A study of possible environmental modulators of neostriatal dopaminergic synapses, with focus on dietary fat and polychlorinated biphenyl 153

Kine-Susann Noren Dervola

2014

Department of Biochemistry

Institute of Basic Medical Sciences

Faculty of Medicine

UNIVERSITY OF OSLO

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 2000

ISBN 978-82-8333-039-7

Cover: Hanne Baadsgaard Utigard.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika Publishing.
The thesis is produced by Akademika Publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

Acknowledgements

The work presented in this thesis was carried out at the department of Biochemistry, Institute of Basic Medical Sciences in Faculty of Medicine at the University of Oslo. I am grateful to the University of Oslo for providing research facilities and financial support making this PhD project possible.

I want to thank my supervisors Prof. Dr. Ivar Walaas and Prof.em. Dr. Frode Fonnum for excellent supervision and guidance in addition to many interesting discussions. Secondly, I want to thank Dr. Øivind Hvalby, Dr.Vidar Jensen and Dr Espen Borgå Johansen for the cooperation in planning of experiments and discussions.

I also want to thank Karen Anita Kvestad from the Institute of Marine Research for introduction and help with GC-analysis, as well as Grete Wøien for taking care of the experimental animals and performance of the behavioural tests.

I also want to thank Dr. Bjørg Roberg, Marit Nielsen, Inger-Lise Bogen, Mattis Wigestrand and Ngoc-Trang Thi Huyn for good team-work and cooperation.

Life would not be the same without the fantastic support from my family and my dear friends and officemates.

Innhold

Summary

Environmental substances like polychlorinated biphenyls and dietary fatty acids have been suspected to influence cognitive functions in humans, although much of their specific effects on synaptic functions are still unclear. There are many contradicting studies of the cognitive benefits of polyunsaturated fatty acids. In some studies it is emphasized that children with cognitive impairments such as ADHD, may have impaired fatty acid metabolism, implicating that the genetic status may be of importance. Ingestion of polychlorinated biphenyls have been associated with negative effects on cognitive processes, although the effects seems to be highly dependent on their molecular structure exposure time, duration, doses. In this thesis, the effect of dietary fat content and polychlorinated biphenyl 153 was investigated in neostriatal dopaminergic synapses and included measurements of related neurotransmitters like e.g glutamate and serotonin.

We included both male and female rats of two different genotypes namely the Wistar Kyoto rats and the spontaneously hypertensive rats as an animal model of ADHD, to investigate if genetic and hormonal status may influence the effect of these compounds. Initially the dietary study involved two distinct diets, one with a low level of dietary fatty acids (5%, w/w) with the other one supplemented with omega-3 polyunsaturated fatty acids (equal amounts of EPA and DHA) containing a high level of fatty acids (21%, w/w). The omega-3 supplementation led to gender dependent improvement in behaviour in the male SHRs, and included reduced levels of reinforcer-controlled activity, impulsiveness and inattention, with no or opposite effects in the female SHRs. Further biochemical studies on the neostriatal dopaminergic system, involved in reinforcement of behaviour, confirmed that the male SHRs had a reduction of the dopamine content in concert with enhanced homovanillic acid and calculated turnover ratio of dopamine, all of which were absent in the female SHRs. To further investigate if the effects achieved by omega-3 supplementation (this time with 80% DHA and 20% EPA), could be specific for omega-3 fat, we included an extra diet composed of high levels of fatty acids (21%, w/w) containing mainly lard which is rich in both saturated (40%) and polyunsaturated fats (60%) but with low levels of omega-3. In this second round we discovered that in the male WKY rats, both the lard and omega-3 enriched diets gave reduced levels of dopamine, tyrosine hydroxylase and vesicular monoamine transporter-2, without

changing the extracellular dopamine metabolite homovanillic acid in neostriatum. In contrast to the lard enriched diet, omega-3 enrichment induced an additional 2-fold increase in dopamine turnover ratio in the male WKYs, as well as a significant decrease in the levels of the dopamine transporter. Another finding was that the young male SHRs at p30 were hypodopaminergic compared to age and gender matched WKYs. In these male SHRs, the 40% lower dopamine turnover ratio was reversed 2-fold, to a similar extent by both lard and omega-3 enriched diets, without any significant effects on the protein levels.

These findings show that dietary fatty acid composition may strongly influence the neostriatal dopaminergic system in a gender and genotype dependent way, with both type of fatty acids as well as the amounts influencing the synaptic responses.

Polychlorinated biphenyls (PCBs) can be separated into ortho- and non ortho-substituted PCBs, where most of the ortho-substituted being neurotoxic and possibly interrupt cognitive functions. In this thesis we employed the ortho-substituted PCB 153, which is one of the most abundant PCB found in mammalian milk.

Biochemical studies were performed on dopamine and serotonin neurotransmitters as well as amino acids in the neostriatum of both genders from the Wistar Kyoto (WKY) and the spontaneously hypertensive rat (SHR) genotypes. Exposure to PCB 153 led to increases in homovanillic acid and 5-hydroxyindoleacetic acid in all groups except the female SHRs, whereas levels of dopamine and serotonin neurotransmitters as well as amino acids were unchanged in all genotypes and genders. PCB-153 also induced a decrease in the neostriatal D5 receptor in both genders and genotypes, without changing the D1 receptor. In contrast, levels of the dopamine transporter were reduced in the male WKYs, together with an insignificant reduction of the mean in the male SHRs. In addition, a gender-specific decrease of the PSD-95 protein occurred in the PCB-exposed male rats. Levels of tyrosine hydroxylase and vesicular monoamine transporter-2 were unchanged in all animals examined. Therefore, postnatal PCB exposure had major effects on both dopamine and serotonin turnover as well as specific PCB-sensitive synaptic proteins. Differences occurred between the effects obtained in both genotypes, as well as between genders. Altogether, this set of studies shows that both PCB 153 and dietary fatty acids, environmental compounds suspected to influence cognitive functions, may modulate neostriatal dopaminergic synapses in distinct gender and genotype dependent ways.

List of Publications

Paper I. **Kine S. Dervola,** Bjørg Å. Roberg, Grete Wøien, Inger Lise Bogen, Torbjørn H. Sandvik, Terje Sagvolden, Christian A. Drevon, Espen Borgå Johansen and S. Ivar Walaas (2012)

Marine omega-3 polyunsaturated fatty acids induce sex-specific changes in reinforcer-controlled behaviour and neurotransmitter metabolism in a spontaneously hypertensive rat model of ADHD *Behavioral and Brain Functions* 2012, 8:56 (10 December 2012)

Paper II. **Kine S. Dervola**, Frode Fonnum, Øivind Hvalby and S. Ivar Walaas (submitted)

Dietary lipid compositions and amounts impact on rat neostriatal dopamine turnover and metabolism.

Paper III. **Kine S. Dervola**, Vidar Jensen, S. Ivar Walaas, Øivind Hvalby, Espen B. Johansen and Frode Fonnum (Submitted)

Dynamic synaptic changes induced by polychlorinated biphenyl 153 in the rat brain

Paper IV. **Kine S. Dervola**, Espen B. Johansen, S. Ivar Walaas and Frode Fonnum (Submitted)

Gender dependent and genotype sensitive dopaminergic changes induced by polychlorinated biphenyl 153 in the rat brain.

Abbreviations

5-HIAA	5-Hydroxyindole acetic acid
ADHD	Attention deficit hyperactivity disorder
cAMP	Cyclic adenosine monophosphate
COMT	Catechol-o-methyl transferase
DAT	Dopamine transporter
DHBA	3, 4-hydroxybenzylamine
DHA	Docohexaenoic acid
D1/5R	Dopamine receptor D1/D5
EPA	Eicopentaenoic acid
GABA	γ-amino butyric acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
LFA	Low fatty acid
LTD	Long term depression
LTP	Long term potentiation
MOA	Monoamine oxidase
n-3	Omega-3
P	Postnatal day
PCB	Polychlorinated biphenyl
PUFA	Polyunsaturated fatty acids
SHR	Spontaneously hypertensive rats
SFA	Saturated fatty acids
TH	Tyrosine hydroxylase
VMAT-2	Vesicular monoamine transporter-2
WKY	Wistar Kyoto rats

1. Introduction

1.1 Environmental modulators of cognitive function

Neuronal circuits communicate through the use of neurotransmitters and receptors. The functional dynamics of neurons are changing trough out the life cycle in response epigenetics and frequency of stimulations, stress, nutrition or other environmental factors like *neuro-active* toxins or pharmaceuticals (Bowers *et al.*, 2010;Johannessen, 1991;Zainuddin & Thuret, 2012;Timmermans *et al.*, 2013;Bliss & Cooke, 2011;Takesian & Hensch, 2013). Disturbances to functional neuronal processes could lead to more or less maladaptive strategies of living and to psychological disorders. Among several neurotransmitters, glutamate, dopamine are believed to be important in cognitional processes. For glutamate, changes in plasticity like long-term potentiation (LTP) or long-term depression (LTD) which is dependent upon glutamatergic signalling, has been associated with memory and learning (Bliss & Collingridge, 1993). Studies have implicated that dopaminergic neurons might be important mediators of reward and involved in regulating aspects of cognitive functions (Schultz, 2007;Nieoullon, 2002). Changes in glutamatergic and dopaminergic neurotransmission have also been suggested to be involved in one of the most common cognitive childhood disorder named, attention deficit hyperactivity disorder (ADHD). The symptoms of this disorder involve severe attention deficit, but also impulsivity and hyperactivity. The world-wide prevalence of ADHD is ~5 % and although it is mainly genetic, twin-studies show only ~76 % heredity, leading to proposals of environmental impact. Environmental factors which have been suggested include maternal smoking, alcohol, poor diet as well as organic environmental toxins, although, no clear effect or single explanation has been found in epidemiological studies (Banerjee *et al.*, 2007). In this thesis I am going to focus on two carefully selected environmental factors that have been proposed to influence synaptic cognitional processes by modulating synaptic functions; 1) Dietary lipids and 2) Polychlorinated biphenyls (PCBs). Since earlier studies have implicated that both lipids and PCBs might influence cognitive functions (Winneke, 2011), we chose to focus on the dopaminergic dynamics of striatum (involved in reward and reinforcing mechanisms), and the glutamatergic dynamics in hippocampus (involved in memory formation). We also wanted to explore if a rodent model with ADHD-linked gene pool (SHR) inhabit the same sensitivity for environmental factors as

its control (WKY), and also if there is gender-related responses. *In this thesis we therefore studied neurologic effects of dietary lipids and PCB on the rat model (SHR) of cognitive deficits (ADHD) and its control (WKY).*

