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Phosphodiesterase type 4 inhibition does not restore ocular dominance plasticity in a Ferret Model of Fetal Alcohol Spectrum Disorders

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Abstract

There is growing evidence that deficits in neuronal plasticity account for some of the neurological problems observed in fetal alcohol spectrum disorders (FASD). Recently, we showed that early alcohol exposure results in a permanent impairment in visual cortex ocular dominance (OD) plasticity in a ferret model of FASD. This disruption can be reversed, however, by treating animals with a Phosphodiesterase (PDE) type 1 inhibitor long after the period of alcohol exposure. Because the mammalian brain presents different types of PDE isoforms we tested here whether inhibition of PDE type 4 also ameliorates the effects of alcohol on OD plasticity. Using in vivo electrophysiology we show that inhibition of PDE4 by rolipram does not restore OD plasticity in alcohol-treated ferrets. This result suggests that contrary to PDE1, PDE4 inhibition does not play a role in the restoration of OD plasticity in the ferret model of FASD.

Introduction

There is growing evidence that deficits in neuronal plasticity account for some of the neurological problems observed in fetal alcohol spectrum disorders (FASD) (Rema and Ebner, 1999; Medina et al., 2003; Medina et al., 2005; Powrozek and Zhou, 2005; Medina and Krahe, 2008). Ocular dominance (OD) plasticity is a well-known and established model of neuronal plasticity in which changes in sensory experience promote functional and anatomical changes in the primary visual cortex (Hubel and Wiesel, 1970; Hubel et al., 1977). In higher mammals, neurons in primary visual cortex receive afferents from the left and right eyes in an alternating columnar fashion. If the eyelid of one eye is closed (monocular deprivation) during a period of development, this eye loses its ability to evoke cortical responses and later its respective ocular dominance columns shrink while the columns representing the normal (open) eye expand (Hubel and Wiesel, 1970; Hubel et al., 1977). This type of plasticity, has been widely used to study the mechanisms that underlie neural plasticity in general and shares common mechanisms with learning and memory (Berardi et al., 2003). We have recently shown that OD plasticity is permanently impaired after early alcohol exposure (Medina et al., 2003; Medina and Ramoa, 2005). After a period of monocular deprivation the visual cortex of alcohol-exposed animals fails to reorganize its connections and the deprived eye remains functional (Medina et al., 2003; Medina and Ramoa, 2005). In this respect this model has a unique role to play to test therapeutic agents that could restore plasticity in models of FASD.

Phosphodiesterase (PDE) inhibitors have been considered promising candidates as plasticity enhancers (Blokland et al., 2006; Lynch, 2002; Rose et al., 2005). They prevent the breakdown of cAMP to 5'-AMP, maintaining activation of protein kinases and thus increasing transcription of the cyclic AMP response-element-binding protein (CREB), leading to the expression of plasticity-related genes (Beavo, 1995; Bito et al., 1996; Chen et al., 2003; Deisseroth et al., 1996; Finkbeiner et al., 1997; Frank and Greenberg, 1994). Recently, we demonstrated that inhibition of PDE type 1 by the alkaloid vinpocetine successfully restored OD plasticity in a ferret model of FASD (Medina et al., 2006). However, so far, it is not known whether this restoration is solely due to PDE type 1 inhibition or if blockade of other isoforms would restore OD visual plasticity as well. Like the PDE1 isoform, PDE4 also regulates intrinsic cAMP levels and is highly expressed in the cerebral cortex and hippocampus (Rose et al., 2005). The importance of this isoenzyme for learning and memory has been demonstrated in genetic and pharmacologic studies (Rose et al., 2005). Mice lacking the subunit PDE4d show better performance in the Morris and in the radial arm mazes [54]. Furthermore, PDE4 inhibitors have been considered potential plasticity enhancers and have been tested in several different models of neurological conditions (Rose et al., 2005; Tully et al., 2003; Dyke and Montana, 1999). In particular, PDE4 inhibition by rolipram [(±)-4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone] has been shown to lower the threshold for LTP induction in the hippocampus induction, (Otmakhov et al., 2004), to reinstate LTP duration in aged mice (Barad et al., 1998), and to re-establishing LTP in a transgenic model of Alzheimer's disease (Gong et al., 2004). In addition, rolipram treatment successfully restored long-term memory in a mouse model of Rubinstein-Taybi syndrome (Bourtchouladze et al., 2003). While Rolipram has been extensively used to improve hippocampal plasticity, its effect on visual cortex plasticity is unknown. In the present study we test whether Rolipram can restore OD plasticity in a ferret model of FASD.

