1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Prenatal alcohol exposure reduces 5-HT concentration in mouse intestinal muscle and mucosa.

Katarzyna A Dylag[1], Sara V S Fidalgo[2], Paul R Gard[2] and Bhavik Anil Patel[2]

[1] Jagiellonian University Medical College, Krakow, Poland

[2]School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton UK

Corresponding author: Katarzyna Anna Dylag,   katarzyna.anna.dylag@doctoral.uj.edu.pl

Jagiellonian University Medical College, ul. sw. Anny 12 31-008 Krakow

Abstract

The influence of prenatal alcohol exposure on the serotoninergic system in the brain has been well studied, however its influence on the serotoninergic system in the gastrointestinal system remains unknown. The objective of the study was to use a mouse model of prenatal alcohol exposure to investigate the effects on serotonin and its metabolites and precursors in colonic tissue. This study used treatment of mouse breeding harems with 5% ethanol with saccharin via drinking water throughout pregnancy and compared the results with a saccharin control group. Tryptophan, serotonin (5-HT) and 5- hydroxyindoleacetic acid (5-HIAA) concentrations were measured in the longitudinal muscle myenteric plexus (LMMP) and mucosa of intestinal tissue by high-performance liquid chromatography (HPLC). Decreased 5-HT concentrations in mucosa and LMMP (females only) were observed in prenatally exposed mice compared to controls. Increases in mucosal and LMMP tryptophan concentration were only observed in prenatally exposed female mice. In conclusion, prenatal alcohol exposure causes a decrease in conversion of tryptophan to 5-HT in both muscle and mucosa although the effect is more pronounced in females. The observed sex difference may be related to changes associated with the estrous cycle.

Keywords: prenatal alcohol exposure, gastrointestinal, serotonin, tryptophan, colon, myenteric plexus

Abbreviations:

5-HT – serotonin

5-HIAA- 5- hydroxyindoleacetic acid

HPLC- high-performance liquid chromatograph

LMMP – longitudinal muscle myenteric plexus

1. Introduction

In 1973 Jones et al. described the characteristics of Fetal Alcohol Syndrome (Jones, 2011). It is now recognized that prenatal alcohol exposure causes a wide range of neurobehavioral deficits, encompassed by the term fetal alcohol spectrum disorders (Hoyme et al., 2016). Fetal alcohol syndrome is considered the most severe form of these disorders and is characterised by growth retardation, such as low birth weight, lack of weight gain over time, disproportional low weight to height and a characteristic pattern of facial anomalies, such as short palpebral fissures, thin vermillion border and flattened philtrum. There are also specific neurological signs such as impaired fine motor skills, neurosensory hearing loss and cognitive impairment (Kodituwakku, 2010; Mukherjee et al., 2006; Willford et al., 2004).

The effect of prenatal alcohol exposure on the serotonergic system in the brain has been studied specifically. Tajuddin et al. described a decrease in serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrocortex of the offspring of ethanol-fed rats (Tajuddin and Druse, 1988). It has also been demonstrated that there is a remarkable deficit of 5-HT_1 receptors in both motor and somatosensory cortex of rats prenatally exposed to alcohol (Clausing et al., 1996) and that serotonin uptake is decreased in the motor cortex (Tajuddin and Druse, 1988). The serotonergic system of the gastrointestinal tract is based on analogous mechanisms to the serotonergic system of the brain (De Ponti, 2004) however the effects of prenatal alcohol exposure have not been studied.

Clinical evidence suggests that prenatal alcohol exposure results in long-lasting effects on the gastrointestinal tract. In a Finnish cross-sectional study the morbidity of gastrointestinal system among individuals with FASD was as high as 26% (Autti-Ramo et al., 2006). Werts et al. reported constipation being a common problem among children prenatally exposed to alcohol (Werts et al., 2014) while Kvigne et al. emphasized the high prevalence of feeding problems and diarrhoea (Kvigne et al., 2009). There is also evidence that individuals with fetal alcohol spectrum disorders experience gastrointestinal motility disorders presenting as chronic pseudoobstruction syndrome (Uc et al., 1997). However limited studies have explored the mechanisms behind these functional changes in gastrointestinal motility.