1.2 ADHD

ADHD is highly common and normally diagnosed during childhood, although diagnosis in adults have become more common the last decades (Polanczyk *et al.*, 2007). According to World health organization and American Psychiatric Association, ADHD are defined by 18 different symptoms of inattention, hyperactivity and impulsivity. The symptoms must been present for at least six months and represent a level that is maladaptive in relation to the age, as well as onset of symptoms before the age of seven years. The common symptoms of ADHD can be followed by severe comorbidities that might lead to reduction of life quality (learning problems, conduct disorder, anxiety, speech problems, increased chance of drug addiction and increased risk taking) (Larson *et al.*, 2011;Reinhardt & Reinhardt, 2013). ADHD is a male-dominated disorder, with three times more boys being diagnosed than girls. Further, symptoms differ between the genders with males usually express more severe hyperactivity and females often are more likely to be mainly inattentive (Weiss *et al.*, 2003). Because of different combinations of symptoms expressed among the patients, ADHD diagnosis consists of three sub-diagnoses. Primarily we have the typical ADHD combined (ADHD-C) subtype with all the main symptoms including attention deficit, hyperactivity and impulsivity, affecting boys three times more than girls. Secondly we have the primarily inattentive (ADHD-PI) subtype, characterized by inattention and a lack of focus leading to increased daydreaming, mind wandering and forgetfulness. The ADHD-PI subtype diagnosis is more frequent among girls than ADHD-C, since girls often show less external hyperactivity and impulsiveness. The last subtype is the hyperactive and impulsive subtype (ADHD-H), where the individuals express mainly these symptoms without being inattentive (Weiss *et al.*, 2003). Twin-studies have given evidence that this is a multifactorial and primarily inheritable disorder due to several polymorphisms; while a small part are thought to be environmental (Faraone et al., 2005). Although increased amounts of polymorphisms have been found in ADHD patients, the variability was so large that no single genetic

marker has been identified. Therefore it has been suggested that the polymorphisms associated with ADHD might hold increased sensitivity for epigenetic and environmental factors (Thapar *et al.*, 2013). First choice medications (psychostimulants) are ineffective in 20% of all cases, and some patients may require special combinations of medication (Wigal, 2009). This shows that the underlying mechanisms are complex and incompletely understood. It is important to gain more understanding about the underlying neurobiology of ADHD. In this thesis I will concentrate my work around neurochemical parameters believed to be important in ADHD, both in normal rats (WKY) and the rat animal model of ADHD-C (SHR), as well as exploring gender-dependent responses to our interventions.

Neurotransmitters of interest in ADHD

In mammalian brain, neurotransmitters can be divided into amino acid transmitters (like glutamate and GABA), catecholamine's (dopamine and noradrenaline), indoloamines (serotonin), acetylcholine or neuropeptides. The catecholamine's like dopamine and noradrenaline are derived from the amino acid tyrosine while the monoamine serotonin is derived from the amino acid tryptophan. With regard to ADHD especially neurons involving dopamine, noradrenaline, serotonin, glutamate and GABA is of interest, since abnormalities have been associated with these neurotransmitter systems (Bralten *et al.*, 2013;Yang *et al.*, 2013;Edden *et al.*, 2012;Gold *et al.*, 2014;Sagvolden *et al.*, 2005a). Although all these neurotransmitter systems are tightly connected and might influence each other, I have chosen to focus this thesis mainly on the dopaminergic neurotransmitter system, while including some serotonergic and amino acidergic analyses.

1.3 The dopaminergic neurotransmitter system

The dopamine producing neurons are mainly present in the substantia nigra, the ventral tegmental area and hypothalamus. These neurons have widely projecting axons and are forming four important signalling pathways namely the nigrostriatal-,

mesocolimbic-, mesocortical- and tuberoinfundibular pathway (Dahlstrom & FUXE, 1964;Anden *et al.*, 1964). Dopamine has an important modulatory role in the brain where it influences motor function and reward processes leading to motivation and different forms of learning (Nieoullon, 2002). The respective dopaminergic pathways innervate a variety of brain tissues. Here they modulate different functional aspects, such as motoric control (nigrostriatal system), feelings of reward and desire (mesolimbic), motivation, emotional and cognitive control (mesocortical) as well as hormonal release (tuberoinfundibular). Diseases and conditions which involves the these systems, includes Parkinson disease, schizophrenia, restless leg syndrome and ADHD (Morales & Root, 2014). Drugs targeting the dopaminergic neurons might therefore modulate a diverse set of systems that might influence several brain functions.

Dopamine cycle

Dopamine is mainly synthetized from L-tyrosine by the stereospecific enzyme, tyrosine hydroxylase (TH). TH is exclusively present in catecholaminergic neurons and represents the rate-limiting step in the biosynthesis of dopamine (Bademci *et al.*, 2012). After production, dopamine is concentrated into synaptic vesicles by the proton gradient- and ATP-dependent vesicular monoamine transporter-2 (VMAT-2), for storage and release (Varoqui & Erickson, 1998). The VMAT family in mammals include two isoform of the vesicular monoamine transporter; VMAT-1 and VMAT-2, where VMAT-2 is the only isoform expressed in the brain (Sudhof, 2004;Weihe *et al.*, 1994).

After an excitatory stimulus, exocytosis of vesicles releases dopamine into the synaptic gap where it diffuses and may stimulate five types of G-protein coupled receptors (D1-D5) (Missale *et al.*, 1998). The dopamine receptors are divided in two main groups called D_1-class and D_2-class receptors, based on their ability to activate adenylyl cyclase. Activation of adenylyl cyclase induces formation of cyclic adenosine 3`,5`-monophosphate (cAMP) and may lead to phosphorylation of phosphoprotein-32 and inhibition of dephosphoryllation (Greengard 2001). The D_1-class receptor group includes the D_1- and D_5-receptor and are bound to G-proteins called G_{olf} or G_s that are

cAMP inducing. The D_2-class receptors, including D_2-, D_3- and D_4-receptor, are coupled to G_i where stimulation inhibits cAMP formation (Missale *et al.*, 1998). The distribution and combination of dopamine receptor subtypes in different brain parts are also characteristic. This demonstrates that the different brain parts and functions are most likely dependent on diverse dopamine receptor subtypes and their unique properties (Beaulieu & Gainetdinov, 2011).

Action of extracellular DA is terminated by presynaptic reuptake by the dopamine transporter (DAT), which is a sodium coupled symporter (Sudhof, 2004). Free dopamine not capsuled by vesicles may be candidates for degradation by monoamine oxidase (MAO) and Catechol-*O*-methyltransferase (COMT) to homovanillic acid (HVA). The balance between TH-activity, DAT, VMAT-2 and the receptors, are therefore important factors controlling dopamine signalling.

Dopamine-receptor ligands

The subgroups of dopamine receptors can be distinguished by ability to induce cAMP formation and their ways of dealing with guanosine triphosphate. In addition they have characteristic agonists and antagonists as well as individual affinity for dopamine. The D_1- and D_5-receptor have SKF-38393 and SCH-23390 as a characteristic agonist and antagonist respectively, and their affinity for dopamine is micromolar and submicromolar respectively. In addition the D_5-receptor is the only subtype that does not regulate guanosine triphosphate. The characteristic agonists for D_2-, D_3- and D_4-receptor are bromocriptine, 7-OH-DPAT and CP-226269 PD respectively, whereas their affinity for dopamine is micromolar, nanomolar and submicromolar respectively. Ligands for these receptors are mainly used as research tools for investigation of dopaminergic function, although some of them potentially can be used as therapeutics (Mailman *et al.*, 2001).

DAT ligands

Many drugs like psychostimulants, antidepressants as well as recreational drugs like cocaine, are targeted for DAT (Wang *et al.*, 2013). Ligands of DAT might be divided in two groups, inhibitors and substrates where the inhibitors will block monoamine

uptake without being translocated, whereas substrates are actively translocated and triggers DAT-mediated release of dopamine by a reverse translocation cycle (Schmitt *et al.*, 2013). Both groups of DAT ligands are contributing to increase in extracellular dopamine and might lead to addiction.

Dopamine and ADHD

Dopamine is the precursor of noradrenaline, dysregulation of dopamine- and noradrenaline seems to be highly associated with ADHD. Methylphenidate and d-amphetamine are first choice pharmaceuticals for treating ADHD, and works by modulating the monoaminergic system as well as inhibiting DAT (Dopheide & Pliszka, 2009). Methylphenidate might also be an cognitive enhancer in healthy individuals (Linssen *et al.*, 2014). Based on the dynamic development theory it is hypothesized that altered dopmainergic function plays a central role by failing to modulate non-dopaminergic targets primarily including glutamatergic and GABAergic neurons (Sagvolden *et al.*, 2005a). According to this theory three of the main dopaminergic pathways can potentially be dysfunctional, giving rise to the three main symptoms respectively. The involved dopaminergic signalling pathways are; 1) the mesolimbic, where impairment results in altered reinforcement of behaviour and deficient extinction mechanisms, 2) the mesocortical pathway where impaired signalling results in attention deficiencies and poorer behavioural planning and 3) the nigrostriatal dopaminergic pathway where impairment will cause altered motor function (hyperactivity/hypoactivity) and nondeclarative habit learning and memory (Sagvolden *et al.*, 2005a).