Material and Methods

Ferrets received 3.5 g/Kg alcohol i.p. (25% in saline) or saline as control every other day between postnatal day (P) 10 to P30, which is roughly equivalent to the third trimester equivalent of human gestation (Clancy et al., 2001; Medina et al., 2003; Medina et al., 2005). This alcohol treatment leads to a blood alcohol level (BAL) of approximately 250 mg/dl after 1–5 hours after injection (Medina et al., 2003; Medina and Ramoa, 2005).

Following a prolonged alcohol-free period (10–15 days), four groups of ferrets had the lid of the right eye sutured closed (monocular deprivation) for four days and were examined for ocular dominance changes at the end of the period of deprivation. One day before the start of the monocular deprivation all animals were administered (i.p.) with Rolipram or vehicle (DMSO solution). Three different doses of Rolipram were used in this study: 0.5, 1.25 and 2.5 mg/Kg. The selection of this dose range is based on previous studies using Rolipram to investigate hippocampal plasticity (Barad et al., 1998; Crowe et al., 2009; Rutten et al., 2007; Zhang and O'Donnell, 2000). The treatment with Rolipram or vehicle was done daily until the electrophysiology experiment (total of 5 days of treatment, Figure 1a). Table I summarizes the number of animals used in our study.

Quantitative single-unit electrophysiology was performed to assess OD changes in the left hemisphere as described elsewhere (Medina et al., 2003; Mower et al., 2002). Briefly, animals were anesthetized with sodium pentobarbital (35mg/kg, Abbott Laboratories, North Chicago, IL) and single-unit recordings were conducted using a microelectrode lowered into the binocular region of the primary visual cortex. Ocular dominance, spontaneous activity and number of spikes per stimulus were quantitatively determined for each cell by presenting a computer-controlled bar of light to each eye. Each stimulus presentation consisted of the bar of light moving across the receptive field at the optimal orientation in one direction and back across in the opposite direction (Figure 1b). Spikes were collected during the 10 stimulus presentations by a computer using Spike 2 software (Cambridge Electronics Design, Cambridge, UK) and peristimulus histograms were generated. Spontaneous activity was determined by recording activity in the absence of stimulation. At the conclusion of the experiment, ferrets were killed with 0.6ml/kg of a solution of euthasol (Demalva labs, Virginia) containing pentobarbital sodium (390mg/ml) and phenytoin sodium (50mg/ml). To quantify cortical neuron OD, an index was calculated for each cell using the following equation: $LE/(LE+RE)$, where LE stands for response to stimulation of left eye and RE for right eye. An OD index of 1.0 indicates that a neuron is responsive only to the left eye, and an OD index of 0.0 indicates responses only to the right eye. To quantify the changes in ocular dominance after monocular deprivation, we calculated a contralateral bias index (CBI) for each animal as $((P_{0.00-0.19} - P_{0.80-1.00}) + (P_{0.20-0.39} - P_{0.60-0.79})/2 + 100)/200$, where P_{A-B} denotes the percentage of cells with OD indices between *A* and *B*. A CBI of 0 indicates that the ipsilateral eye dominated the responses in every neuron tested whereas a CBI of 1 indicates that the contralateral eye dominated every neuron. Table I displays the number of cells collected from each experimental group. Comparison of the CBIs displayed on Figure 3 was done by a Univariate ANOVA using Exposure (Saline or Ethanol), and MD (with or without MD) as factors. In addition, a second Univariate ANOVA was done considering each group independently (Saline, Saline+MD, Ethanol, Ethanol+MD, Ethanol+MD+0.5 Rolipram, Ethanol+MD+1.25 Rolipram, Ethanol+MD+2.5 Rolipram). Post hoc analysis was done by Bonferroni test.

Visual stimulation and Spontaneous activity was analyzed by a Univariate ANOVA using Exposure (Saline or Ethanol) and Treatment (0, 0.5, 1.25 or 2.5 mg/Rolipram) as factors. A second ANOVA was done combining all Rolipram treatments into one group. Thus, in this case the factor Treatment was either vehicle or Rolipram. All procedures described in this paper were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Results

Following three days of deprivation, extracellular recordings were conducted in the binocular region of the primary visual cortex of ferrets. To quantify OD of cortical neurons, we calculated an ocular dominance index as described in the methods section. An ocular dominance index of 1.0 indicates that a neuron is responsive only to the left (open) eye, and an ocular dominance index of 0.0 indicates responses only to the right (deprived) eye.