5-HT is a key signaling molecular within the colon, where it is located within the enterochromaffin (EC) cells present within the mucosal epithelium and neurons within the myenteric plexus. Numerous studies have shown that intestinal serotonin is a key pro-kinetic molecule that drives motility (Bertrand and Bertrand, 2010; Gershon, 2004; Mawe and Hoffman, 2013). The aim of

this study was to use a mouse model of prenatal alcohol exposure to investigate the effects on 5-HT and its metabolites and precursors in gut tissue.

2. Material and Methods

2.1 Animal Husbandry

All procedures were licensed under the UK Animals (Scientific Procedures) Act 1986 and EU directive 2010/63/EU and complied with the ARRIVE guidelines. They were approved by the University of Brighton animal welfare and ethics review board. C57BL/6J mice were maintained at 19.0 ± 1 °C, 55 % humidity and fed on either a breeding diet (RM3 (E) 801002 chow, Special Diet Services) (breeding harems) or a maintenance diet (RM1 (E) 801002 chow, Special Diet Services) (off-spring) *ad libitum*. The mice were maintained on a 12-hour light/ dark schedule, lights on 0700h (60 Lux at cage level).

2.2 Prenatal Alcohol exposure (PAE)

Breeding harems were established with one male to 3-4 females. Following an adaptation of the maternal ethanol consumption model described by Kleiber et al. (Kleiber et al., 2011), the harems received fluid *ad libitum* under a two-bottle choice. The alcohol exposure groups had 24 hour access to both a bottle of 5% ethanol sweetened with 0.066% saccharin solution and a bottle of tap-water. In order to eliminate the influence of saccharine on the experiment results, the control group had 24 hour access to a bottle of 0.066% saccharin solution and a bottle of tap-water. The volume of liquid consumed from each of the two bottles in each cage was recorded daily. It was assumed that the consumption was approximately equal between different mice within each cage in order to estimate individual alcohol consumption. Offspring were weaned at 20 days and group-housed in same-sex, littermate cages with free access to food and tap-water. The offspring have not been exposed to alcohol since weaning. Thus, ethanol exposure was from pre-conception, throughout gestation and throughout lactation. It must be remembered that there is evidence of a detrimental effect of ethanol on the fetus in the very early stages of pregnancy, but also in the last trimester in humans, which equates to the first few post-natal days in rodents. This animal model of PAE was previously described by Allen et al.(Allan et al., 2003a)

2.3 Blood alcohol concentration determination

Blood alcohol concentration was determined by gas chromatography. Briefly, blood was collected by cardiac puncture post mortem from a small sample of male and female ex-breeders between 1100h and 1300h. Plasma samples were deproteinated with 10% trichloroacetic acid spiked with 1 % ethanol and centrifuged at 9400 g for 10 minutes. The supernatant was filtered before gas

chromatography analysis. Standards and samples, both with an internal standard (1% propan-1-ol), were analysed on a Perkin Elmer Clarus 500 gas chromatograph with a Zebron Phase (ZB-waxplus column), equipped with a flame ionization detector. The optimal operating conditions were as follows: oven temperature 35°C and flame ionisation detector temperature 150°C, with the injection temperature 250°C and the capillary temperature 250°C; hydrogen and air pressure were set at optimal conditions of 58psi and 60psi, respectively.

2.4 Tissue sampling and analysis

Colonic mucosa and LMMP tissue samples were harvested from the progeny of the breeding harems under alcohol exposed and control conditions at 3-6 months of age and stored at -80 Celsius.

To measure levels of tryptophan, 5-HT and 5-HIAA, tissue samples were placed in 0.1M perchloric acid. The mixture was then centrifuged at 13,200 g at 4 C for 10 min and the resultant supernatant was analyzed using HPLC. HPLC apparatus consisted of a Jasco HPLC pump (Model: PU-980) and Rheodyne manual injector equipped with a 20 μl loop. A Kinetic ODS 2.6 mm 100 mm x 2.1 mm i.d. analytical column with an in-line guard column (Phenomenex, Macclesfield, UK) was employed. The HPLC system was run at a flow rate of 100 mL min^{-1}. CHI630B potentiostat (CH Instruments, Austin, TX, USA) was used to control the detector voltage and record the current. A 3-mm glassy carbon electrode (flow cell, BAS) served as the working electrode and was used with a Ag|AgCl reference electrode and a stainless steel block as the auxiliary electrode. Amperometric recordings were carried out, where the working electrode was set at a potential of +950 mV vs. Ag|AgCl reference electrode. Control and data collection/processing were handled through the CHI630B software. The stock buffer for the mobile phase was comprised of the following: 0.1 M sodium acetate, 0.1 M citric acid and 27 mM disodium ethylene-diamine-tetra-acetate (EDTA). This was then buffered to pH 3.0. The mobile phase was prepared with the stock buffer mixed with methanol in the ratio of 8 : 2 (v/v) and degassed after mixing.