1.4 The glutamatergic neurotransmitter system

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (Zhou & Danbolt, 2014). It is thought to contribute in memory formation associated processes and are important for neuronal communication, conversely, glutamatergic overstimulation can lead to neuronal damage (Zhou & Danbolt, 2014;Rothman & Olney, 1995;Bliss & Collingridge, 1993). It is therefore important to keep the activity levels in a controlled normalized range. To keep the excitatory glutamate stimuli under control it is important that these neurons are tightly connected

to well-functioning GABAergic neurons, as GABA is the main transmitter for damping excitatory stimulation. Glutamate is also the main neurotransmitter of the hippocampus and are involved in memory formation processes and developmental plasticity. Memory formation has been suggested to involve processes like long-term potentiation (LTP) or long-term depression (LTD) (Bliss & Collingridge, 1993). LTP is the measure of long-term-changes in synaptic plasticity and is mostly studied in the CA1 area at the Schaffer collaterals or in the structural hippocampal layers; stratum oriens and radiatum (Niemi *et al.*, 1998;Herwerth *et al.*, 2012). Although, it is also present in limbic forebrain and the entorhinal area (Racine *et al.*, 1983). In this thesis hippocampus will be the target for measurement of lipid or PCB influenced LTP and excitability.

Glutamate cycle

Released extracellular glutamate in the synapse usually undergo high affinity uptake into astrocytes (Schousboe *et al.*, 1977). In the astrocytes glutamate will react with ammonium by the help of glutamine synthase and adenosine triphosphate (ATP) to form glutamine. This reaction is highly important in detoxifying ammonium, since high concentrations will disturb synaptic function (Nissim, 1999;Sonnewald *et al.*, 1997;Norenberg *et al.*, 1997). Glutamine is not a neurotransmitter and is released from the astrocyte in to extracellular spaces, where it is taken up by the presynaptic terminal and converted back to glutamate by glutaminase. Glutamate are also involved in metabolic pathways like the citric acid cycle (TCA-cycle) and will here be transformed into other amino acids like f.eks GABA (Roberts & Rankel, 1950;Waagepetersen *et al.*, 2005). Glutamate from the cytosol will accumulate in vesicles due to vesicular glutamate transporters (VGlut), which are proton interchanging glutamate ports (Tabb *et al.* 1992). This is due to proton-dependent ATPases in the vesicle membrane that creates a proton-based electrical gradient. For the accumulation of glutamate to happen it is important with positively charged vesicular lumen (full of H^+) in addition to low pH (Tabb *et al.* 1992).

Glutamate Receptors

Glutamate receptors have L-glutamate as a primary activator and are a diverse group, including ionotropic (NMDA-, kainat- and AMPA-receptors) and G-protein-coupled metabotropic receptors (mGluR). The ionotropic glutamate receptors mediate fast excitatory synaptic transmission that are normally not voltage dependent (except NMDA receptors), while the g-coupled mGluR are involved in secondary messenger pathways mediating slow modulatory responses. N-methyl-D-aspartate (NMDA) in addition to AMPA receptors are suggested as important for memory processes and are found in particular high densities in the hippocampus and cerebral cortex(Roberts & Rankel, 1950;Wheal *et al.*, 1998). The NMDA receptors are voltage dependent (requires initial depolarization), ligand gated (requires binding of co-agonists), non-selective cation channels that are composed of two subunits of multiple combinations. Functional NMDA-receptors are normally composed of one NR1 and at least one NR2-subunit (Kew & Kemp, 2005), although these exists as several variants. NR1 can exist in seven splice variants while NR2 can be encoded by four different genes giving a total of 11 different subunits that can be combined. Due to high subunit diversity and their combinations, there can be many variants of this receptor. It's also worth to notice that there are several ways of modulating NMDA receptors as there are 6 known binding sites for different substances that can influence the receptor activity. Activation of the NMDA receptor requires not only binding of the main agonists but also binding of a co-agonist (glycine or d-serine). In addition, the ion channel is during normal resting potential blocked by Mg^{2+} and initial depolarization is necessary for the Mg^{2+} to disassociate allowing influx of Na^{2+}, small amounts of Ca^{2+} and outflow of K^{+}. Immediately after the Mg^{2+} is disassociated it re-binds due to strong attraction to its site in the ion channel, leading to inhibition of further ion flux through the channel. The influx of Ca^{2+} is thought to be the main mechanism behind synaptic plasticity formation, since Ca^{2+} might lead to several intracellular cascades of events. NMDA-receptors are also due to their complexity, slower and requires several factors to be present than the other ionotropic glutamate receptors (AMPA or Kainate) (Meldrum, 2000). AMPA receptors have lower affinity for glutamate than NMDA-R although, when activated, they have much faster kinetics (Meldrum, 2000). Normally, NMDA,

AMPA and Kainate receptors are enriched in postsynaptic densities (PSD) and coupled to PSD associated proteins. This enrichment of NMDA-receptors as well as AMPA-receptors in certain areas leads to increased chances of ion-channel opening when successful stimulation does occurs. Activation of one receptor gives a local depolarization, which removes the Mg^{2+} blockage, leading to increased chances of activation of the neighbour NMDA receptors in the local area.

The glutamatergic system in cognitive processes

LTP can be created by agonists activating the NMDA receptor with continuous patterns of stimulation, which leads to increased plasticity in the synapse, making it more sensitive to stimulation and increase chances of new stimulation (Bliss & Collingridge, 1993). The relationship between AMPA and NMDA receptor has also been suggested to be important(Williams *et al.*, 2007). In addition it is suggested that dopamine stimulation of D1 subunit could change NMDA-receptor sensitivity. This is thought to be a synergistic regulation since it leads to decreased dephosphorylating of the NR1-subunit of the NMDA-receptor (Snyder *et al.*, 1998).

1.5 PCB and lipids as modulators of cognitive processing and association with ADHD

Although high, the concordance of monozygotic twins mentioned earlier is not perfect, suggesting that environmental factors may act in additive or interactive ways with the genetic influence (Caspi & Moffitt, 2006;Willcutt *et al.*, 2007). Identifying possible causes and increasing our understanding of the mechanisms behind ADHD is important in treatment and prevention of symptom development. Although individuals with ADHD do not always express the same polymorphisms, there are some that are frequently represented. Some of these polymorphisms have been found in genes coding for serotonin- and dopamine- transporters and receptors, glutamate receptors and solute carrier protein (9A9) (Akutagava-Martins *et al.*, 2013). Several studies of blood parameters and early clinical studies have implicated that ADHD patients may have a dysregulation in the fatty acid metabolism, but also that lactational exposure of

PCBs could induce changes in gene expression of fatty acid-metabolism proteins and proteins involved in dopaminergic signalling (Sazonova *et al.*, 2011).

PCB

While PCB was widely used in industrial products since the 1920s, suspicion of adverse effects in wild animals and in humans was first raised in the 1970s. Although they are forbidden, their persistence in the environment and in organisms combined with their lipophilic properties has led to bioaccumulation. Although the levels are declining in nature, they are still high in some organisms on top of the food chain. It is also worth noticing that different PCB-congeners and mixtures can give a diverse spectrum of toxicity. Industrial mixtures of different PCBs types have shown to influence a variety of gene expressions also involved in the dopaminergic transmission system (Sazonova *et al.*, 2011).The effect of PCBs is highly dependent on the type of PCB, dosage and development stage of the exposed individual (Sazonova *et al.*, 2011).

PCB-congeners have 209 different possible structures and are hence named PCB 1 to 209. The different PCBs are distinguished by the amount of chlorination and whether they are in *para*-, *meta*- or *ortho*- positions on the biphenyl structure (figure 1.). The chlorination determine the mechanism of action and the persistence and toxicity in biological systems (Safe, 1984). PCBs can be divided in to two main groups, namely coplanar (non-*ortho* substituted) and non-coplanar (*ortho*-substituted) PCBs. The coplanar PCBs have no chlorine atoms in *ortho*-position leading to a planar relationship between the two phenyl rings. The non-coplanar PCBs have chlorine atoms in at least one *ortho*-position, forcing the two phenyl rings into different plane.

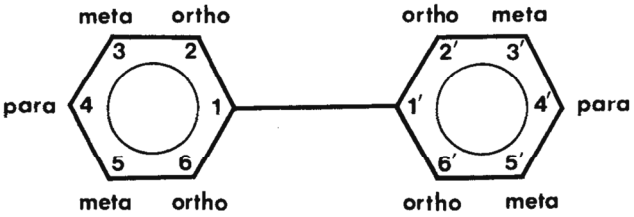

Figure 1: Structure of the PCB biphenyl skeleton. Chlorine atoms are bound to the biphenyl structure in meta-, ortho- or para-position (2-6). There are 209 theoretically possible congeners (including isoforms). The persistence and toxicity is determined by the number of chlorine atoms and their positions (ortho-, meta- or para-) also determine their toxicity (Safe 1984).

Non-*ortho* PCBs are very similar to the structure of dioxins, and might therefore induce similar effects as dioxins. The dioxin-like PCBs are able to induce increased liver enzyme production by binding to the aryl hydrocarbon-receptor (AhR). AhR inducing PCB-molecules includes mostly non-ortho PCBs with 4-6 chlorine atoms in *meta-* and *para-* positions. The AhR is coupled to heat shock protein 90 (HSP90) and are present in the intracellular fluid. When PCB binds to the AhR, Hsp90 is released and the PCB-AhR-complex is transported inn to the nucleus. The release of Hsp90 uncovers the DNA binding site for aryl hydrocarbon receptor nuclear translocator, which is a transcription factor that can bind to DNA. Most of the activated genes are coding for enzymes involved in biotransformation, and could lead to increased turnover (detoxification) or bio activation (increased toxicity) of xenobiotics (Hansson *et al.*, 2006). Also genes coding for enzymes responsible for thyroid hormone conjugation and metabolism of retinol seems to be influenced (Durham & Brouwer, 1989). Exposure to large amounts of non-*ortho* PCB-molecules might in addition result in chlor-acne and hyperpigmentation (Luecke *et al.*, 2010). They have also been suspected to be involved in carcinogenesis, disturbed hormone balance and impaired immune function (Yoshizawa *et al.*, 2007;Davis & Safe, 1990). PCB exposure might also lead to changes in tissues and liver dysfunction caused by necrosis and fibrosis, probably due to changed contents of lipids, cholesterol, porphyrin and retinol.

