Low-dose chronic prenatal ethanol exposure alters serum aminopeptidase activity in adolescent mice.

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Rat and mouse models of prenatal alcohol exposure have been used for approximately 30 years. Such studies are invaluable because they allow the experimenter to control for confounding variables such as nutrition, genetic background, variable drinking patterns etc.
The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models

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In rats, typical oral ethanol doses used range from 3 – 16g/kg/day, with a mode of approximately 5g/kg/day. These give rise to blood alcohol concentrations of 100-700 mg/100ml.

In mice, typical oral ethanol doses used range from 5 – 24g/kg/day, with a mode of approximately 16g/kg/day. Giving rise to blood alcohol concentrations of 100-700 mg/100ml.
16 g/kg/day (mouse dose) would be equivalent to approximately 1L pure alcohol per day in an average human female, i.e. over 5 standard bottles of vodka or whisky per day.

This has to be seen in the light that small animals such as mice eliminate ethanol approximately 5 times more rapidly than humans.
Timing of the dosing is also important.

- Is drinking in the first trimester more dangerous than the third?
- Is infrequent binge drinking more dangerous than constant lower dose?
In the mouse, the first 10-12 postnatal days equate to the human third trimester.
Our model of prenatal alcohol exposure in mice involves ad libitum consumption of 5% \( v/v \) ethanol in drinking water by the breeding harems pre-conception and throughout pregnancy and lactation until weaning at post-natal day 21.

In mice, this equates to an intake of approximately 10g/kg/day \( \approx 1 \) bottle table wine per day in humans, taking into account differences in ethanol elimination.
Our research is focused on the brain renin-angiotensin system, its role in cognition and potential as a target for the treatment of prevention of cognitive disorders.
A schematic representation of the renin angiotensin system illustrating the synthesis and metabolism of the angiotensins and the role of insulin-regulated aminopeptidase (IRAP) as a target for angiotensin IV. (Adapted for Gard et al., 2011).
Using the novel object recognition test, we have reported previously that angiotensin IV is able to improve learning and memory in mice.
Furthermore there is good evidence that manipulation of the brain renin-angiotensin system can improve cognition in humans.
Using our mouse model of prenatal alcohol exposure we investigated the behaviour and cognitive abilities of the offspring at 3-6 months of age (adult).
The effects of pre-natal alcohol exposure and 24-hour pre-treatment with ang IV (5μg/kg s.c) on the behaviour of male and female mice in the novel object recognition test. Data shown as mean ± S.E.M., n=at least 8 (**p<0.01 paired t-test).
Pre-natal alcohol exposure therefore appears to:

- Abolish the precognitive effect of angiotensin IV in males
- Reduce the exploratory behaviour in male and female off-spring, without consistently reducing total locomotor activity.

The elevated-plus maze was therefore used to assess the effect of pre-natal alcohol exposure on anxiety-like behaviour.
Administration of anxiolytic drugs increases the proportion of time spent on the open arms.
The effects of pre-natal alcohol exposure and 25-hour pre-treatment with ang IV (5μg/kg s.c) on the behaviour of male and female mice on the elevated plus maze. Data shown as mean ± S.E.M., n=at least 8 (*p<0.05 and **p<0.01).
We believe that our schedule of low-dose prenatal alcohol exposure produces off-spring which at 3-6 months of age (adult) demonstrate an impaired pro-cognitive response to angiotensin IV (in males only); demonstrate decreased inquisitive behaviour in both males and females, and demonstrate decreased anxiety-like behaviour following treatment with angiotensin IV (males only).

The sex difference is intriguing…….
An examination of sex differences in the effects of early-life opiate and alcohol exposure

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Research shows that FASD is more prevalent in young boys than in young girls (on average 12.9 out of male 1000 births compared to 10.4 out of 1000 female births); however, interestingly, there is no sex difference in the rate of FASD diagnosis when the children are diagnosed later in life [80].

But why is angiotensin IV not enhancing cognition in these male prenatal alcohol exposure animals?
FASD & Aminopeptidases
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= IRAP

