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Effects of Developmental Alcohol Exposure on Potentiation and Depression of Visual Cortex Responses

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Background: Neuronal plasticity deficits are thought to underlie abnormal neurodevelopment in fetal alcohol spectrum disorders and in animal models of this condition. Previously, we found that alcohol exposure during a period that is similar to the last months of gestation in humans disrupts ocular dominance plasticity (ODP), as measured in superficial cortical layers. We hypothesize that exposure to alcohol can differentially affect the potentiation and depression of responses that are necessary for activity-dependent sprouting and pruning of neuronal networks. ODP is an established paradigm that allows the assessment of activity-dependent depression and potentiation of responses in vivo.

Methods: Mouse pups were exposed to 3.6 to 5 g/kg of ethanol in saline daily or every other day between postnatal days 4 and 9. Visual cortex plasticity was then assessed during the critical period for ODP using 2 techniques that separately record in layers 4 (visually evoked potentials [VEPs]) and 2/3 (optical imaging of intrinsic signals [OI]).

Results: We discovered a layer-specific effect of early alcohol exposure. Recording of VEPs from layer 4 showed that while the potentiation component of ODP was disrupted in animals treated with alcohol when compared with saline controls, the depression component of ODP (Dc-ODP) was unaltered. In contrast, OI from layers 2/3 showed that Dc-ODP was markedly disrupted in alcohol-treated animals when compared with controls.

Conclusions: Combined with our previous work, these findings strongly suggest that developmental alcohol exposure has a distinct and layer-specific effect on the potentiation and depression of cortical responses after monocular deprivation.

Key Words: Ocular Dominance, Visual Development, Monocular Deprivation, Visually Evoked Potentials, Fetal Alcohol Syndrome.

ALCOHOL CONSUMPTION DURING pregnancy can lead to a wide variety of neurological problems in offspring. These alterations are collectively referred to as fetal alcohol spectrum disorders (FASD; Hannigan and Armant, 2000). FASD can be associated with deficits in visual processing (Burden et al., 2009; Coffman et al., 2013; Uecker and Nadel, 1996), impaired visual-motor integration (Chiodo et al., 2009; Vaurio et al., 2011), and altered saccadic eye movement (Paolozza et al., 2013). Some of these deficits can be caused by major peripheral alterations such as microphtalmia and hypoplasia of the optic nerve (Strömland, 1985; Strömland and Pinazo-Durán, 2002). However, problems in visual acuity and contrast sensitivity may also be caused by alterations in the visual cortex resulting in amblyopia (Vernescu et al., 2012). Deficits in visual acuity and contrast sensitivity can be seen also in animal models of FASD (Hug et al., 2000; Lantz et al., 2014). There is growing evidence that impaired neuronal plasticity underlies many of the sensory deficits seen in FASD (see Medina, 2011). For instance, recent studies by Sowel group showed that, during infancy and adolescence, control subjects presented a strikingly plastic cortex, with a robust increase in volume followed by loss, while FASD subjects displayed a less plastic cortex, with mostly volume loss (Lebel et al., 2012). One possible explanation of these findings is that developmental alcohol exposure preferentially disrupts processes related to the increase of neuronal connections rather than their elimination.

Neuronal plasticity encompasses the strengthening (potentiation) and weakening (depression) of neuronal responses, which can ultimately lead to an increase or elimination of neuronal connections, respectively. The ocular dominance plasticity (ODP) model relies on cortical changes that occur after monocular deprivation (MD) during a critical period of development (Hubel and Wiesel, 1970; Hubel et al., 1977). In normal animals, a few days of MD lead to depression of responses in neurons driven by the deprived (closed)

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eye (referred here as depression component of ODP [Dc-ODP]), and a potentiation of responses in neurons driven by the experienced (open) eye (referred to here as the potentiation component of ODP [Pc-ODP]) (Fig. 1). The time course of Pc-ODP and Dc-ODP in the mouse has been well described (Crozier et al., 2007; Smith et al., 2009). After 2 to 3 days of MD, predominantly Dc-ODP of the closed eye responses takes place. In contrast, Pc-ODP of the open eye responses is only seen after 5 days of MD (Fig. 1*B*).

Previously, recordings of visually evoked potentials (VEPs) using a single implanted electrode have been used to assess ODP and study plasticity deficits in mice before and after MD (Dolen et al., 2007; Yashiro et al., 2009). The recorded field potential represents the summation of the responses from many neurons, but is primarily composed of responses from layer 4, as indicated by the depth of the electrode implantation (~430 nm), as well as the VEP size and polarity (largest negative polarity VEP is a result of direct thalamic input into layer 4 of the primary visual cortex; Heynen and Bear, 2001). Optical imaging of intrinsic signals (OI) has historically also been used to investigate ODP in rodents (Kalatsky and Stryker, 2003; Lantz et al., 2012; Lehmann and Lowel, 2008). The origin of these intrinsic signals lies in the changes in oxyhemoglobin and hemoglobin with tissue activation, and these signals are now considered to arise predominately from superficial cortical layers such as layer 2/3 (Bonhoeffer, 1995; Frostig and Chen-Bee, 2009; Lee et al., 2012; McCurry et al., 2010; Smith et al., 2009).

The Medina laboratory pioneered the use of ODP to investigate neuronal plasticity deficits in FASD. We showed that alcohol exposure from postnatal day 10 (P10) to P30 in ferrets or between P5 and P9 in mice, periods that are roughly equivalent to the last months of human gestation, leads to a permanent impairment in ODP (Lantz et al., 2012; Medina et al., 2003). However, it is still unknown whether the neuronal plasticity impairments seen after alcohol exposure are due to a specific disruption of Pc-ODP, Dc-ODP, or both. Because potentiation and depression rely on different mechanisms, it is critical to tease apart alcohol's effect on each of these processes to understand the underlying causes of neuronal plasticity deficits in FASD.

