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The chick embryo as a model for the effects of prenatal exposure to alcohol on craniofacial development

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ABSTRACT

Prenatal exposure to ethanol results in fetal alcohol spectrum disorder (FASD), a syndrome characterised by a broad range of clinical manifestations including craniofacial dysmorphologies and neurological defects. The characterisation of the mechanisms by which ethanol exerts its teratogenic effects is difficult due to the pleiotropic nature of its actions. Different experimental model systems have been employed to investigate the aetiology of FASD. Here, I will review studies using these different model organisms that have helped to elucidate how ethanol causes the craniofacial abnormalities characteristic of FASD. In these studies, ethanol was found to impair the prechordal plate—an important embryonic signalling centre—during gastrulation and to negatively affect the induction, migration and survival of the neural crest, a cell population that generates the cartilage and most of the bones of the skull. At the cellular level, ethanol appears to inhibit Sonic hedgehog signalling, alter levels of retionoic acid activity, trigger a Ca^{2+}-CamKII-dependent pathway that antagonises WNT signalling, affect cytoskeletal dynamics and increase oxidative stress. Embryos of the domestic chick *Gallus gallus domesticus* have played a central role in developing a working model for the effects of ethanol on craniofacial development because they are easily accessible and because key steps in craniofacial development are particularly well established in the avian embryo. I will finish this review by highlighting some potential future avenues of fetal alcohol research.

1. Introduction

Ethanol is a teratogen that causes a range of birth defects subsumed under the term fetal alcohol spectrum disorder (FASD) (O'Leary, 2004; Riley and McGee, 2005). The prevalence of FASD in a typical Midwestern US community has recently been estimated as between 2.4% and 4.8%, but it can reach over 10% in certain high-risk communities (May et al., 2014). Prenatal exposure to alcohol (PEA) is thought to be the leading cause of behavioural and cognitive deficits in the Western world (Abel and Sokol, 1986), placing an increasing financial burden on its health systems (Lupton et al., 2004).

Severely affected individuals—suffering from fetal alcohol syndrome (FAS)—are characterised by facial dysmorphologies, growth retardation and CNS dysfunction resulting in cognitive, motor and behavioural problems (Jones and Smith, 1973; Sampson et al., 1997; Mattson and Riley, 1998). However, the clinical manifestations of FASD can vary greatly, depending on the frequency, dose and timing of ethanol exposure, as well as on other lifestyle factors and the genetic makeup of the mother (May et al., 2014). Thus, animal models are indispensable if we want to understand the aetiology and pathophysiology of FASD, as they allow us to eliminate or control at least some of those variables (Wilson and Cudd, 2011).

The characterisation of the mechanisms by which ethanol impairs development is more challenging than for other teratogens that have specific molecular targets. The effects of ethanol are highly pleiotropic, and it is thought to bind to a large number of proteins (Harris et al., 2008; Howard et al., 2011). Research into the effects of PEA may therefore also help to shed light on the cell biological basis of ethanol cytotoxicity.

1.1. Experimental models of FASD

A broad range of different model systems have been employed to study the effects of ethanol on the developing organism. Tissue culture experiments have the advantage of being inexpensive, quick and technically straightforward and of providing a homogenous response of the cultured cells to easily adjustable experimental conditions (Tyler and Allan, 2014). However, cells in culture are likely to behave differently from those in an intact organism, and many of the effects of PEA are likely to involve complex interactions between different tissues that can only be

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modelled at the organismic level.

Small non-vertebrates such as the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* are amenable to genetic studies and their embryonic development is well characterised at the cellular level. Thus, these model systems lend themselves to the characterisation of the effects of ethanol on single cells and/or molecular pathways. Due to their genetic accessibility and their short generation time they are ideal for screens for genetic modifiers that may influence the cellular effects of ethanol. However, there are obvious fundamental differences in tissue and organ composition between these non-vertebrates and humans; thus, they are not well suited to assess the structural and functional effects caused by PEA at the tissue level. The formation of complex organs such as the brain, cranium, heart, kidneys or limbs typically requires spatiotemporally controlled interactions between different tissues that can only be modelled in organisms that are reasonably similar to humans in terms of their embryonic development.

Vertebrates that have been used to study the effects of PEA include zebrafish and frog larvae, mice, rats and non-human primates (Wilson and Cudd, 2011). Anamniotes such as amphibians and fish have the advantage of easy accessibility and large numbers of offspring, but they are structurally less similar to humans than 'higher' vertebrates. Mammals have the advantage of being more closely related—and thus more similar—to humans, but their manipulation and maintenance is usually technically more demanding and more expensive, and litter size tends to be significantly smaller.