2.5 Data Analysis

The peak areas for the responses for 5-HT, 5-HIAA and tryptophan were measured from the chromatograms and converted to concentration using calibration plots as previously shown (Parmar et al., 2011). The concentration of tryptophan, 5-HT and 5-HIAA in colonic LMMP and mucosa from male and female offspring were analysed using 2-way analysis of variance followed by Tukey post-hoc analysis. P values less than 0.05 were deemed to indicate significant differences.

3. Results

3.1 Alcohol intake and blood alcohol levels

Seven breeding cages were monitored: three control cages and four PAE cages. Daily fluid consumption (per animal) was recorded over 10 weeks and plotted as a daily average over a weekly period and a measure of alcohol intake per weight of animal was calculated for female breeders; the average ethanol intake for the female breeders was 8.9±0.4 g/kg/24hours. The total volume of fluid ingested was significantly lower in the alcohol group compared to control (p<0.0001, unpaired t-tests). Only 3 of the 5 blood samples tested had measurable blood alcohol concentrations, with values of 1.885 mg/dL, 6.549 mg/dL and 4.793 mg/dL, giving a mean value (± s.e.m.) of 2.65± 1.31 mg/dL. Progeny weight was determined prior to sacrifice: overall there was no difference in weight between control and PAE animals. Data from other publications using similar ethanol dosing schedules have measures blood alcohol concentrations at the following the period of maximum intake. For example Brady et al. (Brady et al., 2012a) and Allan et al. (Allan et al., 2003b) report peak blood alcohol concentrations of 80-120 mgl100ml using similar doses of alcohol. Our quoted values were taken at the time of lowest expected blood alcohol concentration and are given to illustrate that there is constant low-dose exposure on the fetus.

3.2 Mucosa

Tissues were harvested from 4 male and 4 female mice of the saccharine control group and 4 male and 3 females of the prenatal alcohol exposure group (??)

Synthesis of 5-HT

The highest mean concentration of tryptophan in mucosa was observed in the group of female mice exposed to alcohol (67.78 μmol/l +6.74) followed by females from the saccharine group (35.99 μmol/l +11.09), males exposed to alcohol (31.25 μmol/l +16.86) and males from the saccharine group (25.45 μmol/l +16.00). There was a statistically significant difference in mean mucosal tryptophan concentration between females from alcohol and control group (p<0.05) and males and females within the alcohol exposed group (p<0.05) [Figure 1A].

A difference in 5-HT/tryptophan ratio was observed in both sexes. The mean ratios in alcohol exposed group and saccharine group were 0.0018 (+0.0015) vs. 0.078 (+0.046), respectively in females (p<0.001) and 0.0017 (+ 0,0008) vs. 0.12 (+ 0,048) in males, respectively (p<0.01) [Figure 1D].

Levels of 5-HT

Among the female mice there was a significant (p=<0.05) decrease in mean mucosal 5-HT concentration in the ethanol-exposed group in comparison to control group (0.13 μmol/l + 0.10 vs. 3.06 μmol/l + 1.99). The same effect was observed in male mice, the mean 5-HT concentration in the

exposed group (0.044 µmol/l + 0.01) was more than 100-times lower than in the control group (3.15 µmol/l + 2.29) [Figure 1B].

Turnover of 5-HT

There was no significant difference in mean 5-HIAA concentration in the mucosa between alcohol exposed and control group or between male and female mice in both groups [Figure 1C].

In both sexes there was a significant difference in 5-HIAA/5-HT ratio. In males the mean 5-HIAA/5-HT ratio was 25.2 (+7.32) in the alcohol group and 0.525 (+ 0.37) in the control group, p<0.001 while in females the ratios were 31.27 (+13.28) and 0.97 (+0.87), respectively (p<0.05) [Figure 1E].

3.3 LMMP

Synthesis of 5-HT

The difference in tryptophan concentration in muscle tissue between the alcohol exposed and the saccharin group was only observed in females (81.26 µmol/l +19.15 vs. 37.48 µmol/l +16.66 respectively p<0.05). The concentrations in males were: 41.86 µmol/l +20.99 and 14.1 µmol/l +8.02, respectively [Figure 2A].

