Mechanisms of alcohol damage in utero

Ciba Foundation symposium 105

1984

Pitman
London
Mechanisms of alcohol damage *in utero*
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Mechanisms of alcohol damage in utero

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1984

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Introduction: what do we know of the mechanisms of alcohol damage in utero?

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Abstract. The newly established role of alcohol as a human teratogen important in the aetiology of mental deficiency poses the problem of how maternal ingestion of alcohol damages the developing central nervous system. Two main approaches to the identification of possible mechanisms are suggested: clinical field studies, especially of relatives of affected children, and the use of experimental animal models. The evidence so far provided by each approach, and the limitations of each, are considered. Four possible mechanisms of alcohol-induced damage, originating at different stages of antenatal development, are outlined. These mechanisms are related to the fetal alcohol syndrome, the high incidence of spontaneous abortion or fetal death, retarded fetal growth, and ill-defined behavioural problems in childhood.


It has been established beyond reasonable doubt that alcohol is a teratogen in the human. Several hundred cases of the fetal alcohol syndrome (FAS)—a characteristically recognizable pattern of mental defect and craniofacial malformation that probably originates, despite its name, from damage during the embryonic period—have been reported, and many people believe that other less well defined problems of prenatal development may be due to maternal ingestion of alcohol. It is only recently, however, that most paediatricians have come to realize the extent of the problem. For example, only in the last year have cases been reported from the UK (Pratt 1982, Poskitt et al 1982, Beattie et al 1983). Ethanol is a commonly used substance; moreover, its biochemistry and pharmacology have been studied in some depth. It has therefore become imperative, and possible, to try to establish the mechanisms by which this widely used stimulant causes antenatal damage.

A recent survey suggests that alcohol teratogenesis is a major cause of mental deficiency originating before birth, accounting for some 8% of all cases of mild mental retardation (Hagberg et al 1982). The evidence suggests
that most mental retardation has antenatal causes, and that much brain
damage is due to the effects in utero of an unknown number of environmental
agents of no known mechanism of action. In some cases, several agents may
operate in concert. The sort of teratogenesis that can be produced in animals
by a single dose of a known toxic agent seems to be the exception rather than
the rule in the human. The range and variety of potentially damaging agents
in the human is wide, including, for example—in addition to ethanol or
acetaldehyde—vitamin A, thyroid and other hormones, methotrexate and
other cytotoxic drugs, and analogues of nutrients such as folate, as well as
ionizing radiation. Such diverse agents are likely to act by several different
mechanisms. The danger of prenatal damage from methotrexate or X-rays is
fairly obvious but it seems likely that other agents, whose mode of action is
not so self-evident, remain to be identified. Of the group of potentially
damaging agents, alcohol seems to be the best-established example. It is
important to study it, therefore, as a prototype of a probable series of other,
as yet unidentified, human teratogens.

Since the antenatal effects of alcohol in the aetiology of mental deficiency
are so important, we need to consider the mechanism or mechanisms by
which alcohol damages the developing central nervous system (CNS). We
should not forget, though, that the characteristics enabling us to recognize
FAS are mainly outside the CNS, especially the abnormal development of the
mid-face and the high incidence of cardiac and joint anomalies. We should
also bear in mind that general experience with teratogenesis suggests there
are many aetiologies but rather fewer actual mechanisms of antenatal damage
(Melnick 1979). To explain even one mechanism may ultimately help us to
understand several causes.

How are mechanisms likely to be established? There seem to be two main
ways forward: clinical field studies and the use of experimental models. In
clinical field studies the mechanism itself is usually inaccessible, but by
looking not only at the affected children but also at their siblings and other
relatives one can hope to detect (a) individuals with some abnormal reaction
to ethanol and (b) evidence of any kind of antenatal damage from alcohol,
not necessarily FAS. Such damage in relatives is evidently widespread,
although it may be less severe and less easily detected than in the primary
patient. For example, Poskitt et al (1982) reported a high incidence of
medical and social problems affecting 14 out of 17 children of five alcoholic
mothers.

To detect individuals with an abnormal reaction to ethanol we need to
decide which of the possible variants in alcohol metabolism or known genetic
markers provide the best indicators. A related problem is the question of
what the likely 'risk' factors for FAS are.