To address this question, mice were exposed to alcohol or saline, using a binge drinking like paradigm, during a period roughly equivalent to the third trimester of human gestation. After an ethanol - (EtOH) (OI) free period of ~2 weeks, we recorded VEPs and OI responses to assess changes in Pc-ODP and Dc-ODP.

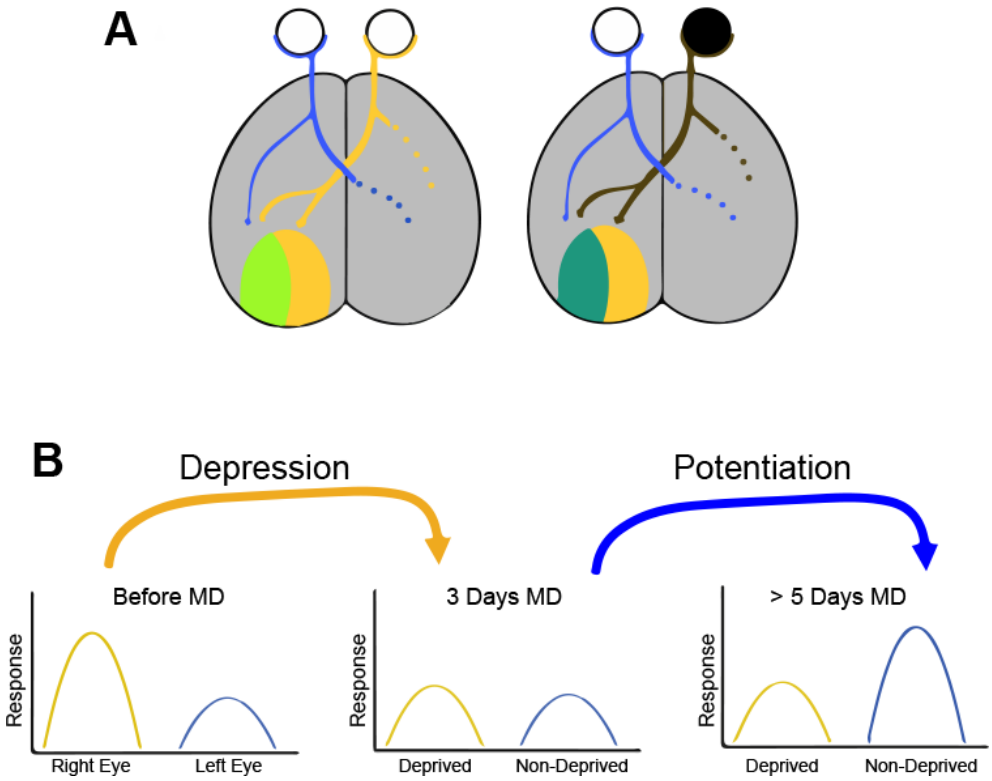

Fig. 1. Ocular dominance plasticity (ODP) in mice. (**A**) The mouse visual cortex is divided into 2 sections, a monocular section and a binocular zone, responding to input from both eyes. In the binocular zone, neurons present different degrees of binocularity, from cells that are equally responsive to stimulation of either eye, to cells that respond almost exclusively to one eye. When both eyes are receiving normal input, the binocular zone is dominated by responses to the contralateral eye (light green). If this eye is sutured closed during the critical period, then the binocular zone shifts, responding primarily to input from the ipsilateral, open eye (bluish green). (**B**) The shift in eye dominance in the binocular zone of V1 occurs through 2 temporally different mechanisms. First, there is a depression of inputs from the deprived eye that occurs after approximately 3 days of monocular deprivation (MD) (Dc-ODP). This is followed by a potentiation of the nondeprived eye inputs (Pc-ODP), after approximately 5 days of MD.

MATERIALS AND METHODS

Experiment 1 (Carried Out in the Medina Laboratory)

Animals. All procedures described here were approved by the IACUC. Visibly pregnant C57/BL6 female mice were obtained from a commercial supplier (Harlan, Indianapolis, IN) and singularly housed in the University of Maryland animal housing. Pregnant dams were checked daily until pups were born. Day of birth was designated as P0. A total of 27 animals were used from 6 litters.

Ethanol Exposure. Pups received a single injection of 5 g/kg of alcohol (25% EtOH in normal saline intraperitoneally [i.p.]) or saline as a control on days P5, P7, and P9. According to our previously published studies, this protocol leads to blood alcohol levels of 411 mg/dl (±43) at 1 hour postinjection (Lantz et al., 2012).

Electrode Implantation. Electrode implantation was carried out as described previously (Lantz et al., 2014). Electrodes were implanted in P21 to P22 mice. Mice were anesthetized with i.p. ketamine 120 mg/kg (Bioniche Pharma, Lake Forest, IL) and xylazine 9 mg/kg (Akorn, Inc., Decatur, IL). Once anesthetized, 2% lidocaine jelly (Akorn, Inc.) was applied locally on the scalp at the incision site. Burr holes were drilled 1.0 mm caudal from bregma and 2.0 mm lateral from the midline, and ground electrodes were implanted. Tungsten microelectrodes (FHC, Inc., Bowdoin, ME; impedance 0.3 to 0.5 M ohms) were implanted in bilateral burr holes drilled at 3.00 mm lateral of midline and 0.00 mm of lambda, at a depth of 0.43 mm. Electrodes were secured with cyanoacrylate glue (Elmers, Westerville, OH). A nail glued over the rostral portion of the skull was used to secure each animal's head during recording. After surgery, each animal was monitored until recovery of righting reflexes and was then given 0.05 mg/kg of buprenorphine (Stokes Pharmacy, Mt. Laurel, NJ) for postsurgical analgesia.