The primary visual cortex of normal ferrets is characterized by a large number of neurons responsive to visual stimulation of both eyes, with the majority of neurons responding to stimulation of the contralateral eye (Issa et al., 1999; Krahe et al., 2005; Medina et al., 2003; Medina and Ramoa, 2005). Figures 2a and 2b show the typical ocular dominance profile in Untreated and Ethanol treated ferrets that received normal visual experience. Note the high proportion of binocular cells and contralateral eye dominance. As expected, monocular deprivation of control animals resulted in a marked shift towards the ipsilateral (open) eye (Figure 2c).

Monocular deprivation had relatively little effect on OD in Alcohol treated ferrets (Figure 2d), confirming our previous findings that early alcohol exposure induces a long-lasting impairment in OD plasticity (Medina et al., 2003; Medina and Ramoa, 2005). To test whether a PDE type 4 inhibitor would be able to restore OD plasticity, we assessed the effects of Rolipram on monocular deprived Alcohol treated animals. Surprisingly, Rolipram did not restore OD plasticity in Ethanol treated animals even when higher doses were used. Figures 2e–g show that several neurons remained responsive to the contralateral (deprived) eye after four days of monocular deprivation, regardless of the dose used. Note that the OD profiles in animals treated with Rolipram (Figure 2e–g) are similar to the OD profile observed in animals that only received alcohol (Figure 2d). The CBI values of Alcohol treated animals that received Rolipram confirm these results (Figure 3). A CBI of 0.0 indicates that the ipsilateral eye dominated all cells measured while a CBI of 1.0 means that the contralateral eye dominated all cells (see methods). Figure 3 shows the mean ($\pm$ SEM) and distribution of CBI scores for the left hemisphere of individual animals from all groups. The ANOVA showed a significant effect of MD ($df=1$, $df\ error=39$, $F=23.315$, $P<0.001$) as well as an interaction between MD and exposure type ($df=1$, $df\ error=39$, $F=5.5$, $P<0.05$). As expected, four days of visual deprivation (right eye) induced a shift in CBI values (towards 0.0) in control animals (Oneway ANOVA, $df=6$, $df\ error=36$, $F=5.7$, $P<0.001$; Saline vs. Saline +MD, $P<0.001$, Bonferroni), but not in Ethanol treated ones (Ethanol vs Ethanol+MD, $P=1.0$, Bonferroni). Likewise, the majority of Ethanol treated animals that received Rolipram did not show a shift in CBI values, regardless of the doses tested ($P=1.0$ for all comparisons, Bonferroni).

We also made use of extracellular single-unit recordings to verify whether Rolipram treatment resulted in abnormal visually driven activity. Figure 4 shows the quantitative assessment of responses to visual stimulation (Figure 4a) and levels of spontaneous neuronal activity (Figure 4b) in monocular deprived animals that received Saline, Ethanol or Ethanol + Rolipram treatments. No significant differences in mean maximal responses nor in spontaneous activity were observed between groups (Oneway ANOVA; maximal visual responses, $df=4$, $df\ error=27$, $F=0.96$, $P=0.45$; spontaneous activity, $df=4$, $df\ error=27$, $F=2.19$, $P=0.097$). However, an Oneway ANOVA considering all Rolipram doses combined indicated a significant reduction in spontaneous activity levels of Rolipram treated animals when compared to levels observed in monocular deprived Saline and Ethanol treated animals that did not receive Rolipram: Ethanol+Rolipram+MD (0.59 ± 0.065) vs. Saline+Vehicle +MD (0.78 ± 0.099) vs Ethanol+Vehicle+MD (0.73 ± 0.12); ($df=2$, $df\ error=29$, $F=4.8$, $P=0.036$). Another indication of the action of the drug comes from the fact that all animals that received Rolipram presented vomiting minutes after its administration, a common side

effect observed in ferrets (Robichaud et al., 2001) and humans (Bertolino et al., 1988). None of the control animals displayed such behavior. Taken together our results indicate that PDE type 4 inhibitors do not play a role in restoring OD visual plasticity in the ferret model of FASD.