The second need is to define forms of damage, short of typical FAS, that
may be associated with the drinking of smaller amounts of alcohol than are
generally consumed in chronic alcoholism. Do we look for microcephaly, IQ
deficit, or something more subtle, such as learning or behavioural difficulties?
Do surveys like those of Little (1977), Olegård et al (1979) and Darby et al
(1981) begin to suggest ways forward? If we could give even tentative answers
to these questions, a hypothesis about the mechanism could be tested in the
field.

Experimental animal models provide a valuable means of looking at those
mechanisms that are difficult to study clinically. We should not expect,
however, to reproduce too closely in animals the full aetiological pattern of
alcohol-related antenatal damage seen in humans. It will be enough if there is
a general similarity of mechanism. Problems arise partly from uncertainties of
dosage and partly from variability between species and strains in responses.
Timing may be critical, and it is difficult to mimic the continuous presence of
alcohol so often seen in human alcoholism. The major problem is the one
experienced generally in studies of alcoholism: it is difficult to reproduce
experimentally the pathological changes that develop in the human over
periods of many years.

Is it reasonable to assume that the brain damage reported in characteristic
FAS is the end-result of an extended pathological mechanism in the mother
and fetus and that, if its effects are severe enough, it also produces the
recognizable facial malformation (but perhaps does so only in the human or
only in primates)? If so, is the brain damage due to embryonic nerve cell loss
and disrupted migration? Can the damage associated with human maternal
alcoholism be reproduced in experimental animals by agents other than
alcohol that are known to have pathological effects in the human? If it can,
then the problem is of how ethanol produces such effects. Answers to these
questions would enable us to focus our attention on a small number of the
many pathological effects attributed to ethanol. Since its antenatal effects
seem to be closely related to a cytotoxic action, either of ethanol itself or of its
first metabolite, acetaldehyde, it looks as if a cytological or metabolic basis
should be sought for the mechanism.

These considerations highlight a more general problem. How many
mechanisms are there of ethanol damage? It seems unlikely that the one
which leads to the characteristic pattern labelled ‘FAS’ is unique. Mechan-
isms are likely to be stage-dependent. The association of fetal death and
spontaneous abortion with maternal alcoholism suggests that alcohol may act
by a lethal mechanism (probably operating via some chromosomal aberration)
earlier than it acts to produce characteristic FAS. If so, what happens if
the amount of alcohol drunk is reduced? Is it an all-or-none type of effect, or
are there as yet unrecognized early effects of smaller amounts of ethanol?
Alcohol acting later than the stage which leads to FAS seems to produce a
brain growth deficit and more subtle effects such as learning difficulties. Is the mechanism here a temporary disturbance of brain structure which leads to a deficit of interneuronal synapses? If, as seems likely, there is more than one mechanism, the field and experimental approaches, as set out above, need appropriate extension.

The experiential doctrine of teratogenesis that there are likely to be few mechanisms but many aetiologies should alert us to the possibility that occasional cases of 'FAS' are not actually due to maternal alcoholism. Other agents that might trigger one of the putative alcohol mechanisms include hydantoin, cytotoxic drugs, tobacco, and addictive drugs. The more causes we can identify, the closer we can get to seeing how the damage actually occurs. Not only is there an overlap of symptomatology, for example with the fetal hydantoin syndrome (Hanson & Smith 1975), but I and others (Pratt 1980, 1982, Lipson et al 1981, Ammann et al 1982) have noted the close similarity between FAS and other conditions that are generally associated with microcephaly, mental retardation and structural anomalies of brain development. These may be confused with FAS; moreover, maternal drinking might be a causal factor in some of them at least. There is evidence for this in a series of cases of congenital aplasia of the thymus with mental retardation (Ammann et al 1982).