Assessment of Ocular Dominance. Awake animals were habituated on the experimental setup for 45 minutes 1 day prior to the experiment. VEPs were recorded using XCell-3 amplifiers (FHC, Inc.; 1 for each recording electrode), a 1401 digitizer (CED, Cambridge, UK), and Spike 2 software (Cambridge Electronics Design, Cambridge, UK). XCell-3 amplifiers were set with a low cutoff of 0.1 Hz and a high cutoff of 100 Hz. Visual stimulations were presented to each eye individually using a monitor placed 18 cm from the nose of the animal (mean luminance 27 cd/m^2, area of 15 × 31 cm) and controlled by a custom program using MATLAB (MathWorks, Natick, MA). Stimuli consisted of full field ordinal sine wave 2 Hz reversing gratings, at 0.05 cycles per degree with 100% contrast. To avoid stimulus response potentiation (Cooke and Bear, 2010), drifting gratings were presented at different angles in the experiments performed before (45°) and after (135°) MD. VEP responses were then averaged from 100 stimulation presentations, and amplitudes were recorded using peak to trough measurements. At the end of the first day of recording, animals were anesthetized using vaporized isoflurane (Baxter, Deerfield, IL) and small portions of the upper and lower right eyelids were trimmed. The eyelids were then sutured and covered with tissue glue (CP Medical, Portland, OR). Animals were then returned to the animal colony and remained monocularly deprived for 3 or 5 to 10 days, until post-MD VEPs were recorded. After deprivation, animals were briefly anesthetized using vaporized isoflurane (Baxter), and the sutured eye was opened. Animals were then placed in the experimental setup, and post-MD VEP recordings were performed immediately after recovery from light anesthesia. Eyelids were checked daily for any sign of opening during the period of MD. Animals with partial eye lid opening during the period of deprivation were discarded. We also discarded animals where responses from both eyes decreased or increased more than 2-fold from the pre-MD day of recording.

Statistics. Data are reported as mean ± standard error. Statistical analysis was performed using SPSS (IBM, Armonk, NY), and univariate analyses of variance (ANOVAs) were used to compare contralateral bias index (CBI) measurements within experimental groups. For comparison of ipsilateral and contralateral VEP amplitude within each group, paired *t*-tests were employed.

Experiment 2 (Carried Out in the Majewska Laboratory)

Animals. All procedures were conducted in strict accordance with the University of Rochester's Committee on Animal Resources. C57/BL6 breeding pairs were maintained in-house under a 12-hour light/dark cycle and supplied chow and water ad libitum. Breeders were checked daily until the birth of pups (P0), when the male was separated from the dam and litter. A total of 19 mice from 4 litters were used for experiments. Litter size was between 6 and 9 pups.

Ethanol Exposure. Pups (P4) were weighed, toe-clipped, and randomly assigned to a saline vehicle control or alcohol (EtOH) treatment group. From P4 through P9, pups received 2 daily subcutaneous (s.c.) injections, spaced 2 hours apart. Pups were returned to the dam immediately following each injection. EtOH-treated animals received a total of 3.6 g/kg EtOH in 2 doses of 1.8 g/kg EtOH, delivered using 20% v/v 200 proof EtOH in 0.9% NaCl. The 20% EtOH was prepared fresh within 30 minutes of each dose. Control animals received an equivalent volume of 0.9% NaCl. Blood alcohol levels at 1.5 hours after the second dose on P4 were (356 ± 10 mg/dl, mean ± SEM). Animals were weighed on P4 to P9, P14, P21 (weaning), and P27. Weights between saline vehicle control and alcohol-treated animals were not significantly different at any time point sampled (data not shown).

Optical Imaging of Intrinsic Signals. On P28 ± 1 day, animals were separated into nondeprived or monocularly deprived cohorts. Monocularly deprived animals were anesthetized using vaporized isoflurane (5% induction, 3% maintenance), and right upper and lower eyelids were resected and sutured together using 2 mattress sutures (5.0 Vicryl; Ethicon, Inc., Portland, OR). After 4 days (P31 ± 1 day), nondeprived or monocularly deprived animals were anesthetized with isoflurane (5% induction, 3% maintenance) and i.p. injection of chloroprothixene (2 mg/kg). Monocularly deprived animal eyelids were reopened. The skull over the contralateral visual cortex was exposed, cleared of membranes, covered with 0.5% agarose, and sealed with a coverslip to create an imaging window. Intrinsic signal optical imaging was performed using a DALSA 2M30 CCD camera (Thousand Oaks, CA) and custom acquisition software (Kalatsky and Stryker, 2003). Anesthesia was maintained with isoflurane (0.75%) throughout imaging. Vasculature was illuminated using a green LED (550 nm), and a region of interest containing binocular visual cortex was selected based on characteristic vascular patterns. Intrinsic signal images were then collected using illumination from a red LED (700 nm). Visual stimuli were presented, consisting of white horizontal square-wave bar gratings on a black background moving upward (90°) or downward (270°) at a frequency of 8°/s for 6 minutes. Visually evoked responses to stimuli moving in both directions, presented to the contralateral and ipsilateral eyes individually, were collected. The amplitude of the fast Fourier transform component of the intrinsic signal was averaged for each eye from responses to both stimulus directions and compared between eyes offline using MATLAB to determine ocular dominance (Kalatsky and Stryker, 2003; Tropea et al., 2010). A CBI was calculated by the following equation: CBI = average contralateral response/average ipsilateral response. MD, intrinsic signal optical imaging, and ocular dominance analysis were all carried out by an investigator blind to animal treatment.

