mean paired-pulse ratio (PPR) of 0.99 ± 0.06 , ($n=12$ cells) with an interstimulus interval of 100 msec and a stimulus intensity of that which produced ~50% of the maximal EPSC amplitude (Table 1). However, synaptic responses showed depression upon delivery of a train of ten synaptic stimuli at 10 Hz ($n=6$ cells) (Fig 3C), supporting the conclusion that this synapse shows synaptic depression.

Chronic alcohol self-administration and nursery rearing alter spontaneous excitatory synaptic activity

We next compared membrane and synaptic properties of AIC neurons from the four groups of animals (Table 1). AIC neurons from animals exposed to chronic ethanol had a significantly more depolarized mean membrane potential relative to neurons from control monkeys ($p<0.01$, unpaired t test). Furthermore, this depolarized membrane potential was associated with a significant reduction in membrane resistance in the ethanol-exposed animals ($p<0.05$, unpaired t test). We then compared evoked synaptic responses. Comparison of the EPSC decay time yielded no significant difference between controls and ethanol-exposed monkeys. However, in comparing paired synaptic stimulations with an interstimulus

interval of 100 msec, we found that ethanol-exposed monkeys showed a significantly reduced PPR. In other words, while control animals showed no facilitation or depression upon paired stimulation, monkeys that chronically self-administered ethanol showed paired-pulse depression, reflecting an increased probability of synaptic glutamate release (Zucker and Regehr, 2002). Furthermore, electrical stimulation of either the white matter or intracortical areas elicited delayed synaptic barrages, defined as those synaptic responses with a peak amplitude occurring 100 – 150 msec after stimulation, in significantly more cells from animals exposed to chronic ethanol (92%, or 23 of 25 cells studied) than control animals (29%, or 6 of 21 cells studied), consistent with increased excitability of AIC circuits following chronic ethanol consumption.

AIC neurons displayed a considerable degree of spontaneous excitatory synaptic activity. We quantified the spontaneous EPSC (sEPSC) amplitude, frequency and decay kinetics in the presence of APV and picrotoxin in AIC neurons from control and ethanol-exposed animals. We found no difference in sEPSC decay time or amplitude. However, we did find significantly lower sEPSC interevent intervals (IEIs) among ethanol-exposed animals, indicating a higher frequency of recorded sEPSCs. Dividing the control and ethanol-exposed groups into MR and NR yielded a significant effect of rearing among ethanol-naïve animals and a significant effect of ethanol-exposure among MR animals (Fig. 4, Table 1). Specifically, we found a significantly greater sEPSC frequency (reduced IEI) in AIC neurons from MR monkeys that chronically self-administered ethanol as compared to MR control monkeys (Fig. 4, $p < 0.05$, unpaired t test). In addition, we found significantly lower sEPSC IEIs among NR control animals compared with MR control animals, indicating NR monkeys exhibit a higher frequency of spontaneous AIC activity (Fig. 4, $p < 0.05$, unpaired t test).

Serotonin differentially modulates AIC glutamatergic synaptic activity in animals that chronically consumed ethanol

Guided by our findings of differential expression of AIC 5-HT_{1A} and 5-HT_{2A} receptor mRNA among animals exposed to various rearing conditions and alcohol exposure, we hypothesized that AIC neurons from each of these groups of animals would show varying responses to agonists for these receptors. Serotonin, released from axon terminals arising from the raphe nuclei, has been shown to affect transmission of several neurotransmitters through heterosynaptic activation of serotonin receptors on neighboring axon terminals (Fink and Gothert, 2007). For example, heterosynaptic activation of serotonin receptors on presynaptic glutamatergic neurons has been shown to modulate glutamate release (Calcagno et al., 2006; Golembiowska and Dziubina, 2002; Maura et al., 1998). We therefore performed whole-cell recordings from AIC neurons and examined both white-matter stimulation evoked EPSCs and sEPSCs before and in the presence of bath applied 8-OH-DPAT, a 5-HT_{1A} receptor agonist. Due to the limited number of animals available, we focused our study on MR animals and 5-HT_{1A} receptors. We isolated acute slices from animals that chronically self-administered ethanol and from ethanol-naïve animals and assessed the differential response magnitude of AIC neurons to 8-OH-DPAT bath application.