I would tentatively suggest four distinct mechanisms of antenatal, alcohol-related CNS damage:

(1) At the time of conception and during the first weeks of development, ethanol (or acetaldehyde) might act as a cytotoxic or mutagenic agent, causing either cell death or subsequently lethal chromosomal aberrations. Stronger support for this hypothesis comes from the experimental than from the clinical evidence. Many workers have shown that ethanol or acetaldehyde are toxic to embryonic and other dividing cells in tissue culture (e.g. Brown et al 1979, Kaufman 1983). A high rate of fetal loss and stillbirths reported Yorg ago (Sullivan 1899) in maternal alcoholism is confirmed by a recent survey (Olegård et al 1979) and by a further study where a significant increase in spontaneous abortions during the second trimester was found for alcohol intakes as low as 15–30 g daily (Harlap & Shiono 1980). More work needs to be done on the incidence of less readily detected spontaneous abortions in the first trimester and, especially, on possible chromosomal aberrations.

(2) During the period roughly 4–10 weeks after conception in the human, I suggest that ethanol (or acetaldehyde) acts cytotoxically, causing excessive cell death in the CNS and abnormalities in nerve cell migration, either from cell damage or, perhaps, due to loss of guiding or 'marker' cells (e.g. the radial glia). From the changes described in FAS at post-mortem (Clarren et al 1978, Peiffer et al 1979) it seems clear that abnormal migration leads, when severe, to agenesis of brain regions, when less severe to heterotopias, or,
when still less severe, to disorganization of tissue structure that is only visible microscopically. Any severe cell loss leads to micrencephaly. A broadly similar pattern of brain damage is reproducible in a susceptible strain of mice given a suitably timed dose of ethanol (Sulik et al. 1981).

In support of mechanism (2), a wide range of abnormalities of cell migration, affecting different regions of the CNS with varying severity and associated with a corresponding degree of micrencephaly, can be produced in mice and rats by single, suitably timed and graded doses of known cytotoxic drugs (Shimada et al. 1982, Dambska et al. 1982). Further, in the human, skull measurements can explain the changed shape of the mid-face which makes FAS a recognizable entity, with early closure of the sphenethmoidal synchondrosis and a consequent shortening of the anterior cranial base (Frias et al. 1982), due to an alcohol-induced deficiency in brain growth.

(3) Later in pregnancy, from 8–10 weeks onwards, ethanol (or acetaldehyde) perhaps again disorganizes or delays cell migration and development. The difference from the effects in (2) would be that damage at this later stage is made good and development is completed in the long run, as has been shown in the rat after alcohol ingestion (Volk et al. 1981). This recovery may not be as complete as it appears, because Volk et al. (1981) and Shimada et al. (1982) found that if nerve cells are not in the right place at the right time synapses will not be formed normally. If the lack of synapses (or formation of the wrong synapses) cannot be made good, this would explain the behavioural problems and neurological deficits reported during infancy and childhood in the progeny of mothers who drink alcohol during pregnancy (Streissguth et al. 1978, Darby et al. 1981).

(4) Alcohol (or acetaldehyde) interferes in various ways with neurotransmitter production in the CNS, leading to neuroendocrine abnormalities, including an effect on the hypothalamus which leads to suppression of growth hormone release (Thadani & Schanberg 1979). The growth hormone deficiency thus produced may account for much of the growth deficit which is probably the most consistent finding in the infants of mothers who have drunk even moderate amounts of ethanol. A better understanding of this mechanism may help us to understand some of the other effects of alcohol, such as addiction in adults.

It is evident from these hypotheses (summarized in Table 1) that we still have to discover whether ethanol acts directly or through an accumulation of its first metabolite, acetaldehyde. Although the amounts of acetaldehyde in the blood are an order of magnitude lower, its toxicity is an order of magnitude greater than that of ethanol.

Are we close enough to an understanding of the mechanisms of antenatal alcohol damage to be able to say whether there is a safe upper limit of alcohol intake in pregnancy? Damage by the first two of the suggested mechanisms...
TABLE 1 Four possible mechanisms by which ethanol or acetaldehyde might damage the human CNS before birth