Statistics. Data are reported as mean ± standard error. Comparisons were performed using Prism VI statistical analysis software (GraphPad, La Jolla, CA). CBI values were analyzed using a 2-way ANOVA with Bonferroni post hoc comparisons.

RESULTS

Mouse pups were exposed to 5 g/kg of alcohol or saline on P5, P7, and P9, mimicking binge alcohol drinking during the third trimester equivalent of human gestation. At P25, ocular dominance was assessed by calculating peak to trough measures of VEPs resulting from stimulation of each eye individually (Fig. 2).

After the initial evaluation of ocular dominance, each saline- and alcohol-treated animal was monocularly deprived for 5 to 10 days. It is well established that in mice 5 days of MD is sufficient to produce a decrease and an increase in the responses of the deprived and experienced eye, respectively (Frenkel and Bear, 2004). At the end of this period, VEPs were recorded to assess the effect of MD on ocular dominance.

After MD, saline-treated animals exhibited a decrease in CBI values from 1.80 ± 0.36 to 0.76 ± 0.32 ($n = 7$). This change indicates a shift from contralateral (deprived) to ipsilateral (experienced) eye dominance. Surprisingly, a reduction in CBI values was also seen in the EtOH-exposed animals, from an average CBI value of 1.46 ± 0.35 to 1.11 ± 0.33 ($n = 11$, $F = 32.309$, df = 1, $p < 0.001$, ANOVA). Despite this difference, the average CBI value for EtOH-treated animals remained above 1, indicating a continued dominance of the contralateral eye (interaction between EtOH and MD, $F = 1.137$, df = 1, $p = 0.004$, ANOVA, Fig. 3*A*). We calculated the percentage of change in CBIs before and after MD. Figure 3*B* shows that the reduction in CBI after MD was higher in saline (57% ± 7) than in alcohol-treated animals (26% ± 6; $t = 3.24$, df = 15, $p = 0.005$), indicating that MD of EtOH-exposed animals resulted in a less plastic ocular dominance response.

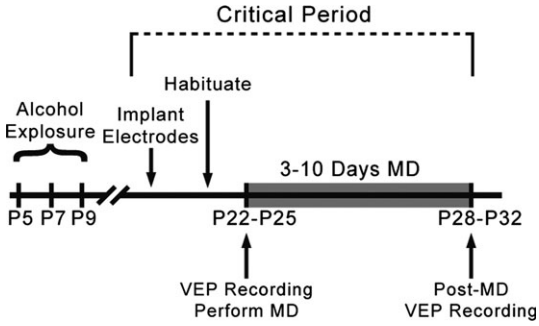

Fig. 2. Experimental design. Animals were exposed to 5 g/kg of ethanol or control saline on P5, P7, and P9. Animals were then implanted with recording electrodes on P21 to P24 and habituated to the recording apparatus 24 hours following surgical recovery. After monocular deprivation (MD) for a short period (3 days) or a longer period (5 to 10 days), the deprived eye was opened and post-MD visually evoked potentials (VEPs) were recorded.

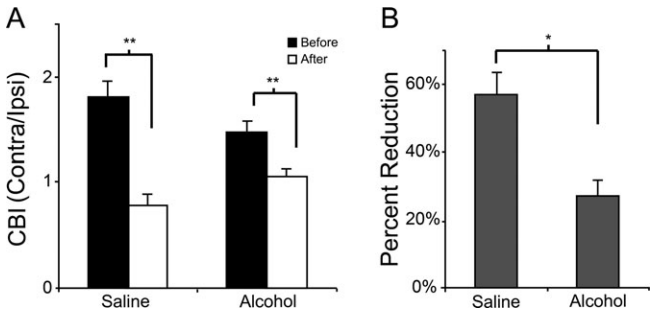

Fig. 3. Effect of 5 to 10 days of monocular deprivation (MD) on contralateral bias index (CBI). (**A**) Both alcohol-(1.46 ± 0.35; 1.11 ± 0.33; $n = 11$) and saline-(1.80 ± 0.36; 0.76 ± 0.32; $n = 7$) treated animals exhibited a significant decrease in CBI values after MD. (**B**) However, the magnitude of this change was larger in saline-(57% ± 7) treated animals than ethanol-(26% ± 6) treated animals. *$p = 0.029$, **$p < 0.001$.

After examining the changes in CBI, we compared the individual changes in eye responses (Fig. 4). In saline-exposed animals, both Pc- and Dc-ODP were observed. This was illustrated by the capacity of MD to reduce contralateral (deprived) eye responses from 156.9 μV ± 13.8 to 94.21 μV ± 7.89 and to increase ipsilateral eye responses from 87.12 μV ± 4.90 to 131.52 μV ± 11.37. Paired *t*-tests indicated that both changes reached statistical significance (contralateral, $t = 5.21$, df = 6, $p = 0.001$; ipsilateral, $t = -3.67$, df = 6, $p = 0.01$). In the alcohol-treated group, we also observed a reduction in contralateral (deprived) eye responses ($t = 2.96$, df = 9, $p = 0.01$), with VEPs decreasing from 125.74 μV ± 13.49 to 96.6 μV ± 6.05. However, this change was not accompanied by a significant increase in ipsilateral eye responses ($t = -1.16$, df = 9, $p = 0.27$), as amplitudes changed only from 89.69 μV ± 12.4 to 97.53 μV ± 10.26. These findings suggest that early alcohol exposure leads to a remarkable impairment in Pc-ODP, but not Dc-ODP. This difference appears to be the cause of the smaller CBI changes seen in alcohol-exposed animals.