We found that bath application of 8-OH-DPAT (2 μ M) reduced the amplitude of white-matter that self-administered ethanol ($51.3 \pm 5.9\%$ of baseline EPSC amplitude, $n=8$; Fig. 5B). However, the magnitude of reduction of the EPSC amplitude was significantly greater for animals exposed to chronic ethanol than for control animals ($p<0.05$, unpaired t test). Paralleling the increased magnitude of EPSC inhibition by 8-OH-DPAT in ethanol-exposed animals, we also found a significant increase in the PPR (baseline= 0.82 ± 0.09 ; 8-OH-DPAT= 1.00 ± 0.14 ; $p<0.05$, Wilcoxon rank test), indicating that a portion of this inhibition was through a presynaptic site of action. By contrast, neurons from control animals, which showed only a moderate decrease in EPSC amplitude in response to 8-OH-DPAT, also failed to show a significant change in PPR (baseline= 0.97 ± 0.25 ; 8-OH-DPAT= 0.95 ± 0.32 ; $p>0.05$) (Fig. 5C), suggesting that the reduction in EPSC amplitude was independent of activation of presynaptic 5-HT_{1A} receptors. These findings indicate that 5-HT_{1A} receptors exerted their effects through heterosynaptic mechanisms that reduced presynaptic glutamatergic excitatory transmission in ethanol-naïve monkeys. Taken together, these findings suggest that animals exposed to chronic ethanol have increased presynaptic 5-HT_{1A} receptor function, possibly mediated by increased receptor expression in AIC.

Discussion

In this study, we examined the effects of chronic ethanol self-administration on serotonergic modulation of glutamate transmission in the AIC of MR and NR rhesus monkeys to identify possible mechanisms underlying the neural adaptations resulting from early-life stress and how they relate to later drinking behavior. The major results of this study are: (1) NR monkeys self-administered more ethanol over the course of the study period than MR monkeys; (2) nursery-rearing alone resulted in a significantly higher sEPSC frequency in AIC neurons, and (3) lower 5-HT_{1A} and 5-HT_{2A} mRNA levels in the AIC; (4) chronic ethanol and nursery-rearing together produced a significant enhancement of serotonergic receptor mRNA expression; and (5) chronic ethanol significantly enhanced sEPSC frequency in AIC neurons, resulting in a more depolarized resting membrane potential. Chronic ethanol also increased the level of 5-HT_{1A} mRNA, which may have produced an upregulation of presynaptic 5-HT_{1A} heteroreceptors (Fig. 6). Based on the role of AIC in motivation and decision-making, our findings are consistent with the hypothesis that AIC hyperactivity produced by early-life stress may play a role in the reorganization of networks associated with anxiety the alcohol use disorders. Based on our findings, we propose a model of AIC synaptic function altered by early-life stress and alcohol exposure whereby hyperexcitable network states are promoted, at least in part, due to modified serotonergic receptor expression and function, causing altered AIC excitability (Fig. 6).

AIC alterations due to chronic ethanol self-administration

Acute ethanol exposure has been shown to decrease miniature EPSCs (mEPSCs) and persistent spiking activity of rodent cortical neurons (Moriguchi et al., 2007; Tu et al., 2007; Woodward and Pava, 2009), but chronic ethanol exposure has been shown to increase sEPSC and mEPSC frequency in hippocampal and amygdalar neurons (Hendricson et al. 2007; Lack et al., 2007; Roberto et al., 2004). Here, we report an increase in presynaptic glutamate release in ethanol-exposed monkeys, as evidenced by: 1) an increased sEPSC frequency in

AIC neurons, 2) a greater ability to evoke large, polysynaptic barrages in postsynaptic neurons from animals chronically-exposed to ethanol, and 3) a significant increase in evoked paired-pulse depression. While no studies have investigated PPRs in cortical neurons following chronic ethanol exposure, it has been shown that human alcoholics exhibit an increase in synaptophysin I in prefrontal tissue, consistent with enhanced synaptic strength (Henriksson et al., 2008). Based on the short latency to evoked excitatory postsynaptic potentials (EPSPs) following stimulation of the white matter tract adjacent to the recording pipette in AIC, the EPSPs likely arose from afferent glutamatergic projections to AIC, which include the extended amygdala and entorhinal cortex, among other areas (Turner et al., 1980; Insausti et al., 1987; Augustine, 1996; Reynolds and Zahm, 2005). Therefore, our findings are consistent with a model in which chronic ethanol exposure produces either an increase in the excitability of the afferent neurons to AIC or an increase in glutamate release at the axon terminal of these afferent neurons. Because our paired pulse results are derived from white matter stimulation, they support the latter interpretation (Fig. 6). Furthermore, we found that neurons from animals exposed to chronic ethanol had significantly depolarized resting membrane potentials and decreased membrane resistance, both of which would contribute to increased excitability.