<table>
<thead>
<tr>
<th>Period of action in human</th>
<th>Suggested mechanism</th>
<th>End-result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Shortly after conception</td>
<td>Cell death or chromosomal errors</td>
<td>Early spontaneous abortion</td>
</tr>
<tr>
<td>(2) About 4 to 10 weeks</td>
<td>Cytotoxic effect causing cell loss and abnormal migration</td>
<td>Regional agenesis, heterotopias or structural disorganization; micrencephaly; characteristic FAS with mental deficiency</td>
</tr>
<tr>
<td>(3) From 8 to 10 weeks onwards</td>
<td>Temporary delays in neuronal migration leading to abnormal synapse formation</td>
<td>Behavioural difficulties in infancy and childhood</td>
</tr>
<tr>
<td>(4) After the first few weeks</td>
<td>Action on hypothalamus to suppress the release of growth hormone</td>
<td>General growth deficit (including that of brain?)</td>
</tr>
</tbody>
</table>

will usually occur before a woman has her pregnancy confirmed by her doctor, so the problem of prevention becomes one for the public health service. There is a need here (Pratt 1981) for educationally based prevention programmes focusing on groups especially at risk. Finally, a question to explore is whether any form of treatment is likely to improve the mental condition of an alcohol-damaged neonate?

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INTRODUCTION

Thadani PV, Schanberg SN 1979 Effects of maternal ethanol ingestion on serum growth hormone in the developing rat. Neuropharmacology 18:821-826
Prenatal and early postnatal exposure to ethanol permanently alters the rat hippocampus

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Abstract. Three separate groups of pregnant Sprague-Dawley rats were (1) fed a liquid diet containing 35% ethanol-derived calories, or (2) pair-fed this diet containing an isocaloric amount of maltose-dextrin instead of ethanol, or (3) fed laboratory chow ad libitum. Their offspring were killed after reaching at least 60 days of age, and their brains were processed with the Timm’s or horseradish peroxidase histochemical techniques. Both procedures revealed that intrauterine exposure to ethanol produced a dramatic change in the topography of hippocampal mossy fibres. An aberrant distal infrapyramidal mossy fibre terminal band was present at mid-temporal levels (where it does not normally occur). Pair-fed controls did not show the aberrant band. Rats exposed to ethanol after birth (using an artificial rearing procedure) showed even greater aberrations in the mossy fibre terminal field than rats exposed to ethanol in utero. Thus, postnatal exposure to ethanol (equivalent to the third trimester in the human) produced more striking changes in the mossy fibre terminal field than exposure to ethanol during the entire length of gestation in the rat (equivalent to the first and second trimesters in the human).


Although it has long been suspected that alcohol consumption during pregnancy could have harmful effects on the developing fetus, a clearly-defined fetal alcohol syndrome (FAS) has been documented only within the last decade (Jones et al 1973, Streissguth et al 1980). Central nervous system (CNS) dysfunction is the most devastating consequence of maternal consumption of ethanol. CNS neuropathology and mental retardation have been observed in fetuses and in children born to alcoholic mothers (Clarren et al 1978, Peiffer et al 1979). Moreover, while prenatal exposure to ethanol can sometimes cause external dysmorphology in animals (Streissguth et al 1980, Sulik, this volume), ethanol exposure during gestation can cause CNS damage even in the absence of external developmental abnormalities (Barnes
ETHANOL EXPOSURE ALTERS RAT HIPPOCAMPUS


One of the basic questions related to FAS is whether there are critical periods of brain development when ethanol exposure may be especially dangerous. We have chosen to investigate this question by examining the rat hippocampus after ethanol exposure during the prenatal and early postnatal periods. The rat hippocampus is a very useful model system for studying CNS abnormalities. The hippocampus and dentate gyrus possess a relatively simple cytoarchitectural organization, each consisting primarily of a single type of neuron—the pyramidal cell in the hippocampus and the granule cell in the dentate gyrus. Both cell layers are tightly packed and spatially isolated (Fig. 1A). Each region has a simple dendritic organization and is characterized by afferents which are segregated into specific laminae along the dendrites, allowing the detection of subtle changes in afferent organization. Furthermore, prenatal exposure to ethanol has been shown to affect the hippocampus, producing developmental delays (Davies & Smith 1981) and long-lasting alterations in dendrites (Abel et al 1983) as well as loss of pyramidal cells (Barnes & Walker 1981).

