Introduction

Autism is a neural developmental disorder characterized by impaired social interaction and communication, and by restricted and repetitive behavior. The social dysfunction represents a key feature of autism, and this debilitating aspect of this disease is still largely untreated by current medication, depriving the patients of one of the most rewarding aspects of human life, namely social interaction. Today, autism affects 1 in 100 newborns and a high percentage (30-40%) of autistic subjects demonstrates co-morbidity with epilepsy. The aim of our project is to evaluate a new animal model for autism with the goal of improving our knowledge of this heterogeneous disorder. We hope this will ultimately lead to improved treatment of patients.

Our rodent model is developed by early prenatal subchronic administration of the antiepileptic drug valproate (VPA) to pregnant rats. The approach is based on the first VPA study by members of our academic team (Anne Sabers and Arne Møller), who found that the administration of VPA to rats during pregnancy unexpectedly produced an enhanced number of neocortical cells in the offspring (Sabers et al., 2012 submitted). This neuropathological finding underscores the risk of VPA in epileptic patients. VPA is known to represent a higher risk of teratogenic effects in pregnancy compared to newer available antiepileptic drugs (Spina and Perugi 2004). However, despite clinical guidelines recommending the avoidance of VPA during pregnancy, it is still commonly used in developing countries due to its low cost. The “fetal valproic acid syndrome” in the human clinic is characterized by a constellation of somatic malformations and long-term cognitive dysfunction in the offspring. The developmental and cognitive deficits, which arise from VPA administration during pregnancy, include the autistic spectrum disorders (ASD) and epilepsy.

Our current hypothesis

It is well known from the literature that the major pharmacological and biochemical effects of VPA are related to its enhancement of the GABA system. In addition, VPA may produce an antagonism of the glutamate system and influence the histone system, which in turn may influence the expression of various genes. The most parsimonious explanation of enhanced neocortical cell numbers may be that it reflects the influence of VPA on the endogenous GABA system in the embryonic rat brain.

During the very early embryonic stage, GABA is present and functions as an endogenous paracrine neurotrophic factor. This occurs prior to the development and maturation of the GABA receptors. It has been shown by several groups that the presence of GABA as a paracrine excitatory factor facilitates and mediates the migration of early neuroblast cells, originating from the deep ventricular zones, into an accumulation of cells in the final superficial zones in the brain. Owing to the newly established inhibitory influence of the GABA receptors (type A and C), the migrating cells receive a stop signal which leaves them at their final destinations in the respective layers in neocortex, hippocampus, amygdala and other areas of the central nervous system.

This role of GABA and its transition from inactive to active receptors is illustrated in figures 1-2, taken from the papers by Denter et al., 2010 and Wang and Kriegstein 2009.

In contrast to the proposed developmental disorders, it has been shown that the exposure of high doses of VPA and other GABA-mimetic compounds to rats in the early postnatal period produces apoptosis leading to a major decline in cortical and hippocampal pyramidal cells (Bittigau et al., 2002; Ikonomidou 2009). The early postnatal period in the rat, represented by days 5-16, comprises the “brain growth spurt phase” where the first neuronal networks are pruned and established. This pruning phase is highly dependent on neuronal excitation and activity. The exposure to high doses of inhibitory GABA agonists (or glutamate antagonists) during this phase may inhibit neuronal activity resulting in increased vulnerability of inactive neurons to apoptosis.

Thus, both the timing and the dose of VPA are crucial for the final outcome of teratogenic effects in the offspring. Our initial data (Sabers et al. 2012, submitted) suggests that our low dose regimen of VPA produces a dominant effect in the early migration phase of the prenatal period.
VPA is teratogenic in most animal species tested so far, and produces various neuropathological changes when administered either pre- or post-natally. In fact, the timing and dose levels of VPA exposure to the rats during various periods of the pregnancy may be used to produce different pathological disturbances in the offspring brain. Indeed, offspring of female rats injected with just one high neurotoxic dose of VPA (300 or 600mg/kg) on the 12.5th day of gestation, which lasts 21 days in the rat exhibit brain abnormalities at autopsy.