Serotonin control of presynaptic release (i.e., 5-HT_{1A} receptor mediated heterosynaptic reduction of axonal excitability and/or synaptic glutamate release) in AIC, which appears to be enhanced after chronic ethanol consumption, may represent a strategy by which the brain compensates for the ethanol-induced increases in synaptic strength and neuronal excitability. While this suggestion is a parsimonious conclusion based on our observations, further investigation will be needed to determine if the altered serotonergic modulation of glutamate release is a compensatory central nervous system (CNS) response or a direct result of chronic ethanol exposure. A parallel study using brain tissue from the same monkeys revealed no difference in tissue content of 5-HT or a metabolite of 5-HT, 5-HTIAA; therefore, the effect of ethanol intake on the 5-HT system is likely at the receptor level and not in the levels of available ligand (Huggins et al., 2011).

Activation of 5-HT_{1A} receptors has been shown to reduce excitatory synaptic responses in several brain areas by presynaptic mechanisms (Schmitz et al., 1995; Cheng et al., 1998; Schmitz et al., 1998; Schmitz et al., 1999; Murakoshi et al., 2001; Torres-Escalante et al., 2004). Here, we have demonstrated similar presynaptic inhibition by 5-HT_{1A} receptors in a monkey model. In slices from ethanol-naïve animals, we found a modest reduction in evoked EPSC amplitude and no significant change in PPR in response to 8-OH-DPAT application. In contrast, in slices from animals that chronically self-administered ethanol, we found decreased EPSC amplitude and a significant increase in PPR. The lack of significant change in PPR in the ethanol-naïve monkeys is consistent with decreased expression or function of 5-HT_{1A} receptors on presynaptic terminals in AIC; accordingly, reduced magnitude of glutamate release suppression may have been below the threshold for detecting changes in PPR. Alternatively, these effects may be explained by the predominant, proximal axonal location (near the axon hillock) of 5-HT_{1A} receptors on glutamatergic cortical neurons (Azmitia et al. 1996; DeFelipe et al. 2001; Czyrak et al. 2003; Wedzony et al. 2007).

In addition to 5-HT_{1A} receptors, 5-HT_{2A} receptors are also densely expressed in cortical neurons (Amargos-Bosch et al. 2004; Santana et al. 2004) and have been shown to play a role in enhancing EPSCs in rat cortical neurons (Aghajanian and Marek, 1997). We found that chronic ethanol consumption increased 5-HT_{2A} receptor mRNA levels. Activation of 5-HT_{1A} has been shown to reduce serotonin release (Amargos-Bosch et al., 2004), so it is possible that increased 5-HT_{1A} receptor expression decreased serotonin levels in the synaptic cleft, prompting a compensatory increase in postsynaptic 5-HT_{2A} receptor expression. Indeed, paradigms of learned helplessness and chronic stress have been shown to similarly reduce serotonin levels, resulting in higher 5-HT_{2A} receptor densities in rodent prefrontal cortex (Ferretti et al., 1995; Dwivedi et al., 2005).

Alterations due to chronic ethanol consumption in NR monkeys

Early-life stress caused by maternal separation is an established model of future disruptions in social and emotional behavior. In nonhuman primates, nursery-rearing has been shown to produce several alterations in physiology and behavior, including increased anxiety and social aggression, decreased social competence, disruptions in sleep-wake patterns, reduced HIAA CSF levels, and increased ethanol consumption (Higley et al., 1996a, Higley et al., 1996b, Barrett et al., 2009). Notably, both increased stress reactivity and sleep disorders associated with early adverse rearing conditions may play a role in the development of alcohol use disorders (Stein and Freidmann, 2005; Barr et al., 2009). Studying the nursery-rearing model is valuable for understanding the neurodevelopmental adaptations that may occur in children exposed to early-life stress who develop addictive behaviors later in life. In this study, both MR and NR animals consumed sufficient volumes of alcohol to classify them as heavy drinkers, and the NR animals consumed modestly, yet significantly, more alcohol than MR animals.