The mossy fibre axons of the granule cells are the major intrinsic afferent pathway connecting the dentate gyrus with the pyramidal cells of hippocampal field CA3. Mossy fibre axons possess characteristic swellings along their length and make giant connections en passant with restricted portions of the apical dendrites of the pyramidal cells (Blackstad & Kjaerheim 1961). These unusual presynaptic terminals contain exceptionally high levels of zinc (Crawford & Connor 1972), which can be stained with the Timm’s sulphide silver histochemical technique (West et al 1981a). The mossy fibres terminate primarily along a hook-shaped suprapyramidal terminal field in the stratum lucidum of CA3 (Figs. 1A, 2A). A small infrapyramidal bundle is present near the hilus but a distal infrapyramidal terminal field of mossy fibres is normally present only in the rostral third of the hippocampus (West et al 1981a). However, we now present evidence that prenatal exposure to ethanol causes abnormal distal infrapyramidal mossy fibres to develop at midtemporal levels, where only a few mossy fibre boutons normally occur (West 1983). This finding is especially significant because these rats show no signs of external dysmorphology.

Methods and results

Timm’s histochemical technique

Pregnant Sprague-Dawley rats were given free access to a liquid diet (Bio-Serv PR-11) containing 35% ethanol-derived calories from days 1–21 of
FIG. 1. (A) Hippocampal formation drawn from a horizontal section taken ventral to the occipital bend of the hippocampus at plane b-b in B. The following abbreviations are used in this and subsequent figures. CA1 and CA3 refer to the major hippocampal field designations. a, b, and c indicate the approximate positions of the CA3 subfields; G, granule cell layer; H, hilus; HF, hippocampal fissure; P, pyramidal cell layer; SO, stratum oriens; SR, stratum radiatum; SP, suprapyramidal; IP, infrapyramidal. (B) Drawing of the lateral view of the rat brain illustrating the relative position of the hippocampus. Plane a-a designates the approximate position of the (dorsal) appearance of the normal distal infrapyramidal mossy fibres in horizontal sections. Plane b-b designates the approximate position of the horizontal sections illustrated in A and subsequent figures.

gestation. A pair-fed control group was given a liquid diet in which ethanol was replaced by an isocaloric amount of maltose–dextrin. Another group of pregnant rats was fed laboratory chow and water ad libitum. At birth the pups from ethanol-fed and pair-fed mothers were cross-fostered to normal mothers and weaned at 22 days of age. After reaching two to ten months of age, rats of either sex were randomly selected from each of the three groups and their
FIG. 2. Photomicrographs of Timm-stained hippocampal sections, all from the same mid-temporal level (plane b-b in Fig. 1B) along the septo-temporal axis. (A) Normal adult rat. Note the heavy suprapyramidal band of mossy fibres in stratum lucidum and lack of significant distal infrapyramidal staining on the stratum oriens side of the pyramidal cell layer. (B) Adult rat exposed to a liquid diet containing 35% ethanol-derived calories during days 1–21 of gestation. Note the dense staining of the aberrant infrapyramidal terminal field on the stratum oriens side of the pyramidal cell layer (white arrows). (C) Adult rat from a pair-fed litter. PYR, pyramidal cell layer; LUC, stratum lucidum. Black arrowheads denote the border between CA1 and CA3. Scale bar, 250 μm. (From West & Hodges-Savola 1983.)
brains were processed with the Timm's sulphide silver technique (West et al 1981a, b, West & Hodges-Savola 1983).

The quantitative analysis of the distribution of the distal infrapyramidal mossy fibre field in hippocampal subfield CA3a was done by locating the first appearance (i.e. in the most dorsal section) of the dark brown mossy fibre staining in the right hippocampus (plane a–a in Fig. 1B). The dorsal–ventral distribution of the distal infrapyramidal terminal field of mossy fibres was determined by defining the position of the last tissue section in which a dense patch of mossy fibre staining (continuous with that in the more dorsal tissue sections) could be observed. The significance of the differences between the treatment groups was analysed by one-way analysis of variance, followed by analysis of the between-group differences using the Neuman-Keuls test (West & Hodges-Savola 1983).