A 5-HT/Tryptophan ratio was significantly lower in alcohol exposed groups in both sexes. In females the ratios were 0.0003 +0.0002 and 0.042 +0.02, respectively (p<0.01). In males the ratios were 0.0003 + 0,0002 and 0.054 +0.02, respectively (p<0.05) [Figure 2D].

Levels of 5-HT

Similarly, the difference in 5-HT concentration in muscle between alcohol and control group was noted only in females (0.02 µmol/l +0,0 vs. 1.3 µmol/l +0.72, respectively, p<0.05). The concentrations observed in males were: 0.01 µmol/l +0.01 and 0.05 + 0.02, respectively [Figure 2B]

Turnover of 5-HT

There was no difference in 5-HIAA concentration in muscle between sexes or exposure groups. [Figure 2C]

The 5-HIAA/5-HT ratio was significantly higher in exposed females than in control females (37.96 +23.22 vs. 0.81 +0.39, respectively, p<0.001). Interestingly, there was also a difference between alcohol exposed females and alcohol exposed males (37.96 +23.22 vs. >50.86 + 22.36 respectively, p<0.05) [Figure 2E].

4. Discussion

The dose of alcohol used in the current study is lower than that used in many prenatal alcohol exposure models. The average ethanol intake per breeding female was approximately 9g/kg/day. Taking into account the different apparent volumes of distribution for ethanol (total body water) in female humans and mice (0.63 l/kg v. 0.8 l/kg) and the greater rate of ethanol elimination in mice (Cederbaum, 2012), the ethanol intake in the current study equates to a human daily ethanol intake of approximately 8g, the equivalent of less than one bottle of table wine. Similar daily consumptions in mice have been reported as resulting in blood alcohol concentrations averaging 80 to 120 mg/100ml (Allan et al., 2003b; Brady et al., 2012b). Our results identified concentrations significantly lower than these, which could be explained by rodents drinking primarily during the dark phase (which in the current would be 1900-0700h) compared to blood sampling some 5 hours later. Allan et al. (Allan et al., 2003b) monitored blood alcohol concentrations of B6SJL/F1 mice over a 24 hour period (with lights on 0700-1900) and found that administration of 14 g/kg/day of ethanol, resulted in peak blood alcohol concentrations of 140 mg/100ml at approximately 0100h and minimum concentrations of 50 mg/100ml between 0900-1200h. Our blood collection was conducted at the time of lowest blood alcohol and it revealed very variable blood alcohol concentrations, although a mean of approximately 0.265 mg/100ml clearly indicates that our 9g/kg/day average-model results in lower-dose exposure to alcohol than the 14/g/kg/day.

This is the first study to evaluate the influence of low-dose prenatal alcohol exposure on the serotoninergic system in colonic tissue

We observed a decrease in 5-HT in the mucosa among prenatally exposed male and female mice together with an increased concentration of tryptophan in the mucosa of the females. In the LMMP, we observed a significant decrease in 5-HT in females but not in males albeit the trend was similar. There were increased tryptophan levels in female mice only. The decreased 5-HT synthesis in both mucosa and muscle could result from decreased tryptophan hydroxylase and/or amino acid decarboxylase function and result in further tryptophan accumulation. If this decrease is due to tryptophan hydroxylase, this would suggest that both tryptophan hydroxylase 1 and 2 are both altered due to prenatal exposure to alcohol. This finding is in contrast with previous reports which demonstrated a decrease only in tryptophan hydroxylase 1, in the brain. Moreover, in our study we observed no decrease in 5-HIAA, which suggests that serotonin transporter SERT in the intestinal tissue is not affected by prenatal alcohol exposure, while in previous reports (Zafar et al., 2000) the function of SERT was decreased in the brain. This finding might be explained by the differences in development and maturation of serotoninergic system in the brain and in the intestinal tissue (Gershon & Erde, 1981).

That the observed decrease in 5-HT concentration in mucosa is not a result of increased monoamine oxidase activity is demonstrated by the fact that there is no increase in 5-HIAA concentrations nor an increase in 5-HIAA/5-HT ratios.

In muscle the same trend was observed, although the difference in 5-HT concentration between male mice prenatally exposed to alcohol and male controls is not statistically significant. However the tryptophan accumulation is observed in both sexes.