Discussion

Our attempt to restore OD plasticity using a PDE4 inhibitor relies on its effects on the cAMP cascade (Blokland et al., 2006; Ghavami et al., 2006) and on evidence that alcohol exposure during development affects cAMP metabolism (Chen et al., 2006; Karacay et al., 2007). However, while the effects of ethanol on the cAMP metabolism are well described in adult animals, much less is known about its effects during brain development. Recently, it has been shown that an increase in cAMP production can protect the developing brain from alcohol-triggered apoptosis. Application of forskolin, which increases cAMP levels through activation of adenylyl cyclase (AC), reduces alcohol-induced cell death in cultured cerebellar granule neurons (Karacay et al., 2007). Conversely, reduction of cAMP production by deletion of AC1 and AC8 genes enhances cell death in mice exposed to alcohol (Maas Jr et al., 2005; Karacay et al., 2007). Consistently with these results, Chen and colleagues showed that ethanol lowers cAMP levels, resulting in cell death in hypothalamic cell cultures (Chen et al., 2006). Reduced levels of cAMP can disrupt several cellular functions and, in particular, CREB activation (Bito et al., 1996; Josselyn and Nguyen, 2005; Lamprecht, 2005). In fact, early alcohol exposure can lead to a persistent decrease in pCREB levels in the hippocampus (Roberson et al., 2009), which could account for the problems of spatial learning seen in FASD (Clements et al., 2005; Goodlett and Peterson, 1995; Richardson et al., 2002). Therefore, trying to restore OD plasticity in alcohol exposed animals increasing cAMP levels using a PDE4 inhibitor seems to be a reasonable approach.

Surprisingly, however, our results demonstrate that, contrary to PDE1 (Medina et al., 2006), PDE4 inhibition does not restore OD plasticity in ferrets treated with alcohol. While both isoforms can be found in the visual cortex and act on the cAMP metabolism (Bailey et al., 2004; Beavo, 1995; Bender and Beavo, 2006; Rose et al., 2005; Van Staveren et al., 2001), these two types of PDE have distinct mechanisms of activation and can affect different cascades. PDE1 is activated by Ca^{++} and Calmodulin and its inhibition prevents the breakdown of cAMP to 5'-AMP and of cGMP to 5'-GMP. Since PDE4 inhibition affects only cAMP levels one may suggest that the restorative effects seen with vinpocetine are due to changes in cGMP. Another possibility is related to the fact that, while PDE type 1 is activated by Ca^{++} and Calmodulin, PDE type 4 is activated by cAMP itself, working in a feedback mechanism that is only triggered when cAMP levels are high. Therefore, a possible explanation for the lack of a restorative effect after Rolipram treatment is that cAMP levels are already low in FASD. Our recent findings that CREB phosphorylation is decreased in the ferret model of FASD support this hypothesis (Krahe et al., 2008). However, we cannot discard the possibility that the range of the Rolipram doses used here was not appropriate. Considering that this is the first study to investigate the role of Rolipram in visual cortex plasticity, our dose choices were based on studies that have used this drug to improve hippocampal plasticity.

The role of rolipram in learning and memory has been extensively tested and results were obtained with a wide range of doses (Barad et al., 1998; Crowe et al., 2009; Rutten et al., 2007; Zhang and O'Donnell, 2000). Barad and colleagues showed that rolipram at either 0.03 or 0.8 mg/Kg doses facilitated the potentiation and latency of LTP. However, the lowest (0.03 mg/Kg) dose was more efficient than the higher one (Barad et al., 1998). In contrast, many other studies show that very low doses (such as 0.01 to 0.03 mg/Kg) are not as effective as higher ones (0.2–2.0 mg/Kg) (Crowe et al., 2009; Rutten et al., 2007; Zhang and O'Donnell, 2000). For instance, while both 0.05 and 1.0 mg/Kg doses of Rolipram successfully reverse scopolamine induced learning deficits in the radial arm maze, lower doses (0.01 and 0.025 mg/Kg) fail to show an effect (Zhang and O'Donnell, 2000). Similarly, 0.1 mg/Kg, but not 0.01 or 0.03 mg/Kg doses of Rolipram improve object recognition in a model of acute tryptophan depletion (Rutten et al., 2007). Finally, it has been recently shown that only doses in the range of 0.25 to 2.0 mg/Kg are able to improve passive avoidance learning in chicks (Crowe et al., 2009). Collectively, these findings suggest that, although we cannot restore OD plasticity in the ferret of model of FASD, the Rolipram dosage used in the present study seems to be appropriate. However, while unlikely, we cannot discard the possibility that doses of Rolipram lower than 0.5 mg/Kg could have an effect.