The first study (Sabers et al., 2012 submitted) included rats which were exposed to daily injections of VPA during the last 9-12 days of pregnancy, and this treatment continued for the following 23 postnatal days. The selected doses of 20 and 100mg/kg VPA were comparable to doses administered in the human condition (Vorhees 1987; Manent, Jorquera et al. 2007). The chosen dose period in rats corresponds to the 2nd and 3rd trimester of human pregnancy and the data in offspring demonstrated a significantly increased number of neocortical cells in the offspring (Sabers et al., 2012 submitted).

The expected neuropathological changes will be evaluated by stereological cell counting, and presence of biomarkers of degeneration (NAA, GABA and glutamate). Behaviour in offspring will be evaluated by studying social behavior and memory. At various days after birth, eight young rats from various litter groups will be evaluated histologically (slices from prefrontal cortex and hippocampus). The behavioural effects in the offspring of VPA exposed rats will be studied in paradigms for a) juvenile play behaviour in the males at postnatal days 28-32, b) social interactions in the adult female rats (days 50-60) and in c) selected tests for learning and memory abilities such as the object recognition test. The social interaction and
in particular the engagement in juvenile social play with peers is essential for the development of communicative skills, to acquire cognitive skills and for obtaining the rewarding aspects of social competence in adulthood (Vanderschuren et al., 1997; Trezza et al. 2011).

The behavioural studies will be performed according to recommendations and publications by our external advisors and collaborators Dr Louk Vanderschuren in Utrecht and Viviana Trezza in Rome (the Juvenile play model) and Jo Neill’s team in Bradford (the social interaction and the object recognition tests). Freja Bertelsen’s PhD project is to further extend these studies and to validate the VPA rodent model and its possible relevance to autism and epilepsy.

**Results**

Sabers et al., 2012 submitted:

The rat pups exposed to both the low (20mg/kg) and high (100mg/kg) clinically relevant doses of valproate had significantly higher total number of neurons in the neocortex compared to controls (** P< 0.01)

Courchesne et al., 2011. In this small preliminary study, brain overgrowth in males with autism involved an abnormal excess number of neurons in the PFC. (2-8 years, 1m 16 Y). JAMA, 2011; 306(18): 2001-10

Our first results in the rat VPA model have shown a neuropathological enhancement in cortical cell number induced by the prenatal chronic administration of VPA, and this finding is consistent with the clinical results published by Courchesne et al., 2011.

We are currently investigating the effects of daily exposure of pregnant rats to VPA (20 and 100mg/kg ip) on their offspring during the critical brain development periods. The first behavioural result showed that the offspring of the female adult rats exposed to VPA during pregnancy showed superior memory at both doses in the novel object recognition test. These results are consistent with the fact that some patients with autism have an incredible memory.
Concluding comments

Clearly, in order to develop new therapies, we need to have an improved understanding of the neurobiology of social behaviour across species. Many species used in research, including rats and humans, have a highly organized social structure and complex social interactions are an essential part of survival of the species. When this social interaction is disturbed, as in many disorders as in autism and schizophrenia, successful treatment becomes more difficult. Restoration of normal social function must therefore be a key feature of successful therapy. Evaluation of new treatments in valid animal models is a critical stage in the development of improved therapies.

References


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NEW FACE AT CFIN

Freja Bertelsen, PhD student has a bachelor degree in biology and a master degree in biomedical engineering. Her main interest for neurotoxins started in 2009 where she was involved in a research project examining the potential risk when nanoparticles enter the environment, including a project evaluating the accumulation of silver nanoparticles in brain tissue of trout.

Her interest for psychiatric disorders and in particular autism grew when she started as a research assistant at CFIN in the beginning of 2011. Autism is a neurodevelopment disorder, which may also be induced in the fetus by drugs affecting the GABA system (alcohol, antiepileptic drugs) by their neurotoxicant exposure during pregnancy. The major focus of Freja’s work at CFIN is thus how the prenatal exposure of the antiepileptic drug valproate can change the development of the rat brain.

In August 2011 Freja started her PhD at CFIN. The purpose of the PhD project is to establish and optimize a new rodent animal model which may be relevant for autism and epilepsy. She studies the neuropathological, behavioural and biochemical changes induced during various development phases in the valproate rat model testing whether it is relevant and related to the neuropathology of the human autistic brain.

Hopefully this new animal model may help us increase our knowledge of autism and improve prevention and lead to novel treatments of this disease in the future.

The PhD project is financed by Aarhus University.