It is well established that the 2 components of ODP do not have the same time course (Frenkel and Bear, 2004). The potentiation component is observed after 5 days of MD while the depression component can start 24 hours after MD with decreased VEPs reliably recorded after 3 days (Smith et al., 2009). Therefore, we considered the possibility that alcohol could delay the early stages of the depression component. To test this, we recorded from mice after only 3 days of MD (Fig. 5). When we examined CBI values for alcohol-exposed animals, we observed a significant shift in CBI values from 1.67 ± 0.2 to 1.07 ± 0.23 ($n = 4$). The magnitude of these CBI shifts closely mirrored those observed in saline-treated animals with 3 days of MD. The CBIs of these animals shifted from 1.62 ± 0.49 to 0.94 ± 0.18 ($n = 5$, $F = 16.995$, df = 1, $p = 0.001$, ANOVA, Fig. 5*B*). Indeed, when we compared the percent change between pre- and post-MD, we saw no significant difference between control and alcohol-exposed animals ($t = -0.46$, df = 8, $p = 0.65$). These results indicate that after 3 days of MD, alcohol- and

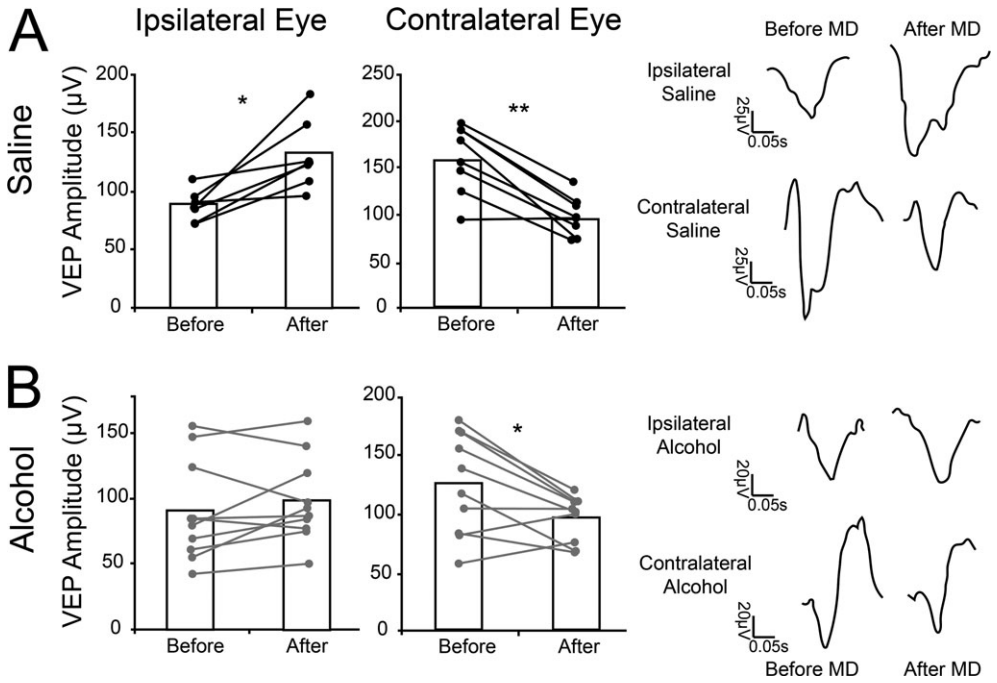

Fig. 4. Changes in individual eye responses after 5 to 10 days of monocular deprivation (MD). After 5 to 10 days of MD, saline-treated animals ($n = 9$) exhibited a significant increase in ipsilateral (87.12 μV $\pm$ 4.90 to 131.52 μV $\pm$ 11.37) eye responses, as well as a significant decrease in contralateral eye responses (156.9 μV $\pm$ 13.8 to 94.21 μV $\pm$ 7.89) (**A**). These bidirectional changes can be seen in traces from a representative animal. In contrast, ethanol-exposed animals ($n = 14$) demonstrated no change in ipsilateral eye responses (89.69 μV $\pm$ 12.4 to 97.53 μV $\pm$ 10.26), yet showed a significant decrease in contralateral eye response (125.74 μV $\pm$ 13.49 to 96.6 μV $\pm$ 6.05) (**B**). *$p = 0.01$, **$p < 0.001$.

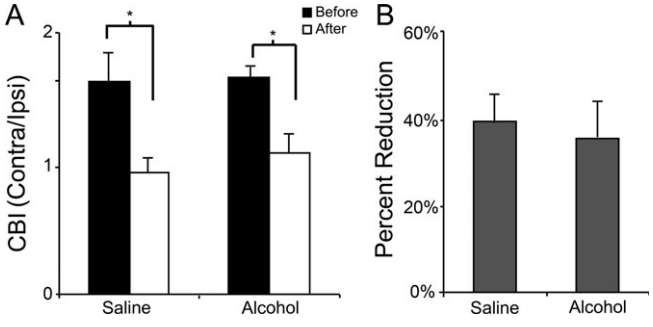

Fig. 5. Effect of 3 days of monocular deprivation (MD) on contralateral bias index (CBI). Both alcohol-(1.67 $\pm$ 0.2; 1.07 $\pm$ 0.23; $n = 4$) and saline-(1.62 $\pm$ 0.49 to 0.94 $\pm$ 0.18; $n = 5$) treated animals exhibited a significant decrease in CBI values (**A**). The magnitude of this change was similar in both groups (**B**). *$p = 0.001$.

saline-treated animals demonstrate a similar magnitude of response changes (Fig. 5).