In conclusion, our results suggest that PDE4 inhibition, for the doses used here, does not restore OD plasticity in the ferret model of FASD. These findings could be explained by: i) the possibility that PDE4 is already inactive in alcohol treated animals due to lower cAMP levels; or ii) the need of activation of different signaling pathways, such as the cGMP cascade. Nonetheless, future studies are needed to investigate the role of other PDEs isoforms in the restoration of OD plasticity in animal models of FASD.

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Figure 1.

Experimental design. (A) Ferrets were exposed to 3.5g/Kg of 25% Ethanol i.p. every other day between P10 to P30 (roughly the third trimester equivalent of human gestation). Animals received either Rolipram (0.5, 1.25 or 2.5 mg/Kg) or Vehicle for 4 days during the peak of the critical period of ocular dominance plasticity. After the first Rolipram dose, animals were monocular deprived (right eye) and ocular dominance plasticity (OD) evaluated by extracellular electrophysiology after 4 days. (B) During electrophysiology experiments, animals were deeply anesthetized, positioned in a stereotaxic frame facing a translucent tangential screen and a microelectrode was lowered into their left primary visual cortex. Under computer control, a moving bar of light (0.5° wide and 20° long) was presented to each eye individually at the optimal orientation. One stimulus presentation consisted of the bar of light moving across the receptive field in one direction and back across in the opposite direction. Evoked spikes were collected by a computer (10 stimulus presentations) and all data were from cells with receptive fields in the binocular region of the visual field.

Figure 2.

Ocular dominance (OD) histograms for neurons from all animal groups tested. An index of 0.0 and 1.0 indicate that a neuron responds only to the contralateral (right) or the ipsilateral (left) eye respectively. Intermediate scores represent binocular cells. The primary visual cortex of Saline (A, n=6 ferrets) and Ethanol (B, n=4) treated animals that had normal binocular visual experience (no MD) were characterized by a large number of binocular cells with several neurons dominated by the contralateral eye. Four days of MD of Saline treated animals (C, n=10) induced a marked shift in OD distribution in the direction of the non-deprived ipsilateral eye. In contrast, the same period of MD in animals treated with alcohol induced only a slight OD shift with most neurons still driven by the deprived (contralateral) eye (D, n=8). Rolipram treatment did not restore OD plasticity in alcohol exposed animals (E, n=5; F, n=6; G, n=4). Note that the OD profile of Ethanol treated animals monocularly deprived for four days is very similar to the OD profile of Ethanol-treated animals that received Rolipram treatment. Error bars indicate $\pm$ SEM.

Figure 3.

Contralateral bias index scores (CBI) are plotted for the same animal groups displayed in Figure 2. Bars represent means ($\pm$ SEM), and each open circle represents an individual animal. In Saline and Ethanol treated animals that were allowed normal binocular vision (no MD) most CBIs were higher than 0.5, reflecting a predominance of the experienced (open) eye. In contrast, CBI values of Saline animals monocularly deprived for 4 days were markedly reduced relative to non-deprived animals, indicating a marked predominance of response to stimulation of the non-deprived eye. Monocular deprivation in alcohol exposed animals did not induce such effect, with CBI values similar to non-deprived animals, indicating impaired OD plasticity. Four days of MD in alcohol exposed animals treated with Rolipram failed to restore OD plasticity. For all doses used, CBI values were similar to values observed in monocularly deprived alcohol exposed animals.

Figure 4.

Visual responses of neurons in the primary striate cortex to a moving bar of light in monocularly deprived animals. (A) Mean maximal response (in spikes per run) of cortical neurons to stimulation at the optimal orientation. (B) Mean spontaneous neuronal activity in the absence of visual stimulation. Error bars indicate $\pm$ SEM.

Table 1

Number of Animals and Cells Used in Each Group

Group	Number of animals	Number of cells
Saline	6	190
Ethanol	4	134
Saline + MD + vehicle	10	308
Ethanol + MD + vehicle	8	193
Ethanol + MD + 0.5 mg/kg rolipram	5	127
Ethanol + MD + 1.25 mg/kg rolipram	6	144
Ethanol + MD + 2.5 mg/kg rolipram	4	110
Total	43	1206