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= IRAP
We determined brain activity of IRAP in the PAE male and female mice at 2 months of age
FASD & Aminopeptidases
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IRAP activity, as determined by hydrolysis of leucine-p-nitroanilide. A represents membrane-bound enzyme from control male mice, B represents membrane-bound enzyme from PAE male mice, C represents cytosolic enzyme from control male mice and D represents cytosolic enzyme from PAE male mice (n=7-10)
IRAP activity, as determined by hydrolysis of leucine-p-nitroanilide. A represents membrane-bound enzyme from control female mice, B represents membrane-bound enzyme from PAE female mice, C represents cytosolic enzyme from control female mice and D represents cytosolic enzyme from PAE female mice (n=7-10)
ApN activity, as determined by hydrolysis of alanine-p-nitroanilide. **A** represents membrane-bound enzyme from control male mice, **B** represents membrane-bound enzyme from PAE male mice, **C** represents cytosolic enzyme from control male mice and **D** represents cytosolic enzyme from PAE male mice (n=7-9)
ApN activity, as determined by hydrolysis of alanine-p-nitroanilide. A represents membrane-bound enzyme from control female mice, B represents membrane-bound enzyme from PAE female mice, C represents cytosolic enzyme from control female mice and D represents cytosolic enzyme from PAE female mice (n=7-9).
AspAP activity, as determined by hydrolysis of β-naphthylamide. A represents membrane-bound enzyme from control male mice, B represents membrane-bound enzyme from PAE male mice, C represents cytosolic enzyme from control male mice and D represents cytosolic enzyme from PAE male mice (n=6)
AspAP activity, as determined by hydrolysis of β-naphthylamide. A represents membrane-bound enzyme from control female mice, B represents membrane-bound enzyme from PAE female mice, C represents cytosolic enzyme from control female mice and D represents cytosolic enzyme from PAE female mice (n=4-6)
ApA activity, as determined by hydrolysis of glutamyl-p-nitroanilide. A represents membrane-bound enzyme from control male mice, B represents membrane-bound enzyme from PAE male mice, C represents cytosolic enzyme from control male mice and D represents cytosolic enzyme from PAE male mice (n=7-8)
ApA activity, as determined by hydrolysis of glutamyl-p-nitroanilide. A represents membrane-bound enzyme from control female mice, B represents membrane-bound enzyme from PAE female mice, C represents cytosolic enzyme from control female mice and D represents cytosolic enzyme from PAE female mice (n=7-8)
ApB activity, as determined by hydrolysis of arginine-p-nitroanilide. **A** represents membrane-bound enzyme from control male mice, **B** represents membrane-bound enzyme from PAE male mice, **C** represents cytosolic enzyme from control male mice and **D** represents cytosolic enzyme from PAE male mice (n=6-9)
ApB activity, as determined by hydrolysis of arginine-p-nitroanilide. A represents membrane-bound enzyme from control female mice, B represents membrane-bound enzyme from PAE female mice, C represents cytosolic enzyme from control female mice and D represents cytosolic enzyme from PAE female mice (n=6-8)
Summary

Prenatal ethanol exposure:
Decreases brain IRAP activity in males and females
Decreases brain ApN activity in males and females
Decreases brain AspAP activity in males and females
Decreases brain ApA activity in males and increases in females
Decreases brain ApB activity in females
These same enzymes are also present in Human plasma.

In mice serum:
Serum ApB activity, as determined by hydrolysis of arginine-p-nitroanilide. (n = 4-8 per group)
Serum AspAP activity, as determined by hydrolysis of β-naphthylamide. (n = 4-5 per group)

P = 0.062
Serum IRAP activity, as determined by hydrolysis of leucine-p-nitroanilide. (n = 8-10 per group)

P < 0.001
Serum ApA activity, as determined by hydrolysis of glutamyl-p-nitroanilide. (n = 6 per group)
ApN activity was not identified in mouse serum, although it is known to be present in Human serum and plasma.
### FASD & Aminopeptidases
*Paul R Gard, School of Pharmacy & Biomolecular Sciences, University of Brighton, UK*

**Summary**

**Prenatal ethanol exposure:**

<table>
<thead>
<tr>
<th>Brain</th>
<th>Serum</th>
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</thead>
<tbody>
<tr>
<td>• Decreases IRAP activity in males and females</td>
<td>• Increases IRAP activity in males and decreases in females</td>
</tr>
<tr>
<td>• Decreases ApN activity in males and females</td>
<td>• ApN activity not detected</td>
</tr>
<tr>
<td>• Decreases AspAP activity in males and females</td>
<td>• (?) Increases AspAP activity in males and unchanged in females</td>
</tr>
<tr>
<td>• Decreases ApA activity in males and increases in females</td>
<td>• Increases ApA activity in males and (?) decreases in females</td>
</tr>
<tr>
<td>• Decreases ApB activity in females</td>
<td>• ApB activity unchanged</td>
</tr>
</tbody>
</table>

Are brain and serum activity inversely related?
FASD & Aminopeptidases
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Brain
- Decreases IRAP activity in males and females
- Decreases ApN activity in males and females
- Decreases AspAP activity in males and females
- Decreases ApA activity in males and increases in females
- Decreases ApB activity in females

All changes suggest decreased angiotensin IV activity in male and female PAE
The questions now are:

- Is there any evidence that angiotensin IV activity is reduced in PAE Humans?
- If so, is the reduction related to PAE or FASD?
- Could plasma or serum enzyme activity be used as a marker of PAE or FASD?
- Could drugs targeted at the renin-angiotensin system be beneficial in FASD?
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