Int. J. Devl Neuroscience 52 (2016) 75–81

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Effects of developmental alcohol and valproic acid exposure on play behavior of ferrets

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ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 11 March 2016
Accepted 15 March 2016
Available online 18 May 2016

Keywords:
Fetal alcohol syndrome
Social behavior
Valproate
Autism
FASD

ABSTRACT

Exposure to alcohol and valproic acid (VPA) during pregnancy can lead to fetal alcohol spectrum disorders and fetal valproate syndrome, respectively. Altered social behavior is a hallmark of both these conditions and there is ample evidence showing that developmental exposure to alcohol and VPA affect social behavior in rodents. However, results from rodent models are somewhat difficult to translate to humans owing to the substantial differences in brain development, morphology, and connectivity. Since the cortex folding pattern is closely related to its specialization and that social behavior is strongly influenced by cortical structures, here we studied the effects of developmental alcohol and VPA exposure on the play behavior of the ferret, a gyrencephalic animal known for its playful nature. Animals were injected with alcohol (3.5 g/kg, i.p.), VPA (200 mg/kg, i.p.) or saline (i.p) every other day during the brain growth spurt period, between postnatal days 10 and 30. The play behavior of pairs of the same experimental group was evaluated 3 weeks later. Both treatments induced significant behavioral differences compared to controls. Alcohol and VPA exposed ferrets played less than saline treated ones, but while animals from the alcohol group displayed a delay in start playing with each other, VPA treated ones spent most of the time close to one another without playing. These findings not only extend previous results on the effects of developmental exposure to alcohol and VPA on social behavior, but make the ferret a great model to study the underlying mechanisms of social interaction.

1. Introduction

Fetal alcohol spectrum disorder (FASD) and fetal valproate syndrome are neurodevelopmental disorders (Lindhout and Schmidt, 1986; May et al., 2014; Ornoy, 2009; Rihtman et al., 2013; Riley et al., 2011) characterized by behavioral and physiological abnormalities that include sensory processing deficits, which in turn are associated with deficits in social interaction and autistic behavior (Carr et al., 2010; Nanson, 1992; Rihtman et al., 2013; Stevens et al., 2013; Williams et al., 2001). While there is extensive evidence that developmental exposure to alcohol or valproic acid (VPA) can remarkably affect social interactions in rodents (Meyer and Riley, 1986; Kelly et al., 2000; Varlinskaya and Mooney, 2014; Burenkova et al., 2014; Yochum et al., 2008; Charles Lawrence et al., 2008), much less is known about the effects of these teratogenic drugs in the social behavior of gyrencephalic species. A more complex cortex leads to more sophisticated behavior, which might be even more susceptible to the effects of teratogens. Therefore, studies of social behavior in gyrencephalic species would be a good addition to the insightful results already demonstrated in the rodent model. Particularly, the ferret is a good candidate because of their social and playful traits (Stockman et al., 1986; Rabe et al., 1985) and because ferrets share elements of cortical organization that are more common in humans than in rodents, such as the presence of ocular dominance and orientation selectivity columns in the visual cortex (Medina et al., 2003, 2005; Krahe et al., 2005), clear tonotopic maps in the auditory cortex (Bizley et al., 2005), and a somatosensory cortex that is less dominated by vibrissae (Juliano et al., 1996; Keniston et al., 2009; Foxworthy and Meredith, 2011).

We have demonstrated that ferrets exposed to ethanol during the "brain growth spurt", a period of increased synaptogenesis that occurs during the first postnatal weeks in rodents, and during the third trimester of gestation in humans, present severe and long-lasting deficits in sensory function (Krahe et al., 2009; Medina et al., 2003, 2005). Some of these deficits, such as the disruption of orien-

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http://dx.doi.org/10.1016/j.ijdevneu.2016.03.007
0736-5748/

tation selectivity, were also observed after exposure to VPA during the same developmental period (Pohl-Guimaraes et al., 2011). It is not known, however, whether these animals also exhibit social interaction deficits. To examine this possibility, here we investigated the social behavior of juvenile ferrets exposed to alcohol or VPA early during development. Specifically, we hypothesize that ferrets exposed to alcohol or VPA during the brain growth spurt will play less with their peers than controls.

2. Materials and methods

2.1. Animals

Lactating ferrets with litters were bought from Marshall Farms (New Rose, NY) and housed in a 12:12 light-dark cycle. Food and water were available ad libitum. Kits received a single i.p. injection of alcohol (ALCOH; 3.5 g/kg alcohol, i.p., n = 8), valproic acid (VPA; 200 mg/kg, i.p., n = 10), or an equivalent volume of saline (SAL; i.p., n = 12) every other day starting on P10 and ending on P30. Regarding cortical development, this period is roughly similar to P4-P10 in rodents and do the third trimester of human gestation (Clancy et al., 2001; Medina et al., 2003, 2005), and encompasses the time when several intracortical, thalamocortical and corticothalamic connections are being formed (Bock et al., 2012; Callaway and Borrell, 2011; Dalva, 2010; McLaughlin and Juliano, 2003). Following a prolonged alcohol- and VPA-free period (21 ± 1.6 days) and well before sexual maturation (30–50 weeks of age)(Ryan and Robinson, 1987), the play behavior of 30 ferrets (6 males and 24 females from 12 litters) was evaluated. Experiments were conducted at Virginia Commonwealth University and all procedures were performed in compliance with the Institutional Animal Care and Use Committee.