To see whether these changes in responses were indeed similar, we looked at the effect of 3 days of MD on the individual eye responses (Fig. 6). This period of MD caused a decrease in contralateral eye responses in alcohol animals, with contralateral eye amplitudes shifting from 126.5 μV $\pm$ 7.56 to 90.7 μV $\pm$ 4.44 ($t = 4.15$, df = 3, $p = 0.02$). In contrast, the increase in ipsilateral (experienced) eye VEP amplitudes after 3-day MD was not significant, 77.43 μV $\pm$ 6.09 to 85.01 μV $\pm$ 10.09 ($t = -0.04$, df = 3, $p = 0.96$, Fig. 6*B*). Curiously, in saline-treated animals, 3-day MD did not significantly alter ipsilateral eye VEP amplitudes; 133.73 μV $\pm$ 44.35 to 163.75 μV $\pm$ 33.71 ($t = -0.78$, df = 4, $p = 0.47$), or contralateral eye VEP amplitudes (169.51 μV $\pm$ 44.3 to 146 μV $\pm$ 22.6; $t = 1.42$, df = 4, $p = 0.22$). There is some variation in the VEP amplitude, and MD induced changes in VEP amplitude within these groups. This can be attributed to our use of animals from multiple litters (litter number = 6), as well as minor differences in electrode implant tolerance, which is normal for this type of chronic implant recording. To account for this variability, most studies using 3 days of MD normalize contralateral responses to the ipsilateral ones (Frenkel and Bear, 2004). This normalization is based on the assumption that the ipsilateral eye responses do not change after short periods of MD. This type of normalization cannot be performed in the 5-day MD paradigm, as contralateral and ipsilateral eye responses change. Figure 7 shows the responses of saline-treated animals after normalization. Note that all animals presented a decrease in contralateral responses. Our results indicate that early EtOH exposure results in a disruption of the potentiation, but not the Dc-ODP, as assessed by VEP recordings.

The results obtained from VEP recording demonstrated that early alcohol exposure affects Dc-ODP, but not Pc-ODP. This finding was intriguing because in our previous study using OI, alcohol-treated animals did not show an ocular dominance shift after 10 days of MD (Lantz et al., 2012). While these prior findings strongly suggested an effect of

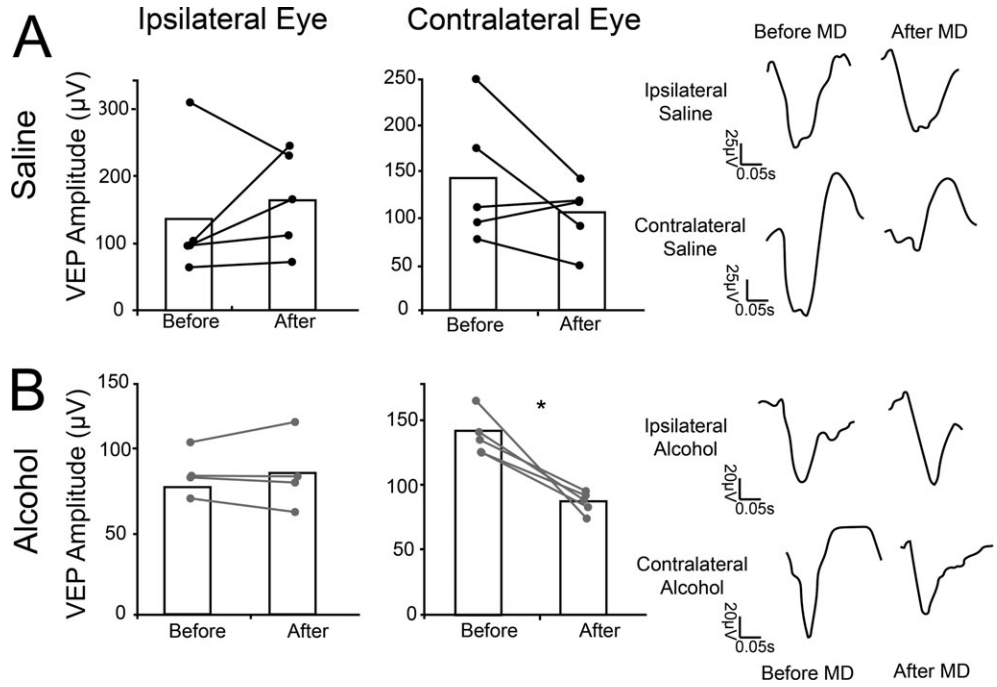

Fig. 6. Changes in individual eye responses after 3 days of monocular deprivation (MD). After 3 days of MD, saline-treated animals exhibited no change in response amplitude of the ipsilateral eye (133.73 μV $\pm$ 44.35; 163.75 μV $\pm$ 33.71; $n = 5$), yet the contralateral eye (169.51 μV $\pm$ 44.3; 146 μV $\pm$ 22.6; $n = 5$) exhibited a trend toward decreased response amplitude (**A**). This change can be seen in visually evoked potential (VEP) responses from a representative animal. Ethanol-exposed animals also showed a significant decrease in their contralateral eye responses (126.5 μV $\pm$ 7.56 to 90.7 μV $\pm$ 4.44, $n = 4$) after MD, but no changes were seen in ipsilateral responses (77.43 μV $\pm$6.09; 85.01 μV $\pm$ 10.09; $n = 5$) (**B**). This change can be seen in VEP traces from a representative animal. *$p = 0.01$.