In body tissue from mammals, birds and fish it has been found that the *ortho*-substituted PCBs are dominating (Mariussen & Fonnum, 2001). Studies have shown that these types of PCBs could lead to changes in behaviour, which involves both hypo and hyperactivity (Eriksson *et al.*, 1991;Holene *et al.*, 1998;Johansen *et al.*, 2011;Johansen *et al.*, 2014). Certain types of ortho-PCBs have also shown to be especially good activators of the Ca^{2+} transporting ryanodine receptor (RyR) (Pessah *et al.*, 2006). Alterations in Ca^{2+} homeostasis and subsequent intracellular second messengers have been proposed to be involved in developmental neurotoxicity (Unni *et al.*, 2004;Tilson & Kodavanti, 1998).

Other changes in brain parameters includes both short and long term changes to the muscarinic cholinergic receptor and dopamine uptake inhibition in vesicles (Eriksson *et al.*, 1991;Mariussen & Fonnum, 2001). Considering the large role of dopamine in ADHD, it is also notable that a recent study have confirmed that especially *ortho*-substituted PCBs might bind to DAT (Wigestrand *et al.*, 2013) with PCB 110 possible being as potent as cocaine. The thyroid and retinol homeostasis was found as one of the *ortho*-substituted PCB 180s most sensitive physiological parameters discovered in WKY rats (Viluksela *et al.*, 2014). Changes in thyroidal hormones might as well lead to severe effects on the thyroid gland, which is highly important in normal development of the brain. Early disturbances in the thyroidal gland can therefore possibly influence hearing, motoric and intellect (Porterfield, 2000).

Also studies on humans have found correlation between psychomotoric impairments and the degree of PCB burden (Gladen *et al.*, 1988). Although, it is important to notice that human studies often includes several confounding factors as they are naturally exposed to a multitude of substances. The prenatal and lactational part of life is considered to be the most vulnerable regarding developing neurological damage due to PCB-exposure (Chevrier *et al.*, 2008;Sazonova *et al.*, 2011;Schantz *et al.*, 2003). This is therefore interesting in regard to ADHD which is believed to be a neurodevelopmental disorder.

Dietary lipids

Dietary fat is one of our most important macronutrient, not only containing lipids but also vitamins. Fatty acids are thought to be important in cellular development and maintenance, and might influence cell membrane fluidity, neuronal function and signalling (Su, 2010;Kim *et al.*, 2011;Cao *et al.*, 2009;Muskiet, 2010). The human diets have been changing gradually since the agricultural revolution approximately 10 000 years ago, with accelerated changes being introduced during the industrial revolution approximately 200 years ago (Cordain *et al.*, 2005). From the nutritional and anthropological point of view, humans have developed on a diet with a fair amount of n-3 FAs, whereas many industrial diets of today contain less n-3 FAs and more n-6 FAs and SFAs (Muskiet, 2010). Both n-3 and n-6 fatty acids are essential and must be provided primarily through diet (Richardson, 2006). Polyunsaturated n-3 FAs like DHA and EPA are abundant in brain tissue and are necessary for proper neurological functions including G-protein signalling (Janssen & Kiliaan, 2014;Litman *et al.*, 2001).

Many studies implicate that cognitive dysfunction in ADHD may be escorted by a lack of n-3 PUFAs, during embryonic development and early life (Antalis *et al.*, 2006;Dopheide & Pliszka, 2009;Peet & Stokes, 2005). This association is believed to root in nutrition sensitive polymorphisms in patients predisposed to ADHD. A recent study shows that children with low scores on cognitional tasks receives improvement on these tasks after DHA supplement, while children with higher scores did not improve (Richardson *et al.*, 2012). This suggests that low scores might be associated with DHA deficiency, but also that the response is highly dependent on genetics. The findings have been varying in older studies and could possibly be due to the absence of genetic considerations. Despite variation, other studies have also found some association with DHA supplementation and improved cognitive performance (Willatts *et al.*, 1998;Bloch & Qawasmi, 2011a;Henriksen *et al.*, 2008;Helland *et al.*, 2008;Birch *et al.*, 2000). The clinical research evidence on n-3 FAs as an ameliorating compound on cognitive disturbances are limited, and further research have been warranted (Peet & Stokes, 2005). We therefore chose to study possible effects of long-term n-3 PUFA supplementation on SHR and WKY rats.

2. Aims of study

The working hypothesis explores the effect of environmental factors such as dietary fatty acids and PCB153 on the neostriatal dopaminergic synapses in different genders and genotype. In addition, we wanted to investigate if the SHR animal model of ADHD have an increased sensitivity to any of these compounds. We want to explore this by studying the dopamine, serotonin and glutamate signalling-dynamics in specific brain structures (striatum and hippocampus, respectively) after introducing a toxin (PCB 153) that is suggested to make the symptoms worse and polyunsaturated fatty acids that is suggested to ameliorate the ADHD symptoms. By introducing a toxin and a suspected ameliorating compound, we aim to identify eventual neurochemical responses in brain areas associated with learning and motivation. The aim of this study was to investigate how these two compounds effected behaviour and related biochemical parameters. We have therefore investigated if:

Polyunsaturated fatty acids:

1) Change ADHD symptoms in the SHR-rat.

2) Have effects on the dopaminergic synapse, by studying the nigrostriatal pathway and if a general increase in n-6 FAs could give the same effect as n-3 FAs.

3) Have same effects on both genders and on the SHR-ADHD model compared to WKY- control rat strain.

PCB 153:

4) Have effects on the dopaminergic synapse, by studying the nigrostriatal pathway, performing analyses in neostriatal tissue and electrophysiological activity measurements of the hippocampus detecting possible changes in dopaminergic modulation of the mesocortical pathway.

5) Have genotype specific effects in the SHR-ADHD animal model and the control WKY-rat. Since our hypothesis suggests that ADHD-genetics and thus SHR-genetics might represent increased vulnerability to environmental factors.

6) Have gender specific effects, especially since ADHD is regarded as a gender dependent disorder.

3. Methodological considerations

3.1 Animal models

The spontaneously hypertensive rat (SHR) is considered one of the best available animal models of ADHD (ADHD-C). It is a genetic model of ADHD and hypertension that have derived natural from the Wistar Kyoto /NHsd (WKY) rat strain by prolonged isolation. In operant behavioural tests the SHRs show hyperactivity, impulsivity and reduced attention compared to WKY-rats(Sagvolden *et al.*, 2009), and also if compared to Sprague Dawley rats. Although many rat strains might show similar behaviour as the WKY, they may not have the same genetic and levels of biological markers. This also comprise outbred WKY strains like Wistar Kyoto from Charles River (WKY/NCrl) which exhibit divergence in behavioural tests, showing inattention compared to WKY/NHsd. The WKY/NCrl is therefore proposed as an animal model of ADHD-PI and cannot be used as a control for SHR-rats (Sagvolden *et al.*, 2009). It is therefore important to select a proper control-strain to avoid misinterpretation of acquired data and spurious results. Since the SHR-rat strain has developed from the

WKY/NHsd, they should be more genetically similar than any other rat strain making them the most proper control for studying the SHR-rats. Exposure of WKY-rats to di-*ortho*-substituted PCB-congeners during development have in some tests shown to result in changed behaviour which is similar to SHR behaviour (Holene *et al.*, 1998).

The SHR-rat model has been extensively studied in details and shows exceptional validity in behavioural symptoms in a variety of behavioural tests. In general, SHR-rats respond well to d-amphetamine, methylphenidate and guanfacine resulting in reduced hyperactivity (Sagvolden *et al.*, 2005b;Sagvolden, 2006). Since there is no known specific genetic and neurological benchmark marker to define ADHD, the validity of the biological mechanisms can therefore not be emphasized too much. Although there are polymorphisms in related gene families that seem to be present more often in ADHD subjects, one cannot state that all subjects with the given polymorphism will exhibit ADHD-like symptoms (Akutagava-Martins *et al.*, 2013).

Since SHR-rats are the currently best validated model of ADHD, we chose to perform our experiments on both the SHR-rat and on its appropriate control; the Wistar Kyoto rat (Sagvolden *et al.*, 2009).

3.2 Lipid enriched diets and PCB exposure

Lipid enriched diets

In our animal facility, the standard rat feed consists of 5% (w/w) FAs (LFA) giving 11% of total calorie intake. A calorie intake of 11% from fat might be considered as fairly low compared to western diets with an average at 35% (Lee *et al.*, 2001). Although several studies have suggested that the type of fat is more important than the amount; the American Academy of Pediatrics recommends to maintain the calorie consumption of fat to 20-30% (Lee *et al.*, 2001). The FAs found in human diets depend on many factors, including geography, socioeconomic status and culture. In the coastal areas or Inuit people there is usually a high intake of n-3 PUFAs compared to inland populations or populations with low marine seafood culture. Therefore it was

important to also investigate the effect of low fat diet (with 11 % of total kcal from ~5% FAs) to a more normal high fat diet (LFA; 40 % of total kcal from ~20% FAs), as well as investigating if there were differences between an western diet (SFA; rich in SFA and n-6 FAs) compared to a Inuit-diet (PUFA; rich in n-3 PUFAs) (Muskiet, 2010).