Among ethanol-naïve monkeys, we have shown an increase in glutamatergic transmission in the AIC of NR monkeys, as demonstrated by increased sEPSC frequency. Similar to ethanol-exposed monkeys, these findings may indicate either an increase in the excitability of the afferent neurons to AIC or an increase in glutamate release at the axon terminal of these afferent neurons. Furthermore, this increased excitation was correlated with a decrease in 5-HT_{1A} receptor mRNA levels. This reduction is supported by studies demonstrating reduced 5-HT_{1A} receptor binding in brains of rhesus monkeys exposed to early-life stress (Spinelli et al., 2011) as well as reduced 5-HT_{1A} receptor binding in the insula of patients with social anxiety disorder (Lanzenberger et al., 2007) or depression (Bhagwagar et al. 2004; Sargent et al. 2000).

In addition to the rearing-dependent differences among ethanol-naïve animals, we also found a significant interaction between rearing and ethanol consumption, indicating significantly greater increases in the gene expression levels of 5-HT_{1A} and 5-HT_{2A} receptors among ethanol-exposed NR animals. These results may suggest an enhanced sensitivity of the serotonin system to stressors in animals that experience early adverse rearing conditions.

The role of the AIC and serotonin in anxiety and addiction potential

The AIC has bidirectional connections with the extended amygdala and entorhinal cortex (Turner et al., 1980; Insausti et al., 1987; Augustine, 1996; Reynolds and Zahm, 2005), receives additional input from the lateral hypothalamus and mediodorsal thalamus (Allen et al., 1991) and projects to the ventral striatum (Haber et al., 1995; Chikama et al., 1997), anterior cingulate (Vogt and Pandya, 1987) and orbital frontal cortices (Ongur and Price, 2000). These inputs and outputs place the AIC in a position to generate conscious motivations by evaluating current and future internal states based on potential interactions with external stimuli (Craig, 2002). Chronic alterations in either the homeostatic set points that drive these internal representations or the processes by which present and future states are evaluated could then affect the activation of cortical neurons encoding these internal representations. Accordingly, insular hyperactivity has been demonstrated in anxiety-prone individuals (Simmons et al., 2006). Rodent models have also shown that early-life stress leads to decreased expression or function of 5-HT receptors later in life (Oreland et al., 2009; Gartride et al., 2003), increased aggression and behaviors consistent with psychopathology (Huot et al., 2001; Ladd et al., 2000) as well as increased propensity for high voluntary ethanol intake (Gustafson and Nylander, 2006; Roman and Nylander, 2005; for review, see Veenema, 2009). Our results demonstrating increased AIC glutamatergic transmission in NR ethanol-naïve monkeys as well as MR ethanol-exposed monkeys are consistent with previous reports in rodents and support a role for the AIC in psychopathology and alcohol seeking (Goldstein and Volkow, 2002; Hyman et al., 2006; Jentsch and Taylor, 1999). We conclude that early-life stress, induced by nursery rearing, resulted in altered expression of components of the 5-HT system and altered excitability of AIC circuits, which rendered the monkeys susceptible to anxiety-like psychopathology, characterized by several behavioral features, including increased alcohol consumption (Barr et al., 2003; Lesch, 2005).

Limitations

Our study was limited in scope for several reasons. First, the number of animals used in this study was limited to the minimum required to reach a level of statistical significance. Second, the amount of alcohol consumed by MR and NR monkeys classifies the monkeys as heavy drinkers, and although NR monkeys did consume significantly more alcohol than MR monkeys, the magnitude of the increased consumption was only modest. Thus, the differences we found in gene expression, electrophysiological response to 5-HT challenge, and excitability of AIC neurons between MR and NR animals exposed to alcohol were likely underestimates. Third, while analysis of mRNA provides useful information regarding how nursery rearing or ethanol exposure affects serotonin receptor expression at the transcriptional level, protein levels measure functional outcome of alterations in transcription, translation and modifications. Therefore, future studies should assess protein levels of serotonin receptors in the AIC following exposure to nursery rearing and alcohol exposure. Our group has assessed 5-HT_{1A} protein expression in the hippocampus of the same animals as those used in the current study and found that ethanol and nursery rearing induced changes in 5-HT_{1A} protein expression in hippocampus, although the direction of the change depended on the specific hippocampal region (Davenport et al., 2005, 2007). Fourth, due to limited tissue availability, we did not study serotonergic function in other brain areas,

so we cannot exclude the possibility that similar changes occur elsewhere in the brain. Fifth, because our recordings were performed blind and cesium was used in the internal solution, we cannot be certain of the neuronal sub-class of each recorded neuron.