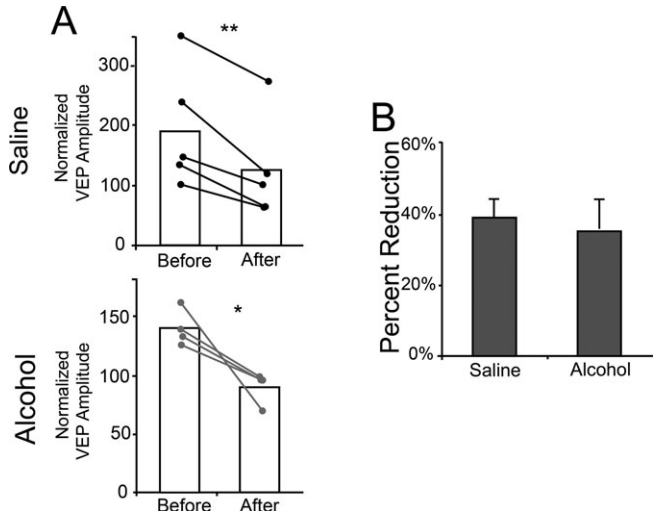

Fig. 7. Normalized contralateral eye responses after 3 days of monocular deprivation (MD). When contralateral eye visually evoked potentials (VEPs) are normalized to ipsilateral eye responses, the decrease in contralateral eye responses for all groups becomes apparent (**A**). With this data transformation, saline- and alcohol-treated animals exhibit a similar percent change in VEP amplitude from before to after MD (**B**). *$p = 0.04$, **$p = 0.007$.

alcohol on both Pc-ODP and Dc-ODP, it is difficult to tease apart the contributions of these individual components using OI. To test whether alcohol affects Dc-ODP using OI shorter periods of MD (<5 days) would be required. This is particularly relevant as VEPs and intrinsic signals record from different layers (2/3 and 4, respectively) which could indicate a layer-specific effect of alcohol exposure.

In an independent experiment, our collaborators in the Majewska laboratory tested the effect of early alcohol exposure on Dc-ODP using OI (see Materials and Methods, Experiment 2). Saline-injected control animals and alcohol-injected animals were either monocularly deprived for 4 days or left nondeprived. A 2-way ANOVA showed a significant main effect of deprivation ($F = 13.85$, df = 1, $p = 0.002$), but not of treatment ($F = 0.1429$, df = 1, $p = 0.71$) or an interaction ($F = 2.316$, df = 1, $p = 0.15$). Post hoc analysis demonstrated that control animals monocularly deprived for 4 days exhibited a significantly lower CBI value (1.134 $\pm$ 0.05 $n = 5$) compared to nondeprived control animals (1.967 $\pm$ 0.32, $n = 3$, Bonferroni test, $p = 0.02$), indicative of Dc-OPD. In contrast, a reduction in CBI values was not observed in animals previously exposed to alcohol. The CBI values were 1.785 $\pm$ 0.07 $n = 4$ for nondeprived EtOH mice and 1.436 $\pm$ 0.15 $n = 7$ for 4-day monocularly deprived EtOH mice (Bonferroni test, $p = 0.67$) (Fig. 8).

DISCUSSION

Here, we showed that early alcohol exposure leads to long-lasting effects on Pc-ODP, but not Dc-ODP in layer 4. Surprisingly, the effect of early alcohol exposure on ODP as

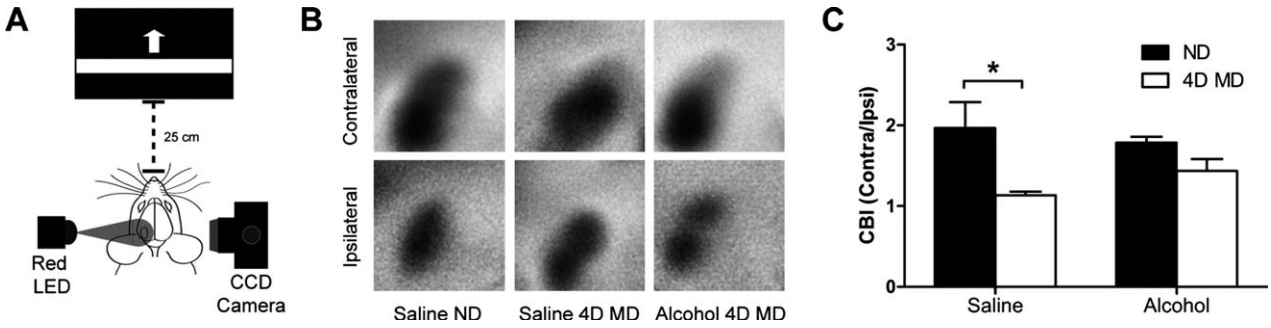

Fig. 8. Changes in ocular dominance after 4 days of monocular deprivation (MD) assessed by optical imaging of intrinsic signals. Mice were treated with 1.8 g/kg × 2 of ethanol or saline vehicle control daily from P4 to P9. Note that in all previous figures animals were exposed to 5 g/kg of ethanol or control saline on P5, P7, and P9. (**A**) Diagram of intrinsic signal optical imaging setup. (**B**) Representative average contralateral response and average ipsilateral response amplitude maps used for contralateral bias index (CBI) calculation. (**C**) Compared to nondeprived saline animals (1.97 ± 0.32, $n = 3$), 4-day monocularly deprived saline animals exhibited a significantly lower CBI (1.134 ± 0.05, $n = 5$). In contrast, after alcohol treatment, 4 days of MD did not result in a significant decrease in CBI (1.785 ± 0.07, $n = 4$; 1.44 ± 0.15, $n = 7$). *$p < 0.05$. Graphed mean ± SEM.