The 20% SFA-rich diet induced a significant increase in the weight of both WKY and SHR rats, compared to the 5% FA-diet. In contrast the 20% PUFA-rich diet induced a small (paper I) or no weight gain (paper II). It is also worth to notice that we changed distributor of the PUFA-diets between paper I and paper II, which also led to a slight change in the n-3 FA composition. In paper I, the n-3 content were present as almost equal amounts of DHA (40%) and EPA (60%), while this ratio was changed to mainly DHA (80%) and EPA (20%) in paper II. Since there are different amounts of fat and different fatty acids present in the diets, the rats could have preferences, making them ingest different amounts of feed. Therefore we also performed fatty acid analysis with Gas chromatography (GC) of the cortical brain tissue, to see if the diets could reach the brain and induce a change in the lipid composition. While there was few changes in the SFA-fed groups with no effect on the n-3 FA proportion, the PUFA-diet induced significant increases in the cortical n-3 FA proportion, giving a higher n-3/n-6 ratio in the cortical tissue.

PCB Exposure

In humans and mammals non-coplanar PCBs have been especially associated with problems in cognition. These PCBs are found in high concentration in both nature and human milk. PCB 153 is the most dominant congener in human milk and has a slow turnover in human tissue of 25 to 30 years (Cerna *et al.*, 2010;Seegal *et al.*, 2011). For this reasons we have chosen to study the di-ortho-substituted PCB congener 153 in this thesis. The total dose of PCB 153 used (9 and 18 mg PCB/Kg bodyweight) is similar to a tissue concentration range found in wild animals (Skaare *et al.*, 2000). The dose was divided in sub-doses of 3 and 6 mg/Kg that were administered orally three times during lactation, to avoid acute toxicity. Because we have recently observed behavioural changes attributed to PCB 153 (Johansen *et al.*, 2011;Johansen *et al.*,

2014), this study focused on whether PCB 153 interfered with aspects of glutamatergic excitatory and dopaminergic/serotonergic modulatory neurotransmission.

3.3 Brain areas selected for analysis

The function of the dopaminergic striatal synapses is linked to various types of learning including reward based learning, habits and procedural skills . The dopamine content of the nigrostriatal synapses is high, compared to other brain areas like cortex and hippocampus. The dopaminergic terminals communicate with cholinergic interneurons and GABAergic efferent neurons mainly through D5 and D1-receptors respectively. Hippocampus is another brain structure known for its importance of information processing leading to learning and memory formation, which seems to be linked to encoding of prediction error of reward, cue outcome-associations of novel responses and mismatch signals (Delgado & Dickerson, 2012). The hippocampal neurons are primary of glutamatergic nature, although they are modulated by dopamine and GABA (Lang *et al.*, 2014;Shohamy & Adcock, 2010). Although not much is known about the function of the anatomical connection between hippocampus and striatal tissue (cortico-striatal circuit), some studies implicates that it is important for reward-based learning (Delgado & Dickerson, 2012;Pezzulo *et al.*, 2014). The dynamic developmental behavioural theory of the neurologic mechanisms of ADHD is based on the hypothesis that altered dopaminergic function leads to impaired modulation of non-dopaminergic (primarily glutamate and GABA) signal transmission. In addition it is suggested a hypofunctioning mesolimbic dopamine branch in ADHD (Sagvolden *et al.*, 2005a). Dysregulation of the dopaminergic system seems to be highly relevant with regard to ADHD. In addition, both striatum and hippocampus seems to be possible areas important for the neuropsychological symptoms of this disorder. Therefore, we chose to study biological parameters primarily in striatum and additionally in the hippocampus.

4. Summary of results

Before treatment

The dopamine turnover of untreated prepubertal (p30) SHR rats (paper III), had a 50% lower turnover than age-matched WKYs, while at adult age (p60) this difference was almost vanished between these two strains. This is due to an age dependent decrease in dopamine-turnover, which was predominant in WKY. A similar age dependent reduction was also found in the serotonin-turnover, although no significant differences was found between WKY and SHR. In general the basal level of dopamine, serotonin and dopamine-related proteins like DAT, VMAT-2 and TH as well as D1R and D5R were the same in both the WKY and SHR rat strains. In paper I, we also observed a higher neostriatal levels of GABA in adult WKY, compared to the SHRs. Since ADHD is also believed to be highly linked to dysfunction in the dopaminergic system, the low dopamine-turnover in the young SHRs might be of high relevance to the symptoms of the SHR rats.

Dietary FA supplementation

Paper I. This study investigated if n-3 PUFA-supplement during development could ameliorate ADHD symptoms in the SHR-rat model of ADHD. Here we gave the SHRs one experimental n-3 PUFA-enriched diet with 20% total fat, while the control SHRs received the standard animal facility diet containing mainly n-6 FAs with 5% total fat. In the behavioural studies, n-3 rich feed led to improved reinforcer induced attention in males, while inducing a reduction in spontaneous locomotion in both genders. Further, a n-3 PUFA effect restricted to males involved a 30% decrease in dopamine-levels and an increased turnover. The turnover was practically doubled in the SHRs that received the n-3 FA supplement. In addition, a similar n-3 FA induced change was observed in the serotonergic system with a 40% reduction of serotonin and a four times increased serotonin turnover, both restricted to male.

Paper II. Further investigation was performed on males of both WKY and SHRs at p30 instead of p60. The feeding procedure was the same as in paper I, by giving diets before and during pregnancy and development. The experimental diets included two high FA-diets with 20% fat, one containing mainly n-6 FAs as and the other containing mainly n-3 PUFAs. They were compared to a control group fed the standard animal facility diet for rats which contained low levels of FAs (5% fat). FA-analysis of cortical tissues was performed and showed that the n-3 FA diet lead to increased n-3 FA in the tissue, while n-6 FA diet did not. Further, we found that both FA-enriched diets could lead to the same dopaminergic modulation in both WKYs and SHRs, as seen in paper I. The n-3 FA was the most potent and led to larger effects on the protein levels including DAT, as well as on the turnover in both SHR and WKY rats. Although the SHRs responded to the FAs in a similar way as WKY, the responses were weaker and did not reach significant levels for all parameters.

Postnatal PCB 153 exposure

Paper III. In this paper we investigated the effect of postnatal exposure of PCB 153 on the dopaminergic striatal system and the LTP obtained in the hippocampus of standard WKY rats. We found that PCB 153 exposure led to a 250 % and 115% increased level of the metabolite of dopamine and serotonin; HVA and 5-HIAA, respectively, without influencing the overall levels of dopamine and serotonin. This led to a 4 and 2 times increase in the calculated turnover rates of dopamine and serotonin, respectively. There were no effects on the general amino acids. At the same time we did find a pathway specific increase in LTP magnitude of the stratum oriens (no effect in stratum radiatum) in hippocampus without effecting the basal synaptic transmission in the same strata.

Paper IV. Further we wanted to investigate if the PCB 153-effect was the same in the SHR as in WKY, and if there were any gender specific effects. In this study we focused mainly on the measurements of dopamine, serotonin and their respective metabolites, in addition to measurements of dopamine associated proteins. Here we did also find an increase in the HVA and 5-HIAA –levels in all but the female SHRs, without effecting dopamine or serotonin levels. When looking at the dopamine associated proteins we found a reduction of DAT in the WKY males only as well as a reduction in D5R in all group without any effects on D1R. Reductions of PSD-95 proteins were restricted to the males of both genotypes. At the same time no effects were seen on TH or VMAT-2. It might be proposed that increased HVA results from inhibited reuptake of dopamine through DAT. Most likely, the measured down-regulation of D5R might either be due to compensatory mechanisms of dopaminoceptive cells or degradation of postsynaptical targets (as seen by a decrease of PSD-95 in males), possibly due to increased dopamine-induced stress. In the overall results we found that there were fewer significant effects in the female rats of both strains. Also, the SHRs of both genders seemed to respond less than the gender matched WKYs. In this set of data we reused the monoamine results of WKY male from paper III, to compare to the females and the SHRs, but there was also added new additional measurements to the male WKY groups for this paper.

5. Discussion of results

Effects of polyunsaturated fatty acids

Mapping of dopamine dynamics during postnatal development in the animals showed that there was a hypoactive dopamine-turnover in SHR compared to the WKY which was larger at p30 compared to p60. This difference is supported by a previous study showing 25% less effective COMT in the SHRs (Masuda *et al.*, 2006), in addition to another work, showing enhanced DAT reuptake in the SHRs (Miller *et al.*, 2012). Both of the findings may contribute to restrained HVA formation and lead to lower turnover rates. In the our first study (Paper I) where we examined (p60) SHR-rats of both genders after receiving diets with either 5% FAs or 20% PUFAs we found gender dependent effects restricted to males. These data made us perform further analyses in paper II on males at 30 only, where we also included both WKYs and SHRs exposed to the experimental diets with the proportion of dietary at 5% (LFA) and 20% w/w (SFA or PUFA respectively).

In the initial study (Paper I) we measured ADHD related symptoms in adult (p60) SHR-rats of both genders receiving diets with either 5% FAs or 20% PUFAs. It was clear that there was a gender-dependent response to the PUFA-diet, where several responses were restricted to males, such as ameliorated impulsiveness, inattention and increased dopamine-turnover, while both genders got a reduction in general movement. The reason for this remains unknown, although it could be due to sex-hormone sensitivity of FA-acid metabolism (Kelly *et al.*, 2014;Petrovic *et al.*, 2014). The behavioural and dopaminergic data of SHRs from paper I, implied that the PUFA-supplement improved behaviour compared to the LFA-diet, with greater effects in males. Because of low levels of FAs in the control diet, it was difficult to conclude whether this behavioural effect was a result of the n-3 PUFAs or a result of increased level of general FAs. Although the uncertainty, a published paper showed that high-fat diet should led to reduced mesolimbic dopamine-turnover and unchanged dopamine-levels (Davis *et al.*, 2008). Since these results were opposite from what we found, we

made a preliminary conclusion that the effect we found in paper I, could be due to the n-3 PUFA supplement.