2.2. Play behavior

The play behavior of juvenile ferrets (7.2 ± 0.2 weeks) was video recorded for 10 min in a glass box (70 × 40 × 45 cm). Animals were kept isolated from each other for 18 h before testing and for the evaluation of the play behavior they were paired with counterparts of the same experimental group (i.e. ALCOH vs. ALCOH, VPA vs. VPA, and SAL vs. SAL). Animal pairs could be from the same or the opposite sex, however they were always from different litters. Before each testing session the glass box was placed in the sink, thoroughly flushed with tap water and dried. We avoided using an alcohol cleaning solution since it could differentially affect animals early exposed to alcohol (Youngentob and Glendinning, 2009).

An observer, who was blind to the treatment, later scored the time animals spent: (1) playing (Play behavior, Fig. 1A), (2) in contact with each other, but without playing (Cold contact, Fig. 1B), and (3) distant from each other (No contact, Fig. 1C). Play behavior was considered as any playful interaction between animals such as biting, jumping and rolling over, pushing, or galloping toward and away from their counterpart. Cold contact, on the other hand, was scored when animals touched or leaned over each other, but without any apparent playfulness. Finally, No contact comprised behaviors made when animals were apart from each other such as walking around, sniffing, grooming, or exploring the testing chamber. Behavioral analysis was conducted within 1 min intervals for the total testing session (10 min). There were no significant effects of sex (univariate ANOVAs, Play behavior, F = 4.31, P = 0.07; Cold contact, F = 0.22, P = 0.81; No contact, F = 2.60, P = 0.15) or litter (univariate ANOVAs, Play behavior, F = 0.17, P = 0.70; Cold contact, F = 0.33, P = 0.59; No contact, F = 1.12, P = 0.33). Moreover, no significant differences between pairs of the same or opposite sex were observed regarding the total time animals interacted with each other (Same sex, 420.6 ± 22.66 s; Opposite sex, 404.12 ± 44.99 s; T-test, p = 0.72).

2.3. Statistical analysis

Data were submitted to univariate or repeated-measures ANOVAs followed by post hoc comparisons to ascertain group differences. Differences between experimental groups were also determined using Wilcoxon Mann-Whitney *U* tests. For all statistical tests, significance was set at P < 0.05 (2-tailed). Data is shown as mean and ± SEM.

3. Results

Table 1 shows offspring weight gain during alcohol and valproic acid treatments. While the mean litter weight increased significantly from P10 to P30 for all experimental groups [age: F(2.7,84.1) = 490; P < 0.001], ALCOH and VPA exhibited a slower growth than SAL [F(2.7,84.1) = 8.8, P < 0.001]. At P30, the mean offspring weight of ALCOH and VPA treated animals was, respectively, 22% and 16% smaller than that of SAL group (Table 1). There was no sex effect or interaction between sex and treatment. At the age of behavioral testing (∼P50) ALCOH and VALP animals still weighted less than SAL ones (data not shown).

Histograms depicting the distribution of the time animals spent playing (Fig. 1D, G and J), in close contact to each other but not playing (Fig. 1E, H and K), and distant from one another (Fig. 1F, I and L) are shown in Fig. 1 for ALCOH (Fig. 1D–F), VPA (Fig. 1G–I) and SAL (Fig. 1J–L) groups. While no differences were found for the distributions regarding the time animals spent without interacting with each other (Fig. 1F, I and L; SAL vs. ALCOH, U = 49,000, P = 0.97; Saline vs. VPA, U = 44,500, P = 0.68; ALCOH vs. VPA, U = 47,000, P = 0.85; Mann-Whitney *U* tests), animals from the VPA group displayed significantly more time in "cold" contact to one another along the testing session than animals treated with alcohol and saline (Fig. 1E, H and K; SAL vs. VPA, U = 13,000, P < 0.01; ALCOH vs. VPA, U = 3500, P < 0.001; Mann-Whitney *U* tests). No differences were observed between SAL and ALCOH groups (Fig. 1E and H; U = 41,000, P = 0.53; Mann-Whitney *U* test). Regarding play behavior, the distribution of the time saline treated animals spent playing was significantly different from that exhibited by the VPA group (Fig. 1D and J; SAL vs. VPA, U = 12,000, P < 0.01; Mann-Whitney *U* test), with VPA treated animals showing less time playing as well as a delay to start to do so compared to the SAL group. Similarly, although the distributions of alcohol and saline treated animals were not statically different (Fig. 1D and G; U = 45,000, P = 0.74; Mann-Whitney *U* test), ALCOH animals seemed to engage in playful behaviors later than SAL ones.

To further explore this scenario, we investigated the behavior patterns displayed by alcohol, VPA, and saline treated animals during the first and last 5 min of the testing period. Fig. 2 illustrates the average time animals spent playing (Fig. 2A–C), in cold contact (Fig. 2D–F), and apart from one another (Fig. 2G–I) for the total time (Fig. 2A, D and G), the first (Fig. 2B, E and H), and the last 5 min (Fig. 2C, F and I) of the testing session. Indeed, animals in the ALCOH and VPA groups play significantly less than saline treated animals during the first 5 min of the testing session (Fig. 2B; univariate ANOVA, F = 8.96; P < 0.01), with ALCOH animals showing an increase in play behavior by the end of the testing period (Fig. 2C). This could explain why for the total time no differences were observed between ALCOH and SAL groups (Fig. 2A). VPA treated animals on the other hand, spent significantly less time playing than the other groups (Fig. 2A–C). That does not mean they completely ignored each other, only that they rather stayed in close proximity to one another without playing (Fig. 2D–F; First 5 min, F = 7.71; P < 0.01; Last 5 min, F = 3.99; P < 0.05; Total time, F = 6.44;