assessed by VEPs seems to be much less robust than what our laboratory has previously observed using OI (Lantz et al., 2012). In our previous study, 10 days of MD did not result in any distinguishable change in CBIs in alcohol-exposed mice, suggesting that both Pc-ODP and Dc-ODP were disrupted. In the present study, we confirmed that early alcohol exposure disrupts Dc-ODP assessed by OI after 4 days of MD (Fig. 8). Importantly, this result was obtained through an independent experiment carried out by the Majewska laboratory and with a slightly different method of alcohol exposure. In both alcohol administration paradigms, alcohol was given between P4 and P9; in the Majewska laboratory, 3.6 g/kg of alcohol was administered every day, while in the Medina laboratory, 5 g/kg was administered every other day during this time frame. Although the paradigms differ slightly, they both result in abnormal ODP. Both the Medina and Majewska EtOH paradigms were chosen to specifically induce aberrant apoptosis via blood alcohol concentration levels over 200 mg/dl for an extended period of time (Ikonomidou et al., 2000; Lantz et al., 2012) during the brain growth spurt. While it is possible that there was more EtOH-induced apoptosis in the Majewska paradigm via the daily treatment with EtOH which may make their paradigm more severe, we believe this difference in daily versus every-other-day treatment is compensated for by the increased EtOH dose given by the Medina laboratory. Interestingly, the fact that OI and VEPs register responses from layers 2/3 and 4, respectively (Cooke and Bear, 2010; Frenkel and Bear, 2004; Frostig and Chen-Bee, 2009; Lee et al., 2012; McCurry et al., 2010; Smith et al., 2009), allows us to hypothesize that alcohol may affect extragranular layers more than the granular layer. This hypothesis is also supported by prior studies demonstrating that Dc-ODP relies on different mechanisms in layer 2/3 than in layer 4 (Crozier et al., 2007). This work can be further extended and confirmed using laminar recordings of VEP and single units in the primary visual cortex to further confirm layer-specific deficits induced by early alcohol exposure.

Dc-ODP begins during the first 24 hours after MD and is clear after 3 days (Frenkel and Bear, 2004). This effect is mechanistically different across cortical layers. For instance, in layer 2/3, Dc-ODP relies on cannabinoid receptors and is independent of AMPA receptor internalization. In contrast, in layer 4 the depression component is independent of cannabinoid receptors and appears to rely on traditional long-term depression (LTD) mechanisms, involving clathrin-dependent AMPA receptor internalization (Crozier et al., 2007; Smith et al., 2009).

Conversely, Pc-ODP is consistently detected after 5 days of MD (Frenkel and Bear, 2004). In contrast to the depression component, potentiation of nondeprived eye responses appears to share similar mechanisms in layer 2/3 and layer 4, as both rely on AMPA receptor insertion at the synapse (Heynen and Bear, 2001).

We observed that early alcohol exposure affects Pc-ODP, but not Dc-ODP in layer 4. The fact that VEPs recordings are derived mostly from layer 4 (Cooke and Bear, 2010), where potentiation and depression rely on insertion and internalization of AMPARs, respectively, suggests that alcohol may affect glutamatergic transmission (Bellinger et al., 2002; Rema and Ebner 1999; Savage et al., 1991), possibly disrupting the NR2A/B ratio which is crucial in maintaining normal ODP (Cho et al., 2009). EtOH's effects on Dc-ODP in layer 2/3, but not in layer 4, suggest a possible action on endocannabinoid transmission. In fact, a series of studies by Basavarajappa's group suggest a strong effect of developmental alcohol on anandamide-CB1 receptor signaling (Basavarajappa et al., 2008; Subbanna et al., 2013).

Pc-ODP and Dc-ODP share similar mechanisms with long-term potentiation (LTP) and LTD, respectively (Crozier et al., 2007; Heynen and Bear, 2001; Yoon et al., 2009). Most studies on the effects of early alcohol exposure in LTP and LTD have been carried out in rats using hippocampal slice preparations. Studies using a variety of alcohol doses, regimens, and time periods showed unequivocally that alcohol can disrupt LTP in hippocampus (Izumi et al., 2005; Puglia and Valenzuela, 2010a,b; Richardson et al., 2002;

Savage et al., 2010; Sutherland et al., 1997). Interestingly, in this region, pharmacological potentiation of AMPA receptor responses reverses the deficits of alcohol-exposed animals in the Morris water maze, a behavior that is dependent on LTP in the hippocampus (Vaglenova et al., 2008). Surprisingly, few studies have investigated the effects of alcohol on LTD. Using rat hippocampal slice preparations, Izumi and colleagues (2005) demonstrated that exposure to alcohol at P0 or P7 (2 injections of 2.5 g/kg s.c.; blood ethanol concentration [BEC] = ~500 mg/dl) can disrupt LTD in rats at P30. In contrast, according to a different study, exposure to moderate levels of alcohol (BEC = ~200 mg/kg) encompassing rat gestation did not affect LTD in vivo (Titterness and Christie 2008). Much less is known about the effects of early alcohol exposure on LTP and LTD in the neocortex. Our findings suggest that EtOH may severely affect LTP but that the effects on LTD are likely layer specific.

In conclusion, we have shown that early alcohol exposure impairs ODP in layer 4 by disrupting the potentiation of the experienced eye responses, while sparing the depression of the deprived eye responses. However, in layer 2/3, depression of responses was disrupted suggesting a layer-specific effect of developmental alcohol exposure. Together with our previous study, these results indicate that animals exposed to early alcohol have impaired plasticity in layer 2/3 (as show by ODP and MD), while they maintain LTD-like plasticity in layer 4 (Lantz et al., 2012). This is the first study to report a disconnect between layer-specific plasticity in the primary visual cortex. These findings contribute to our understanding of neuronal plasticity deficits in FASD.

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