An examination of the Timm's histochemical staining in the hippocampus revealed that intrauterine exposure to ethanol produced a dramatic change in mossy fibre topography. A dense dark-brown band of Timm's-positive material was present in a distal infrapyramidal position (roughly equivalent to hippocampal subfield CA3a) at mid-temporal levels (Fig. 2B). This staining was similar in intensity and colour to the mossy fibre staining in the suprapyramidal terminal band. It was discontinuous from the infrapyramidal staining in CA3c. Strictly speaking, most of the aberrant terminal band could be classified as intrapyramidal. However, there was usually a clear (unstained) zone in the pyramidal cell layer between the two bands. The aberrant band appeared to be a continuous extension, past the occipital bend (into mid-temporal hippocampal levels) of the distal infrapyramidal band of mossy fibres normally present at more dorso-rostral levels (Fig. 3). In many sections,

![FIG. 3. Schematic diagram of the hippocampus (lateral view) illustrating the relative dorsal-ventral distribution of the distal infrapyramidal terminal field of mossy fibres in the three different groups of adult rats tested. (A) Normal rats. (B) Rats exposed to a liquid diet containing 35% ethanol-derived calories during days 1–21 of gestation. (C) Rats exposed to the pair-feeding regimen during days 1–21 of gestation. The striped and black areas represent the distribution, mean and standard error of the mean (respectively) of the distal infrapyramidal terminal field of mossy fibres as detected in serial horizontal sections (see text). The distal infrapyramidal band in the ethanol-exposed group was significantly more extensive than in either the normal controls ($P<0.05$) or pair-fed controls ($P<0.01$). (Modified from West & Hodges-Savola 1983.)](image-url)
there was an area of staining near the proximal end of the aberrant band which appeared to connect the supra- and infrapyramidal bands. Such staining suggests that bundles of suprapyramidal mossy fibres may help to form the aberrant terminal field, being displaced spatially from their customary position.

In pair-fed rats the dorsal-ventral distribution of the Timm's distal infrapyramidal mossy fibre staining was even less extensive than that seen in normal rats (Fig. 3C). At mid-temporal and temporal hippocampal levels, only a few dark-brown granules were found on the stratum oriens side of the pyramidal cell layer in CA3a (Fig. 2C). In other respects, the hippocampus of the pair-fed rats appeared normal.

**Anterograde horseradish peroxidase technique**

Although the Timm’s sulphide silver technique provides considerable information on the termination of afferent fibre systems in the hippocampal formation, this technique was not suitable for studying the origin and morphology of the axons contributing to the aberrant terminal field. Although it was highly likely that the dark-staining distal infrapyramidal area represented a terminal field of mossy fibre axons, we sought confirmation of this finding using an anterograde horseradish peroxidase (HRP) method (West et al 1981a, West 1983). Small iontophoretic injections of HRP were made into the dentate gyrus at mid-temporal levels just ventral to the occipital bend of the hippocampus in normal adult rats and in those exposed to 35% ethanol-derived calories during days 1–21 of gestation. These small injections of HRP labelled the suprapyramidal mossy fibre axons in both groups, as well as substantial bundles of axons crossing into the distal infrapyramidal region in the ethanol-exposed animals (Fig. 4). Individual axons of the distal infrapyramidal mossy fibre projection showed the same characteristic periodic axonal swellings as had previously been observed in axons of the suprapyramidal fibres (Blackstad & Kjaerheim 1961, West et al 1981, West 1983). Thus, the HRP procedure demonstrates that the aberrant terminal band seen with Timm's stain is of granule cell origin, and that the granule cell axons cross over from the suprapyramidal band to form the aberrant band at the same septotemporal level.

**Artificial rearing procedure**

In the studies reported above, ethanol was given during virtually the entire length of the gestation period of the rat. However, the rat is less mature in
FIG. 4. Horseradish peroxidase (HRP)-labelled mossy fibre terminal band in hippocampal subfield CA3a. (A) Normal adult rat. (B) Adult rat exposed to ethanol during days 1–21 of gestation. Note the extensive labelling in the intra- and infrapyramidal regions (arrows). Arrowheads indicate the approximate border between CA1 and CA3. Dark-field illumination. Size differences are a function of differential shrinkage due to the different counterstaining techniques used. Bar, 100 μm.
ETHANOL EXPOSURE ALTERS RAT HIPPOCAMPUS