In paper II, we found that increasing the dietary fat content from 5% to 20%, regardless of whether it contained SFA or PUFA, had large impact on the neostriatal DA system in both male WKY and SHR. Although SFA led to similar effects, n-3 PUFA had an even larger effect than SFAs on the dopamine-turnover and the related synaptic proteins. It should be emphasized that it is a weakness of the study design that we were unable to measure the behavioural parameters of the animals used in paper II, therefore giving no behavioural results for WKY rats receiving n-3 PUFAs or SFAs as well as for the SHRs receiving SFA. Since the neostriatal dopamine system of both WKY and SHR reacted in a similar way with the SFA-diet as with the PUFA-diet, it is highly likely that the fat content of the standard animal facility diet (LFA) is insufficient, particularly considering that normal human diets should preferably contain 20-30% w/w fat (Lee *et al.*, 2001). Since the SHRs received behavioural benefits from increased fat as well as reaching a dopamine turnover similar to WKY, this implies that the SHRs might need higher amounts of dietary fat than the WKY to achieve proper dopamine signalling (Miller *et al.*, 2012;Masuda *et al.*, 2006). The WKY-rats received a greater boost of their dopamine turnover than the SHR after PUFA-supplementation. Before drawing any conclusion of the benefit of SFA and PUFAs in the WKY animals, it would be necessary to measure their behavioural parameters.

Furthermore, the SFA and PUFA-dependent decrease in the levels of both TH and VMAT-2, could explain the decreased dopamine level. Strangely, the cells managed to keep the dopamine signalling and turnover rates stable, while increasing it with the PUFA-diet. Another study also demonstrate that dietary restriction of FAs could lead to an increased amount of TH as well as a VMAT-2 level, most likely leading to higher dopamine levels like we found in the LFA-diet (Bondi *et al.*, 2014). The study also confirmed our results by demonstrating lower VMAT-2 and TH levels due to n-6 or n-3- FA supplemented diets (Bondi *et al.*, 2014;Narendran *et al.*, 2012). The concordance of the TH and VMAT-2 regulation found in paper II and by Bondi et al.

might be due to a TH and VMAT-2 complex on the synaptic vesicles (Cartier *et al.*, 2010;Bondi *et al.*, 2014) therefore a reduction might possibly implicate a decrease in amounts of vesicles. Although reduced VMAT-2 might mean that the vesicle uptake is reduced, the amount of DA may also be reduced. The PUFA induced increase in dopamine turnover might possibly be due to the increased down regulation of DAT, which was larger than in the SFA group. The PUFA induced reduction of DAT have most likely resulted in reduced dopamine reuptake by the presynaptic neuron, leading to increased amounts of extracellular dopamine, giving a Ritalin/amphetamine-like effect. Our discovery of SFA and PUFA-enhanced dopamine turnover was unexpected, as another study has shown opposite effect on dopamine-turnover after high FA-diets (Davis *et al.*, 2008). However, our use of HVA as marker for extracellular dopamine degradation may give a more detailed picture of dopamine available for stimulation of receptors than DOPAC (Jones *et al.*, 1998).

We did not see any changes in the D1/D5-type receptors, suggesting no compensatory effect of these receptors on cholinergic and GABAergic interneurons or the efferent GABAergic neurons (Berlanga *et al.*, 2005) Finally, a significant reduction in PSD-95 was induced only by PUFAs and might implicate changes in postsynaptic glutamatergic neurons and their glutamate receptors (El-Husseini *et al.*, 2000;Rao *et al.*, 1998;Sans *et al.*, 2000). Except for the changes in dopamine levels and turnover, the SHRs failed to reach significant levels of confidence on the TH, DAT and VMAT-2 levels. The lower sensitivity to SFA and PUFA might be a reflection of possible underlying dysfunctions in SHRs dopamine metabolism and regulation (Miller *et al.*, 2012).

Since the SFAs also were able to induce changes in the dopamine system which were similar although slightly less effective, than PUFAs, it could be possible that the SFAs are metabolized less effectively, to some of the same active compounds as PUFAS. However, when we measured total FAs from cortex, we did not find any increase in the n-3 FAs in those only receiving SFA-diet. In contrast, we did find significant increases in n-3 PUFA levels in the group that received PUFA-diet, showing that the feeding was successful and that the lipids were distributed to the brain. For future

studies, it would be interesting to measure different behavioural parameters also for animals which have been treated with the SFA-type diet to find out if n-6 also may induce similar behavioural changes as n-3 PUFA did. Studies on children have also shown that n-3 PUFA supplements might be beneficial for reading and behaviour, especially for underperforming children with lower reading and behavioural scores (Bloch & Qawasmi, 2011b;Richardson *et al.*, 2012). In one of the recent studies the placebo group receiving n-6 FAs did not achieve the same benefits as the group receiving n-3 PUFAs (Richardson *et al.*, 2012). It would also be interesting to investigate what the reduction of PSD-95 might mean to the incorporation, amount and function of glutamate receptors in the neostriatum.

Effects of polychlorinated biphenyls (PCB)

The level of HVA and 5-HIAA are good markers of released dopamine and serotonin, respectively. The PCB-induced increase in HVA-levels in both WKYs and SHRs of both genders, described in paper III and IV, is most likely due to the reduction in DAT-levels. DAT impairment leads to accumulation of extracellular dopamine by inhibiting reuptake and making dopamine a candidate to degradation to HVA. We found a PCB induced reduction of DAT in WKY male together with a tendency of reduction in SHR male and in the females of both strains. A recent study (Wigestrand *et al.*, 2013) demonstrates that PCBs also have the ability to bind to DAT, leading to dopamine-reuptake inhibition. The unaffected dopamine levels found in both WKY and SHR of both genders, were also supported by the finding of unchanged TH levels. High dopamine production and reduced DAT can contribute to accumulation of dopamine in the extracellular space and explain the increase in HVA-levels that we observe. The dopamine degradation process by COMT is necessary for clearance of extra-vesicular dopamine and might be especially important during reuptake inhibition. Clearance of dopamine by COMT have previously shown to be impaired in the SHRs (Masuda *et al.*, 2006), in addition to a recent work showing enhanced reuptake of dopamine by DAT in the SHRs (Miller *et al.*, 2012), both of which might be an explaining factor for the low PCB-induced dopamine turnover found in the

SHRs compared to the WKYs of both genders. High amounts of free dopamine can also be bio-transformed to the toxic metabolite called 6-hydroxydopamine which is damaging to the dopamine producing cells (Zigmond *et al.*, 2002). Excess extra vesicular dopamine have been suspected to result in increased chance of 6-hydroxydopamine formation, and might therefore be disadvantageous (Graumann *et al.*, 2002). Since the degradation of free dopamine by COMT might be impaired in the SHRs in addition to enhanced DAT uptake, these rats could face a greater risk of 6-hydroxydopamine formation and toxicity (Liao *et al.*, 2003;Masuda *et al.*, 2006);(Miller *et al.*, 2012). The female SHRs had the lowest PCB-induced increase in HVA-levels and was the only group that failed to show significant enhanced dopamine-turnover. Like HVA and dopamine, 5-HIAA was also highly increased while serotonin remained stable in both WKYs and SHRs except for the female SHRs. Also here, the female SHRs failed to receive significant enhancement of serotonin turnover, unlike the other groups, as well as it only was a small but insignificant increase in 5-HIAA. This shows that PCB 153 most likely works in the same ways on both of the dopamine and serotonin transmitter systems. Another finding was that the D5 receptor were significantly down-regulated by PCB 153, whereas D1-receptors was unchanged for all strains and genders. A down-regulation of D5 receptors could be regarded as a compensating mechanism by the cholinergic interneurons in response to excess dopamine, by reducing the availability of these targets. In contrast, D1 receptors are located mainly on the efferent GABAergic neurons. The failure of D1Rs to respond to PCB in a similar manner as the D5R, could indicate possible inability of the D1Rs to compensate for increased stimulation of dopamine or alternatively that they are more insensitive to increased dopamine levels (Sunahara *et al.*, 1991). Further research should be performed to investigate if the cAMP-levels in these neurons are changed, as this would be a good indicator of the postsynaptic response to the excess extracellular dopamine levels (Walaas et al 2011).

PSD-95 is highly involved in scaffolding and modulation of postsynaptic receptors, and is especially concentrated in glutamatergic excitatory neurons. The decrease of PSD-95 in males only, could therefore signalise PCB-induced changes on these innervating glutamate neurons (Han & Kim, 2008;Kennedy, 1998) and possible

AMPA and NMDA activation (El-Husseini *et al.*, 2000;Rao *et al.*, 1998;Sans *et al.*, 2000). In paper III we also found a PCB-induced pathway specific increased LTP in hippocampus. Increased LTP has also been found in Alzheimer disease and PSD-95 mutant mice, and were associated with reduced learning abilities. The findings of increased LTP and reduced learning was explained by possible disturbances of bidirectional synaptic plasticity (Gylys *et al.*, 2004;Migaud *et al.*, 1998). An increase in PSD-95 have on the other hand been associated with a possible compensatory mechanism in learning-impaired old rats (Nyffeler *et al.*, 2007). As increased PSD-95 can protect against impairments in the bidirectional synaptic plasticity processes.

In the female rats we also found increased HVA and 5-HIAA, together with stable dopamine and serotonin levels. This findings suggest excess levels of extracellular dopamine levels also in these female-rats. Similar to the males, the female rats also responded to PCB by a reduction in the D5 receptor although the PSD-95 were unchanged. A down-regulation of D5 receptors could also be regarded as a compensating mechanism by the cholinergic interneurons, by reducing availability of these targets in response to excess dopamine. The reason for why we do not find a reduction in PSD-95, in the females could possibly be due to PCBs inability to significantly reduce DAT in these females, as well as a stronger tendency of increased VMAT-2 (WKY and SHR females; $p = 0.37$ and $p = 0.056$ respectively, whereas males; $p = 0.39$ and $p = 0.30$ respectively). Both DAT and VMAT-2 levels are important for efficient clearance of dopmaine from the synapse, and high levels decrease the chance of dopamine-mediated toxicity (Liao *et al.*, 2003).