Again the decreased gastrointestinal muscle 5-HT is probably due to decreased tryptophan hydroxylase activity rather than increased release and metabolism of 5-HT. The results therefore indicate that prenatal alcohol exposure results in decreased synthesis of 5-HT in gastrointestinal muscle and mucosa at 3-6 months of age (young adult), several months after the last exposure to alcohol.

Previous work by Clausing et al. (Clausing et al., 1996) indicated a sex difference in the effects of prenatal alcohol exposure with a decrease in striatal 5HT and 5HIAA seen in females only. On the other hand, Asghari et al. (Asghari et al., 2011) reported differences in tryptophan hydroxylase 1 activity in the brain throughout estrous cycle and López-Contreras et al. reported sex differences in dihydroxyphenylalanine decarboxylase activity in the intestine between the sexes (López-Contreras et al., 2008). There is also evidence for sex differences in serotonergic gastrointestinal system among humans. Viramontes et al.(Viramontes et al., 2001) documented different responsiveness to 5-HT3 antagonist (alosetron) among females and males. Houghton et al. studied 5-HT concentration in platelet-depleted plasma of both IBS and healthy male and females (Houghton et al., 2009). The authors documented the influence of oestrous cycle on 5-HT levels. Due to ethical reasons, direct measurement of mucosal and muscle 5-HT and its metabolites have not been performed on humans. The sex difference observed in the current study may therefore reflect a sex difference consequent to variations in female reproductive hormones.

In conclusion, clinical evidence suggests that children with fetal alcohol spectrum disorders (FASD) are more likely to suffer from disorders associated with decreased gut motility such as constipation or pseudoobstruction syndrome. The results of the current study in a mouse model of FASD indicates that low-dose prenatal alcohol exposure results in decreased conversion of tryptophan to 5-HT, and therefore reduced tissues storage of 5-HT. Such transmitter depletion might be expected to be associated with decreased gut motility. Strategies to overcome the 5-HT deficit, such as monoamine oxidase inhibitors, may therefore be of use in the treatment of these children.

References:

Allan, A.M., Chynoweth, J., Tyler, L.A., Caldwell, K.K., 2003a. A Mouse Model of Prenatal Ethanol Exposure Using a Voluntary Drinking Paradigm. Alcohol. Clin. Exp. Res. 27, 2009–2016. doi:10.1097/01.ALC.0000100940.95053.72

Allan, A.M., Chynoweth, J., Tyler, L.A., Caldwell, K.K., 2003b. A Mouse Model of Prenatal Ethanol Exposure Using a Voluntary Drinking Paradigm. Alcohol. Clin. Exp. Res. 27, 2009–2016. doi:10.1097/01.ALC.0000100940.95053.72

Asghari, R., Lung, M.S.Y., Pilowsky, P.M., Connor, M., 2011. Sex differences in the expression of serotonin-synthesizing enzymes in mouse trigeminal ganglia. Neuroscience 199, 429–437. doi:10.1016/j.neuroscience.2011.10.036

Autti-Ramo, I., Fagerlund, A., Ervalahti, N., Loimu, L., Korkman, M., Hoyme, H.E., 2006. Fetal alcohol spectrum disorders in Finland: clinical delineation of 77 older children and adolescents. Am. J. Med. Genet. A 140, 137–143. doi:10.1002/ajmg.a.31037

Bertrand, P.P., Bertrand, R.L., 2010. Serotonin release and uptake in the gastrointestinal tract. Auton. Neurosci. 153, 47–57. doi:10.1016/j.autneu.2009.08.002

Brady, M.L., Allan, A.M., Caldwell, K.K., 2012a. A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. Alcohol. Clin. Exp. Res. 36, 457–66. doi:10.1111/j.1530-0277.2011.01644.x

Brady, M.L., Allan, A.M., Caldwell, K.K., 2012b. A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. Alcohol. Clin. Exp. Res. 36, 457–66. doi:10.1111/j.1530-0277.2011.01644.x

Cederbaum, A.I., 2012. Alcohol metabolism. Clin. Liver Dis. 16, 667–85. doi:10.1016/j.cld.2012.08.002

Clausing, P., Ali, S.F., Taylor, L.D., Newport, G.D., Rybak, S., Paule, M.G., 1996. Central and peripheral neurochemical alterations and immune effects of prenatal ethanol exposure in rats. Int. J. Dev. Neurosci. 14, 461–9.