terms of brain development at parturition than the human (Dobbing & Sands 1979). In order to determine the effects of ethanol exposure during a period equivalent to the human third trimester we had to expose neonatal rats to ethanol during the first 10 days after parturition. Although there are several methods available for doing this, we chose an artificial rearing procedure (Diaz et al 1982). Newborn rats were given ethanol as a 3% (v/v) solution in the milk formula for days 1–10 of postnatal life via an intragastric cannula chronically implanted into the animal’s stomach. The rats were removed from the apparatus on the afternoon of day 10 and returned to their mothers. At 60 days of age they were killed and their brains were processed using the Timm’s procedure. These rats showed more dramatic alterations in the mossy fibre organization than those seen after exposure to ethanol in utero on days 1–21 of gestation. The aberrant distal infrapyramidal band appeared to be wider than in the pups exposed to ethanol in utero. Furthermore, pups exposed to ethanol postnatally exhibited aberrant intra- and infrapyramidal mossy fibre staining which projected to subfield CA3b in addition to CA3a (Fig. 5). Thus, exposure to ethanol during a period equivalent to the third trimester of pregnancy in the human produced more striking changes than exposure to ethanol for a longer period in utero (days 1–21 of gestation, equivalent to the first and second trimesters in the human).

Discussion

Differentiation of ethanol-induced effects from those produced secondary to malnutrition is always difficult. Aberrant terminal fields of mossy fibres were not observed in any of the pair-fed rats in this study, indicating that these changes were not due to alterations in caloric intake. However, the distribution of the infrapyramidal mossy fibre field was reduced in pair-fed controls (when these were compared to controls fed on laboratory chow). By contrast, ethanol caused an increased distribution of the mossy fibre fields compared to that in pair-fed controls. This finding thus constitutes strong evidence that the effects observed are due to ethanol per se and are not secondary to alterations in the nutritional state of the animal. (Of course, the ethanol effects may be due to production of acetaldehyde rather than to ethanol per se.)

Possible mechanisms of aberrant mossy fibre development

Aberrant mossy fibre connections could develop in a variety of ways. First, the pyramidal cell layer may not be formed as compactly after ethanol exposure. The aberrant terminal field may thus result from a normal
FIG. 5. A horizontal section of the hippocampal formation at a mid-temporal hippocampal level stained with the Timm’s sulphide silver technique. Taken from an adult rat exposed to a milk formula containing 3% (v/v) ethanol (7 g/kg daily) during postnatal days 1-10. The white arrows indicate an aberrant distal (CA3a) infrapyramidal terminal field. White arrowheads indicate additional aberrant mossy fibre staining in hippocampal subfield CA3b. Bar, 500 μm.
complement of synaptic connections made with proximal apical dendrites of pyramidal cells which did not migrate completely into their normal position in the pyramidal cell layer. Second, the normal sequence of pyramidal cell migration may have been disrupted. It has been suggested that a similar mechanism may be responsible for strain-related differences in mossy fibre organization in mice (Vaughn et al 1977). Third, misalignment of some of the pyramidal cells could result in projection of the relevant target position of their apical dendrites towards the infrapyramidal rather than the suprapyramidal side (where the suprapyramidal terminal band is located). Fourth, loss of pyramidal cells (Barnes & Walker 1981) could result in an increase in the ratio of mossy fibres to target cells, producing a relative shortage of synaptic sites on the apical dendrites and leading to mossy fibre synapses being formed on the basal dendrites on the infrapyramidal side of the cell layer. Similar formation of synapses between mossy fibres and basal dendrites could also result from increased formation of granule cells, since the area of the terminal field has been shown to depend on the number of parent neurons rather than target neurons in the rat hippocampus (Gaarskjaer 1978). Fifth, failure of the apical dendrites to develop fully (Davies & Smith 1981, Abel et al 1983) could result in fewer dendritic spines (including thorny excrescences) on the pyramidal cells. Maintenance of an optimal number of synaptic contacts might then again result from formation of mossy fibre synapses with the basal dendrites. Sixth, changes in timing of the formation of commissural and mossy fibre connections could alter the segregation of these afferents, producing aberrant terminal fields (Laurberg & Zimmer 1980). Finally, ethanol exposure might cause a delay in mossy fibre development. This could permit the basal dendrites (which develop later than the apical dendrites) to be more receptive to the mossy fibres (Laurberg & Zimmer 1980). The presence of a few normal mossy fibre terminals in the basal area (West 1983) suggests that ethanol might produce hyperdevelopment of this normally sparse projection.