A recent behavioural study of rats that received PCB 153 (Johansen et al. 2014) shows dose dependent behavioural changes which were sex and strain dependent. This particular study demonstrated that the SHRs are more sensitive to PCB induced behavioural changes than WKYs, at the same time, more behavioural changes was seen in the females than in males, although few changes reached significance. Since the behavioural data shows magnitude of effects regarding sex and strain which were opposite compared to what we found in the biochemical analyses, it is possible that the measured biochemical changes, such as HVA and 5-HIAA, which were greater in

WKY and in male rats, might be of compensatory importance, by showing metabolic effectiveness of the excessive amounts of free neurotransmitters. Effective clearance and compensatory mechanisms might be important if the stimuli of these neurotransmitters exceed to unbeneficial levels. Effective clearance might also possibly reduce the chance of toxic by-product formations, such as 6-hydroxydopamine. Because of possible lower clearing efficacy of free DA from the synaptic gap in the SHRs (Masuda *et al.*, 2006), it is possible that the SHRs have a lower ability to compensate for possible PCB-induced dopamine-toxicity. Also the decrease of PSD-95 as well as D5-receptors which were greater in males than in females, might contribute to lowering postsynaptic targets. From these combined findings it is clear that PCB-153 might influence neurological processes related to cognition at several levels. However, the currently available biochemical and behavioural data of these animals makes it difficult to determine the exact impact on cognitive processes.

To sum up

Taken together, this study shows that both dietary FA and PCB 153 environmental factors may modulate the dopaminergic system and its related proteins. It is also clear that there are some gender specific effects of fatty acids, with males being more receptive than females, but also that genetics influence the effect of these compounds on the dopaminergic system, where we saw a larger effect in WKYs compared to SHRs, which might be due to SHRs suggested hypo-functional dopaminergic system. Since our behavioural data, as well as studies done by others, have showed reduction in ADHD-associated behaviour or cognitive improvements after PUFA-supplemented diets, it is likely to believe that this supplementation was beneficial. Since an increase in general SFA induced similar biochemical changes as PUFAS, although PUFAS was more potent, it looks like both of the 20% FA-diets boosts dopamine signalling compared to the 5% FA-diet. This enhancement might be especial beneficial for the SHRs, which have indications of a hypo-functional dopamine signalling. Although the WKY rats-got double effect of the PUFAS compared to SHRs, still remain to be done extended behavioural tests of these animals in order to underpin the meaning of these biochemical changes. The effect of PCB was distinct from that found in the PUFA

studies. While PCB led to increased HVA and had no effect on dopmaine levels, SFA and PUFA-diets lowered the dopamine levels, while HVA-levels were almost unchanged. Interestingly both PCB, SFA and PUFAs led to increased calculated dopamine turnover rates, with PCB being most potent followed by PUFAs. Excess free dopamine has been suggested to be unbeneficial, as it might increase probability of reactive dopamine species (Graumann *et al.*, 2002). It is clear that PCB is a more potent agent in increasing HVA-levels, compared to n-3 PUFAs and SFA, implying stronger inhibition DAT reuptake of dopamine. The possible low activity of COMT in SHRs might also contribute to higher effect of possible dopaminergic hyperactivity (Masuda *et al.*, 2006).

5. Concluding remarks

In conclusion, both groups of compounds led to increased transmitter turnover, although by distinct mechanisms. Where high FA-diets seemed to reduce the dopamine content, while keeping the HVA more or less stable, PCB had the opposite effect, by keeping the dopamine levels stable and largely increasing HVA levels. These environmental compounds clearly have the ability to modulate neuronal functions associated with cognitional processes, and may modulate behaviour. Studies on specific impacts of these effects on cognitive functions and overall behaviour, still remains limited.

Effects of polyunsaturated fatty acids

1) *Ameliorate ADHD symptoms in the ADHD-animal model SHR by operant testing of behaviour:*

   According to the initial study (Paper I) where we measured ADHD related symptoms in SHR-rats, the n-3 PUFA rich diet reduced ADHD symptoms in a gender dependent manner. Male rats obtained reduced reinforcer-controlled activity, impulsiveness and inattention, with no or opposite effects in the female SHRs. The PUFA-supplement also led to reduced general hyperactivity in SHRs of both genders. These behavioural effects in the male SHRs occurred in concert with biochemical changes of the dopamine synapse. In the second study, similar biochemical changes as found by the n-3 FA enrichment were found to be induced by the SFA-diet, rich on saturated and n-6 FAs. Unfortunately we were unable to obtain behavioural data from the SFA-group. Therefore we cannot conclude if the ameliorating effect was restricted to a certain type of fatty acid.

2) *Have effects on the dopaminergic synapse, by studying the nigrostriatal pathway and if a general increase in n-6 FAs could give the same effect as n-3 FAs:*

   In paper II we introduced three different diets (LFA, SFA and PUFA). From this study we learned that a general increase in the percentage of dietary fat from 5% to 20% could dramatically reduce the overall dopamine level in neostriatum, by lowering the dopamine synthetizing protein TH. Since the dopamine levels were lowered and the HVA levels were stable after SFA- diet, the turnover (HVA/dopamine) were increased compared to the LFA-diet, at the same time the n-3 PUFA-diet was the only one reducing DAT-levels which led to slightly increased HVA levels, increasing the dopamine turnover even more than with the SFA-diet. The reduction of DAT could be thought of as a Ritalin-like effect, by increasing dopamine availability in the synaptic gap.

3) *Have same effects on both genders and have same effects on the ADHD model compared to a control rat strain:*

We did find gender and strain specific responses to the PUFA-diet. Firstly, in paper I, we showed that the PUFA-diet did not decrease dopamine levels in the female rats as it did in the male rats, in addition the HVA levels remained unchanged, resulting in unaffected turnover ratios. This lack of responsiveness was further reflected by the behavioural data, showing reduced reinforcer-controlled activity, impulsiveness and inattention in male SHR, with no or opposite effects in the female SHRs. Furthermore, in paper II, we observed clear strain differences on biochemical parameters, with WKY being more receptive to changes than the SHR. The dopamine associated proteins TH, DAT and VMAT-2, were significantly reduced in WKY; the SHRs only had an insignificant tendency towards the same effects. Despite this, the WKY and SHR responded in the same way regarding the total levels of dopamine, and both strains responded to increased dietary FA with increased dopamine-turnover with n-3 PUFA being more effective than n-6 FAs .

Effects of PCB 153

4) *Have effects on the dopaminergic synapse, by studying the nigrostriatal pathway, performing analyses in neostriatal tissue and electrophysiological activity measurements of the hippocampus detecting possible changes in dopaminergic modulation of the mesocortical pathway:*

PCB 153 had a clear effect on the dopaminergic system which involved an increase in free dopamine that could be degraded to HVA. We also observed PCB 153 mediated effects on hippocampal excitatory synaptic plasticity in the stratum oriens but not in the radiatum. It is clear that PCB153 did not affect the

total measured TH and dopamine levels, as these seemed to be stable, although it had an ability to increase HVA, showing that the amount of TH is able to compensate for the metabolised dopamine. We also discovered a reduction in DAT, which could be a contributing factor to the observed increase in the HVA level. Furthermore we also discovered increased amounts of the metabolic degradation product of serotonin; 5-HIAA, which also might contribute to modulate synaptic signalling. It is also clear that PCB have the ability to affect PSD-95 and D5R, implicating changes in both postsynaptic glutamatergic neurons, GABAergic and cholinergic interneurons.

5) *Have genotype-specific effects in the ADHD-animal model spontaneously hypertensive rat (SHR) and the control WKY rat. Our hypothesis suggests that ADHD-genetics and thus SHR-genetics might represent increased vulnerability to environmental factors:*

However, no clear differences were seen in the responses between WKY and SHR, although SHR seems to respond with lower magnitude and higher variability compared to the WKYs. Another genotypic but also gender-specific effect was found in the female SHRs, which failed to show significant increased turnover of both dopamine and serotonin.

6) *Have gender-specific effects, which was of importance since ADHD is regarded as a gender dependent disorder:*

The males seemed to respond in a greater magnitude as well as with higher p-values compared to the females of both the WKY and SHR genotype. Regarding ADHD, this finding fits well, as the symptomatic display in boys and girls are highly gender dependent (Arnett *et al.*, 2014).

Reference List

Akutagava-Martins GC, Salatino-Oliveira A, Kieling CC, Rohde LA, & Hutz MH (2013). Genetics of attention-deficit/hyperactivity disorder: current findings and future directions. *Expert Rev Neurother* 13, 435-445.

Anden NE, Carlsson A, Dhaltroem A, Fuxe K, Hillarp NA, & Larsson K (1964). Demonstration and mapping out of nigro-neosrtriatal dopamine neurons. *Life Sci* 3, 523-530.

Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, & Burgess JR (2006). Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. *Prostaglandins Leukot Essent Fatty Acids* 75, 299-308.

Arnett AB, Pennington BF, Willcutt EG, DeFries JC, & Olson RK (2014). Sex differences in ADHD symptom severity. *J Child Psychol Psychiatry, doi:10.1111/jcpp 12337*.

Bademci G, Vance JM, & Wang L (2012). Tyrosine hydroxylase gene: another piece of the genetic puzzle of Parkinson's disease. *CNS Neurol Disord Drug Targets* 11, 469-481.

Banerjee TD, Middleton F, & Faraone SV (2007). Environmental risk factors for attention-deficit hyperactivity disorder. *Acta Paediatr* 96, 1269-1274.

Beaulieu JM & Gainetdinov RR (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63, 182-217.

Berlanga ML, Simpson TK, & Alcantara AA (2005). Dopamine D5 receptor localization on cholinergic neurons of the rat forebrain and diencephalon: a potential neuroanatomical substrate involved in mediating dopaminergic influences on acetylcholine release. *J Comp Neurol* 492, 34-49.