De Ponti, F., 2004. Pharmacology of serotonin: what a clinician should know. Gut 53, 1520–1535. doi:10.1136/gut.2003.035568

Gershon, M.D., 2004. Review article: serotonin receptors and transporters -- roles in normal and abnormal gastrointestinal motility. Aliment. Pharmacol. Ther. 20 Suppl 7, 3–14. doi:10.1111/j.1365-2036.2004.02180.x

Gershon, M.D., Erde, S.M., 1981. The Nervous System of the Gut. Gastroenterology 80, 1571–94.

Houghton, L.A., Brown, H., Atkinson, W., Morris, J., Fell, C., Whorwell, P.J., Lockhart, S., Keevil, B., 2009. 5-hydroxytryptamine signalling in irritable bowel syndrome with diarrhoea: effects of gender and menstrual status. Aliment. Pharmacol. Ther. 30, 919–929. doi:10.1111/j.1365-2036.2009.04121.x

Hoyme, H.E., Kalberg, W.O., Elliott, A.J., Blankenship, J., Buckley, D., Marais, A.-S., Manning, M.A., Robinson, L.K., Adam, M.P., Abdul-Rahman, O., Jewett, T., Coles, C.D., Chambers, C., Jones, K.L., Adnams, C.M., Shah, P.E., Riley, E.P., Charness, M.E., Warren, K.R., May, P.A., 2016. Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. Pediatrics 138. doi:10.1542/peds.2015-4256

Jones, K.L., 2011. The effects of alcohol on fetal development. Birth Defects Res. C. Embryo Today 93, 3–11. doi:10.1002/bdrc.20200

Kleiber, M.L., Wright, E., Singh, S.M., 2011. Maternal voluntary drinking in C57BL/6J mice: Advancing a model for fetal alcohol spectrum disorders. Behav. Brain Res. 223, 376–387. doi:10.1016/j.bbr.2011.05.005

Kodituwakku, P.W., 2010. A neurodevelopmental framework for the development of interventions for children with fetal alcohol spectrum disorders. Alcohol 44, 717–728. doi:S0741-8329(09)00179-7 [pii]\r10.1016/j.alcohol.2009.10.009

Kvigne, V.L., Leonardson, G.R., Borzelleca, J., Neff-Smith, M., Welty, T.K., 2009. Hospitalizations of children who have fetal alcohol syndrome or incomplete fetal alcohol syndrome. S. D. Med. 62, 97, 99, 101–3.

López-Contreras, A.J., Galindo, J.D., López-García, C., Castells, M.T., Cremades, A., Peñafiel, R., 2008. Opposite sexual dimorphism of 3,4-dihydroxyphenylalanine decarboxylase in the kidney and small intestine of mice. J. Endocrinol. 196, 615–24. doi:10.1677/JOE-07-0564

Mawe, G.M., Hoffman, J.M., 2013. Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. Nat. Rev. Gastroenterol. Hepatol. 10, 473–86.

doi:10.1038/nrgastro.2013.105

Mukherjee, R. a S., Hollins, S., Turk, J., 2006. Fetal alcohol spectrum disorders: an overview. J. R. Soc. Med. 99, 298–302. doi:10.1007/s11065-011-9166-x

Parmar, L., Morgan, L.D., Patel, B.A., 2011. Intracellular and extracellular sampling to monitor the neurotransmission process using a chromatographic method. Anal. Methods 3, 2770. doi:10.1039/c1ay05520h

Tajuddin, N., Druse, M.J., 1988. Chronic maternal ethanol consumption results in decreased serotonergic 5-HT1 sites in cerebral cortical regions from offspring. Alcohol 5, 465–470. doi:10.1016/0741-8329(88)90084-5

Uc, A., Vasiliauskas, E., Piccoli, D.A., Flores, A.F., Di Lorenzo, C., Hyman, P.E., 1997. Chronic intestinal pseudoobstruction associated with fetal alcohol syndrome. Dig Dis Sci 42, 1163–1167.