Our findings demonstrating enhanced presynaptic inhibition of glutamate release through increased 5-HT_{1A} receptor function may be only one mechanism by which the CNS may adapt to aberrant AIC activity. Indeed, pharmacologically targeting 5-HT_{1A} receptors has been shown to be a promising treatment in reducing both alcohol consumption and anxiety (Schreiber et al., 1993; McKenzie-Quirk and Miczek 2003; Kelai et al., 2006). Buspirone, a partial 5-HT_{1A} receptor agonist, has also been shown to be an effective treatment in alcoholics with comorbid anxiety (Malec et al., 1996). Additionally, FG 5974, a drug with both 5-HT_{1A} receptor agonist and 5-HT_{2A} receptor antagonist properties significantly decreased ethanol self-administration in rats (Roberts et al., 1998), which complements our results showing increased AIC gene expression of both inhibitory 5-HT_{1A} and excitatory 5-HT_{2A} receptors. These results support the idea of specific, brain regional roles of distinct serotonin receptor subtypes in the development and maintenance of alcohol dependence within certain populations of patients. Continuing to identify both the direct and adaptive changes as a result of chronic ethanol exposure will provide a valuable framework for the successful treatment of alcoholism. Furthermore, identifying the neurodevelopmental changes following nursery-rearing will aid in understanding the mechanism by which early-stressed individuals develop later addictive behaviors.

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Highlights

- Nursery-reared monkeys self-administered more ethanol than mother-reared monkeys
- Nursery-rearing increased sEPSC frequency in AIC neurons
- Nursery rearing decreased 5-HT_{1A} and 5-HT_{2A} mRNA levels in the AIC
- Chronic ethanol increased sEPSC frequency and 5-HT_{1A} mRNA level in AIC
- Early-life stress may increase alcohol use disorders due to AIC reorganization

Figure 1.

A, Daily ethanol intake (g/kg) during 12 months of unlimited access to ethanol (4% w/v) in MR and NR monkeys. Two data sets are plotted for MR monkeys: one set with all four MR alcohol exposed monkeys used in the study and one set with three MR alcohol exposed monkeys that were used for gene expression studies. Data are smoothed using weighted average of nine nearest data points. In comparing consumption of NR monkeys with each set of MR, NR monkeys consumed significantly greater ethanol than either set of MR monkeys throughout the study period (two-way repeated measures ANOVA, $p < 0.01$). **B,** Blood

ethanol concentrations during 22-hour free access and corresponding ethanol consumption at the time of sampling (sample taken 6 hours post session start time) revealing a significant correlation ($r^2=0.67$, $p<0.0001$).

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Figure 2.

Relative mRNA expression levels of GAPDH and various 5-HT receptor subtypes for ethanol naïve and ethanol exposed MR or NR monkeys. **A**, Comparison of ethanol's effects on MR and NR groups reveals a significant increase in GAPDH, 5-HT_{1A} and 5-HT_{2A} mRNA levels (n=3, *p<0.05 unpaired *t* test). Chronic ethanol significantly upregulated GAPDH, 5-HT_{1A} and 5-HT_{2A} mRNA levels in the NR monkeys (n=4, *p<0.05 unpaired *t* test), with a significant interaction between chronic ethanol exposure and rearing conditions for 5-HT_{1A} and 5-HT_{2A} mRNA levels (#p<0.05, 2-way ANOVA). **B**, When comparing

rearing conditions, nursery-rearing alone produced a significant decrease in expression of both 5-HT_{1A} and 5-HT_{2A} when compared to MR, ethanol-naive monkeys. No differences were seen between MR and NR animals chronically-exposed to ethanol.

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Figure 3.

Synaptic properties of AIC neurons upon white matter or intracortical electrical stimulation in MR control animals. **A**, EPSCs consisted of APV-sensitive NMDA component and NBQX-sensitive AMPA/kainate component. **B**, Electrical stimulation evoked a rapid synaptic response followed by a delayed synaptic barrage. **Bi**, Single sweep showing white matter evoked short-latency synaptic response followed by delayed synaptic barrage. Arrows show individual synaptic responses that comprise the barrage response. Asterisk shows persistent depolarization resulting from synaptic stimulation. Depolarization persisted for ~1500 msec. **Bii**, Averaged sweeps showing white matter stimulation-evoked and intracortical stimulation-evoked responses from the same neuron. Asterisk shows short-latency synaptic response that was elicited by white matter stimulation but not intracortical stimulation. Arrow shows difference in latency to onset of synaptic barrage arising from white matter stimulation and intracortical stimulation. **C**, Short-term plasticity of white-matter stimulation evoked synaptic responses showing frequency-dependent depression for an individual neuron (**i**) and for the population ($n=6$ cells; **ii**).