I will argue below that avian embryos (in particular those of the domestic chick *Gallus gallus domesticus*) provide an excellent model system to study the effects of PEA that combines the advantages of being comparably similar to human embryos with easy experimental accessibility and cost-effectiveness.

1.2. Manifestations of FASD

The manifestations of FASD are highly variable and may include neurological, craniofacial, limb and heart abnormalities, as well as microcephaly and general growth retardation. High doses of ethanol at early developmental stages cause holoprosencephaly (HPE), the most common developmental defect of the forebrain and midface (Cohen, 2006; Roessler and Muenke, 2010), and some

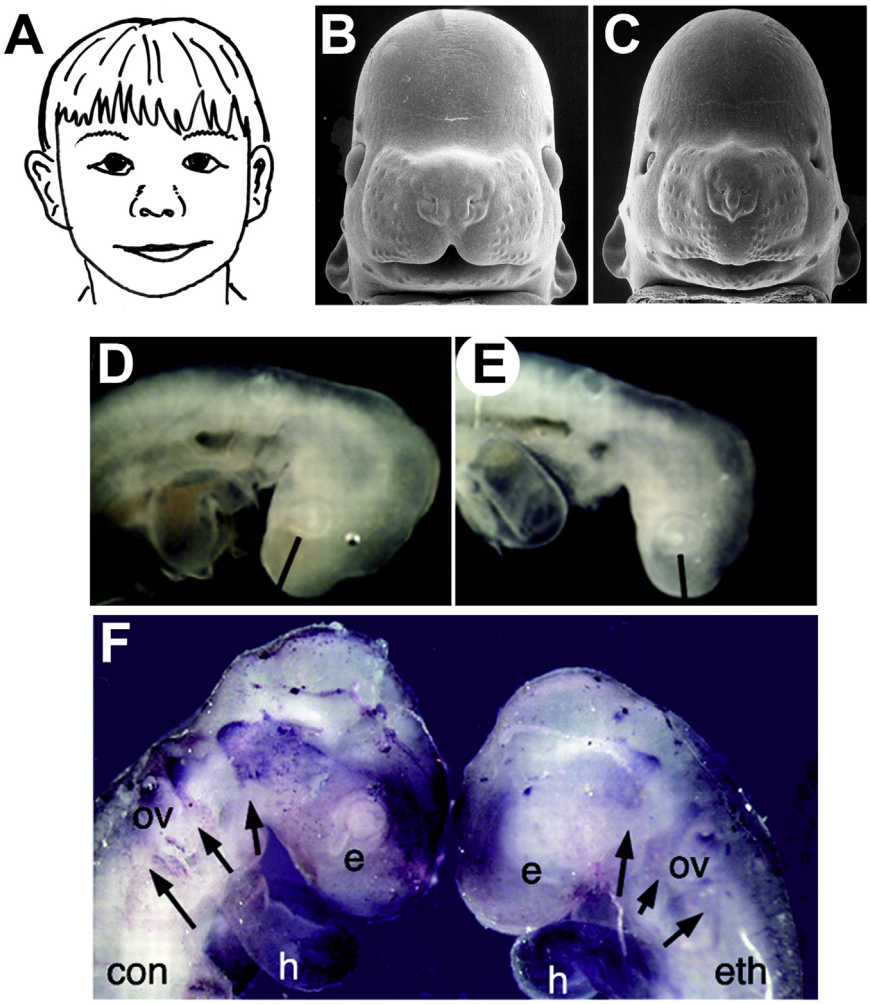

Fig. 1. *The effects of PEA on craniofacial development.* (A) Sketch of infant with facial appearance characteristic of FAS. Note thin upper lip, smooth/indistinct philtrum (no medial groove between nose and upper lip), short nose, short palpebral fissures (width of eye opening), epicanthal folds (skin fold covering the inner corner of the eye), low nasal bridge, minor ear abnormalities and possible micrognathia. (B, C) Scanning electron micrographs of face of normal mouse embryo (B) and of embryo after exposure to ethanol (C). Note characteristic changes of upper lip, nose and eyes. (D) Normal chick embryo; (E) embryo after exposure to ethanol (both after 2.5 days of development; lateral view, head points to the right). Note general microcephaly and shortening of the frontonasal mass (black bar) in ethanol-treated embryo. (F) Chick embryos (lateral view) stained for expression of the neural crest cell marker *HNK1*. Note reduction in neural crest cells streaming out of the hindbrain region (arrows). Abbreviations: con, control embryo; e, eye; eth, ethanol-treated embryo; h, heart; ov, otic vesicle. Panels (B) and (C) were kindly provided by Kathy Sulik, panels (D–F) by Marianne Bronner (Ahlgren et al., 2002).

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