Viramontes, B.E., Camilleri, M., McKinzie, S., Pardi, D.S., Burton, D., Thomforde, G.M., 2001. Gender-related differences in slowing colonic transit by a 5-HT3 antagonist in subjects with diarrhea-predominant irritable bowel syndrome. Am. J. Gastroenterol. 96, 2671–6. doi:10.1111/j.1572-0241.2001.04138.x

Werts, R.L., Van Calcar, S.C., Wargowski, D.S., Smith, S.M., 2014. Inappropriate Feeding Behaviors and Dietary Intakes in Children with Fetal Alcohol Spectrum Disorder or Probable Prenatal Alcohol Exposure. Alcohol. Clin. Exp. Res. 38, 871–878. doi:10.1111/acer.12284

Willford, J. a, Richardson, G. a, Leech, S.L., Day, N.L., 2004. Verbal and visuospatial learning and memory function in children with moderate prenatal alcohol exposure. Alcohol. Clin. Exp. Res. 28, 497–507. doi:10.1097/01.ALC.0000117868.97486.2D

Zafar, H., Shelat, S.G., Redei, E., Tejani-Butt, S., 2000. Fetal alcohol exposure alters serotonin transporter sites in rat brain. Brain Res. 856, 184–92.
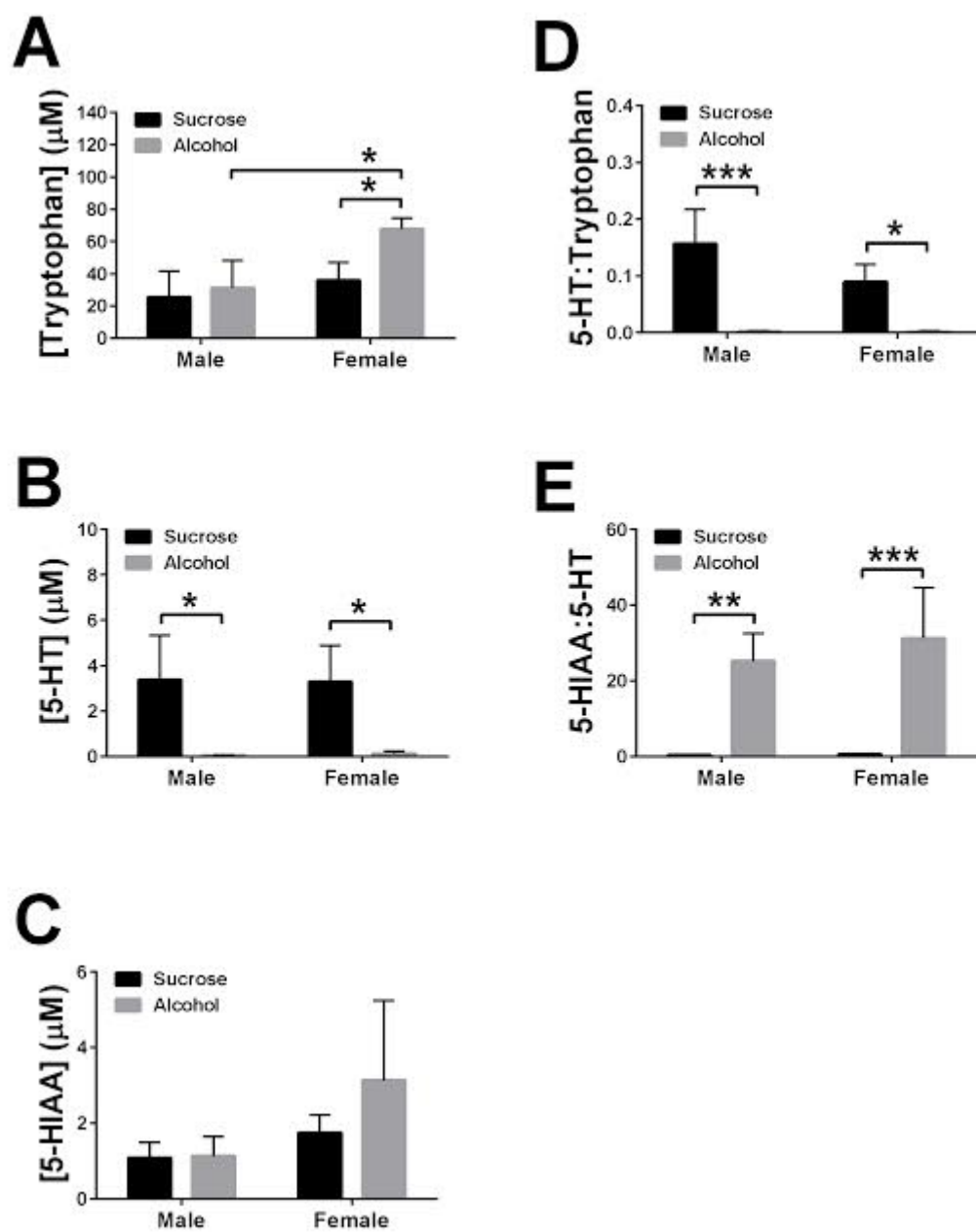
Figure 1. Tryptophan, 5-HT, 5-HIAA concentration and 5-HT/Tryptophan and 5-HIAA/5-HT ratio in intestinal mucosa of mice prenatally exposed alcohol in comparison to control group.
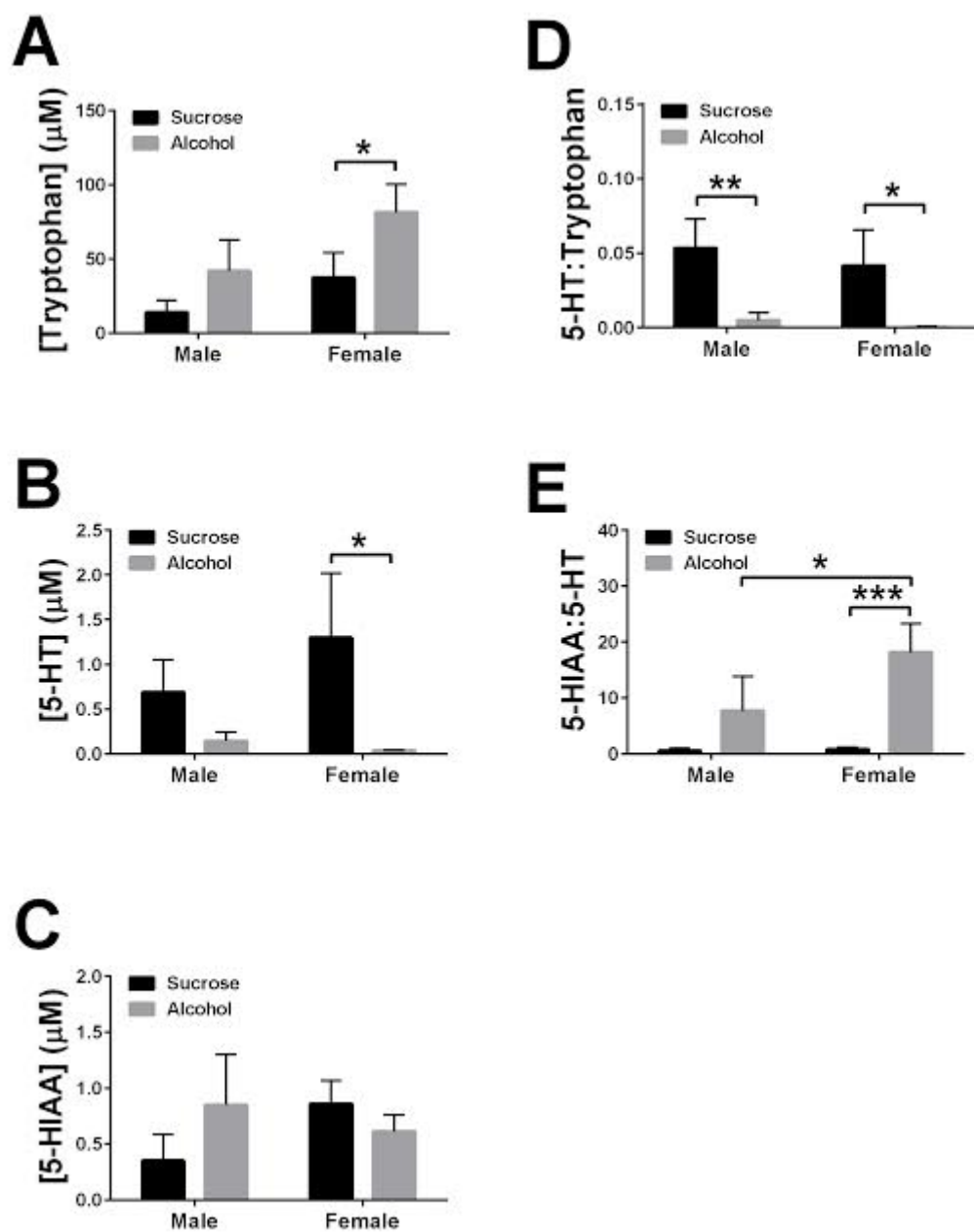
Figure 2. Tryptophan, 5-HT, 5-HIAA concentration and 5-HT/Tryptophan and 5-HIAA/5-HT ratio in intestinal muscle of mice prenatally exposed alcohol in comparison to control group.