Figure 4.

AMPA/kainate mediated spontaneous EPSCs recorded from AIC neurons in MR and NR control and chronic ethanol-consuming monkeys. **A**, MR animals that chronically self-administered ethanol showed significantly greater frequency (lower IEI) of sEPSCs as compared to MR ethanol-naïve controls (* $p < 0.05$). NR ethanol-naïve monkeys also showed significantly lower sEPSC IELs (higher frequency) as compared to MR ethanol-naïve controls (* $p < 0.05$). **B**, Cumulative IEI and amplitude histograms for the population of cells in each group of animals. **C**, Example traces for all four groups are also shown, along with a trace demonstrating that these sEPSC events could be eliminated with the AMPA/kainate receptor blocker DNQX in a MR control animal.

Figure 5.

The 5-HT_{1A} receptor agonist 8-OH DPAT reduced the amplitude of white matter-evoked EPSCs in layer 4/5 AIC neurons. **A–B**, Time course of the effect of 8-OH DPAT (2 μM) on EPSC amplitude recorded from neurons from control MR monkeys (**A**) or ethanol-exposed MR monkeys (**B**). **C**, 8-OH DPAT reduced the amplitude of EPSCs from ethanol-exposed MR monkeys significantly more than EPSCs from MR control monkeys. The magnitude of the decreased EPSC amplitude is paralleled by the magnitude of the increase in the paired pulse depression ratio (PPDR) (*p < 0.05, Wilcoxon rank test).

Figure 6.

Proposed model of synaptic changes in animals exposed to alcohol and/or nursery rearing based on experimental findings. **A–B**, Chronic exposure to alcohol may increase synaptic strength and expression of 5-HT_{1A} and 5-HT_{2A} receptors when compared to MR controls. Nursery rearing may also increase synaptic strength but decrease expression of 5-HT_{1A} and 5-HT_{2A} receptors. **D**, Chronic alcohol exposure may increase 5-HT_{1A} and 5-HT_{2A} receptor expression in nursery reared monkeys, returning serotonergic tone closer to baseline levels.

Table 1

Membrane and synaptic response properties for AIC neurons acutely isolated from control (ethanol naive) and ethanol-exposed animals divided into two rearing conditions

	Membrane Potential (mV)	Membrane Resistance (MΩ)	EPSC decay (msec)	Paired Pulse Ratio	sEPSC amp (pA)	sEPSC ICI (msec)	sEPSC decay (msec)
Mother-Reared Control	-66.1 ± 0.8 (7 cells, 3 animals)	295.7 ± 35.0 (12 cell 3 animals)	21.5 ± 3.0 (7 cells, 3 animals)	1.05 ± 0.07 (7 cells, 3 animals)	5.1 ± 0.7 (7 cell, 3 animals)	194.9 ± 27.6 (7 cells, 3 animals)	3.1 ± 0.7 (7 cells, 3 animals)
Nursery-Reared Control	-70.0 ± 1.8 (8 cells, 3 animals) *	228.7 ± 29.6 (9 cells, 3 animals)	26.2 ± 4.8 (5 cells, 3 animals)	0.95 ± 0.12 (5 cells, 3 animals)	7.6 ± 1.0 (6 cells, 3 animals)	145.6 ± 11.7 (6 cells, 3 animals) *	7.6 ± 0.8 (6 cells, 3 animals)
Mother-Reared EtOH	-58.4 ± 2.4 (11 cells, 4 animals) #	177.2 ± 23.3 (12 cells, 4 animals) #	23.9 ± 3.6 (13 cells, 4 animals)	0.85 ± 0.06 (11 cells, 4 animals) #	7.4 ± 1.3 (5 cells, 3 animals)	106.7 ± 25.2 (5 cells, 3 animals) #	7.6 ± 1.3 (5 cells, 3 animals) #
Nursery-Reared EtOH	-60.8 ± 1.9 (8 cells, 4 animals) #	235. ± 61.0 (7 cells, 3 animals)	22.3 ± 2.8 (8 cells, 4 animals)	0.93 ± 0.03 (5 cells, 4 animals)	6.7 ± 1.9 (2 cells, 2 animals)	111.1 ± 10.7 (2 cells, 2 animals)	6.5 ± 2.6 (2 cells, 2 animals)

Values represent mean ± SEM. Parenthetical numbers represent number of cells studied.

* Between rearing conditions,

between ethanol treatment. p<0.05. unpaired t-test.