Alterations in mossy fibre topography in rats exposed to ethanol in utero are remarkably similar to those observed in rats treated with L-thyroxine (Lauder & Mughnaini 1980) during early postnatal development; rats given hippocampal lesions shortly after birth had similar alterations (Laurberg & Zimmer 1980). The postnatal treatments in these two studies were given after the pyramidal cells had formed but during the period of rapid generation of the granule cells of the dentate gyrus (Bayer 1980). In the present study, the prenatal ethanol exposure covered an interval during which virtually all the pyramidal cells (the target cells for the mossy fibre axons) are formed but before a significant number of granule cells appear (Bayer 1980). We do not know whether the effects we observed are a direct result of the ethanol treatment or represent a secondary effect occurring after an ethanol-induced
change in development. The observation that lesions and increased thyroxine levels both produce similar changes certainly suggests that disruption of a common mechanism could produce aberrant development of mossy fibres. One possibility is that alteration of other systems, such as the hypothalamo–pituitary–endocrine axes which regulate the production of adrenal, thyroid and other hormones, might be responsible for the changes observed after these diverse treatments. Evidence for such endocrine changes has been reported (Taylor et al 1982, and this volume). Thus, mossy fibre formation seems to be altered readily under a variety of conditions, although little is known about the mechanisms involved or the consequences of such alterations.

Functional consequences

Since the mossy fibres represent the only known major output from the granule cells, the alterations found in this work may be detrimental to normal hippocampal function. A shorter prenatal exposure to a concentration of ethanol slightly higher than that used in our work prevents the acquisition of a normal number of hippocampal pyramidal cells (Barnes & Walker 1981). Thus, alterations in neuronal connections (such as those between granule cells and pyramidal cells in the hippocampus) may have a profound effect on a region of the brain which is thought to play a role in memory in humans (Milner 1970). In rats, ethanol exposure in utero produces behavioural deficits indicative of hippocampal damage (Riley et al 1979). Furthermore, the size of the infrapyramidal terminal field of mossy fibres has an inverse relationship with performance of an active avoidance task (Schwegler et al 1981). Therefore, alterations in mossy fibre organization such as those reported here may be responsible for some of the serious impairments in mental function associated with FAS.

Species comparisons

In extrapolating experimental results from rats to humans, it is important to realize that brain development proceeds at a different pace in various species. At birth, the rat brain shows the same approximate degree of development as the human brain at the end of the second trimester of pregnancy. Thus, ethanol exposure during days 1–21 of gestation in the rat is equivalent to exposure up to the end of the second trimester in the human (Dobbing & Sands 1979). Third-trimester brain development in humans occurs during the first 10 days or so of post-partum life in the rat—the period covered by the
artificial rearing procedure. Moreover, during days 1–21 the hippocampal pyramidal cells—the targets of the mossy fibre axons—are being generated and migrating into position (Bayer 1980). Most granule cells (85%) are formed postnatally in the rat (Schlessinger et al 1975).

The effects of ethanol on hippocampal development may differ, depending on the time of administration. Ethanol exposure during the first 10 days of postnatal life produced a wider and more extensive alteration of the mossy fibres than ethanol treatment on days 1–21 of gestation. Thus, ethanol exposure during the third-trimester equivalent appears to be more deleterious than exposure during the first and second trimesters combined (although the total amount of ethanol administered is substantially greater in the latter case). Our preliminary studies seem to indicate that prenatal exposure for only days 1–10 or days 11–21 of gestation (first trimester alone or second trimester alone) does not produce the alterations of mossy fibre development seen when alcohol is given during the entire period of gestation or during the first 10 days of postnatal life. However, Barnes & Walker (1981) observed cell losses in the hippocampus after ethanol exposure during the second-trimester equivalent. It appears that ethanol exposure is more harmful during the late stage of brain development, but exposure during the first and second trimesters is also dangerous. Thus it would appear advisable for women to abstain from drinking alcohol early in pregnancy, to minimize the possibility of abnormal brain development.

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