Birch EE, Garfield S, Hoffman DR, Uauy R, & Birch DG (2000). A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev Med Child Neurol* 42, 174-181.

Bliss TV & Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31-39.

Bliss TV & Cooke SF (2011). Long-term potentiation and long-term depression: a clinical perspective. *Clinics (Sao Paulo)* 66 Suppl 1, 3-17.

Bloch MH & Qawasmi A (2011a). Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry* 50, 991-1000.

Bloch MH & Qawasmi A (2011b). Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry* 50, 991-1000.

Bondi CO, Taha AY, Tock JL, Totah NK, Cheon Y, Torres GE, Rapoport SI, & Moghaddam B (2014). Adolescent behavior and dopamine availability are uniquely sensitive to dietary omega-3 fatty acid deficiency. *Biol Psychiatry* 75, 38-46.

Bowers MS, Chen BT, & Bonci A (2010). AMPA receptor synaptic plasticity induced by psychostimulants: the past, present, and therapeutic future. *Neuron* 67, 11-24.

Bralten J, Franke B, Waldman I, Rommelse N, Hartman C, Asherson P, Banaschewski T, Ebstein RP, Gill M, Miranda A, Oades RD, Roeyers H, Rothenberger A, Sergeant JA, Oosterlaan J, Sonuga-Barke E, Steinhausen HC, Faraone SV, Buitelaar JK, & Arias-Vasquez A (2013). Candidate genetic pathways for attention-deficit/hyperactivity disorder (ADHD) show association to hyperactive/impulsive symptoms in children with ADHD. *J Am Acad Child Adolesc Psychiatry* 52, 1204-1212.

Cao D, Kevala K, Kim J, Moon HS, Jun SB, Lovinger D, & Kim HY (2009). Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. *J Neurochem* 111, 510-521.

Cartier EA, Parra LA, Baust TB, Quiroz M, Salazar G, Faundez V, Egana L, & Torres GE (2010). A biochemical and functional protein complex involving dopamine synthesis and transport into synaptic vesicles. *J Biol Chem* 285, 1957-1966.

Caspi A & Moffitt TE (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* 7, 583-590.

Cerna M, Bencko V, Brabec M, Smid J, Krskova A, & Jech L (2010). Exposure assessment of breast-fed infants in the Czech Republic to indicator PCBs and selected chlorinated pesticides: area-related differences. *Chemosphere* 78, 160-168.

Chevrier J, Eskenazi B, Holland N, Bradman A, & Barr DB (2008). Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *Am J Epidemiol* 168, 298-310.

Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, & Brand-Miller J (2005). Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 81, 341-354.

Dahlstrom A & Fuxe K (1964). Localization of monoamines in the lower brain stem. *Experientia* 20, 398-399.

Davis D & Safe S (1990). Immunosuppressive activities of polychlorinated biphenyls in C57BL/6N mice: structure-activity relationships as Ah receptor agonists and partial antagonists. *Toxicology* 63, 97-111.

Davis JF, Tracy AL, Schurdak JD, Tschop MH, Lipton JW, Clegg DJ, & Benoit SC (2008). Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav Neurosci* 122, 1257-1263.

Delgado MR & Dickerson KC (2012). Reward-related learning via multiple memory systems. *Biol Psychiatry* 72, 134-141.

Dopheide JA & Pliszka SR (2009). Attention-deficit-hyperactivity disorder: an update. *Pharmacotherapy* 29, 656-679.

Durham SK & Brouwer A (1989). 3,4,3',4'-tetrachlorobiphenyl-induced effects in the rat liver. I. Serum and hepatic retinoid reduction and morphologic changes. *Toxicol Pathol* 17, 536-544.

Edden RA, Crocetti D, Zhu H, Gilbert DL, & Mostofsky SH (2012). Reduced GABA concentration in attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry* 69, 750-753.

El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, & Bredt DS (2000). PSD-95 involvement in maturation of excitatory synapses. *Science* 290, 1364-1368.

Eriksson P, Lundkvist U, & Fredriksson A (1991). Neonatal exposure to 3,3',4,4'-tetrachlorobiphenyl: changes in spontaneous behaviour and cholinergic muscarinic receptors in the adult mouse. *Toxicology* 69, 27-34.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, & Sklar P (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57, 1313-1323.

Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, & Tully M (1988). Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J Pediatr* 113, 991-995.

Gold MS, Blum K, Oscar-Berman M, & Braverman ER (2014). Low dopamine function in attention deficit/hyperactivity disorder: should genotyping signify early diagnosis in children? *Postgrad Med* 126, 153-177.

Graumann R, Paris I, Martinez-Alvarado P, Rumanque P, Perez-Pastene C, Cardenas SP, Marin P, Diaz-Grez F, Caviedes R, Caviedes P, & Segura-Aguilar J (2002). Oxidation of dopamine to aminochrome as a mechanism for neurodegeneration of dopaminergic systems in Parkinson's disease. Possible neuroprotective role of DT-diaphorase. *Pol J Pharmacol* 54, 573-579.

Gylys KH, Fein JA, Yang F, Wiley DJ, Miller CA, & Cole GM (2004). Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. *Am J Pathol* 165, 1809-1817.

Han K & Kim E (2008). Synaptic adhesion molecules and PSD-95. *Prog Neurobiol* 84, 263-283.

Hansson MC, Persson ME, Larsson P, Kjellman C, & von ST (2006). Polychlorinated biphenyl load, aryl hydrocarbon receptor, and cytochrome P4501A1 induction in a wild population of Atlantic salmon (Salmo salar) from the Baltic Sea. *Environ Toxicol Chem* 25, 2197-2207.

Helland IB, Smith L, Blomen B, Saarem K, Saugstad OD, & Drevon CA (2008). Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics* 122, e472-e479.

Henriksen C, Haugholt K, Lindgren M, Aurvag AK, Ronnestad A, Gronn M, Solberg R, Moen A, Nakstad B, Berge RK, Smith L, Iversen PO, & Drevon CA (2008). Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. *Pediatrics* 121, 1137-1145.

Herwerth M, Jensen V, Novak M, Konopka W, Hvalby O, & Kohr G (2012). D4 dopamine receptors modulate NR2B NMDA receptors and LTP in stratum oriens of hippocampal CA1. *Cereb Cortex* 22, 1786-1798.

Holene E, Nafstad I, Skaare JU, & Sagvolden T (1998). Behavioural hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. *Behav Brain Res* 94, 213-224.

Janssen CI & Kiliaan AJ (2014). Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration. *Prog Lipid Res* 53, 1-17.

Johannessen JN (1991). A model of chronic neurotoxicity: long-term retention of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) within catecholaminergic neurons. *Neurotoxicology* 12, 285-302.

Johansen EB, Fonnum F, Lausund PL, Walaas SI, Baerland NE, Woien G, & Sagvolden T (2014). Behavioral changes following PCB 153 exposure in the spontaneously hypertensive rat - an animal model of Attention-Deficit/Hyperactivity Disorder. *Behav Brain Funct* 10, 1.

Johansen EB, Knoff M, Fonnum F, Lausund PL, Walaas SI, Woien G, & Sagvolden T (2011). Postnatal exposure to PCB 153 and PCB 180, but not to PCB 52, produces changes in activity level and stimulus control in outbred male Wistar Kyoto rats. *Behav Brain Funct* 7, 18.

Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, & Caron MG (1998). Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc Natl Acad Sci U S A* 95, 4029-4034.

Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, Brooke JC, McLaren DS, Channer KS, & Jones TH (2014). Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. *Life Sci* 109, 95-103.

Kennedy MB (1998). Signal transduction molecules at the glutamatergic postsynaptic membrane. *Brain Res Brain Res Rev* 26, 243-257.

Kew JN & Kemp JA (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl)* 179, 4-29.

Kim HY, Moon HS, Cao D, Lee J, Kevala K, Jun SB, Lovinger DM, Akbar M, & Huang BX (2011). N-Docosahexaenoylethanolamide promotes development of hippocampal neurons. *Biochem J* 435, 327-336.

Lang M, Moradi-Chameh H, Zahid T, Gane J, Wu C, Valiante T, & Zhang L (2014). Regulating hippocampal hyperexcitability through GABAB Receptors. *Physiol Rep* 2, e00278.

Larson K, Russ SA, Kahn RS, & Halfon N (2011). Patterns of comorbidity, functioning, and service use for US children with ADHD, 2007. *Pediatrics* 127, 462-470.

Lee Y, Mitchell DC, Smiciklas-Wright H, & Birch LL (2001). Diet quality, nutrient intake, weight status, and feeding environments of girls meeting or exceeding recommendations for total dietary fat of the American Academy of Pediatrics. *Pediatrics* 107, E95.

Liao PC, Kuo YM, Chang YC, Lin C, Cherng CF, & Yu L (2003). Striatal formation of 6-hydroxydopamine in mice treated with pargyline, pyrogallol and methamphetamine. *J Neural Transm* 110, 487-494.

Linssen AM, Sambeth A, Vuurman EF, & Riedel WJ (2014). Cognitive effects of methylphenidate in healthy volunteers: a review of single dose studies. *Int J Neuropsychopharmacol* 17, 961-977.

Litman BJ, Niu SL, Polozova A, & Mitchell DC (2001). The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: visual transduction. *J Mol Neurosci* 16, 237-242.

Luecke S, Backlund M, Jux B, Esser C, Krutmann J, & Rannug A (2010). The aryl hydrocarbon receptor (AHR), a novel regulator of human melanogenesis. *Pigment Cell Melanoma Res* 23, 828-833.

Mailman R, Huang X, & Nichols DE (2001). Parkinson's disease and D1 dopamine receptors. *Curr Opin Investig Drugs* 2, 1582-1591.

Mariussen E & Fonnum F (2001). The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. *Toxicology* 159, 11-21.

Masuda M, Tsunoda M, & Imai K (2006). Low catechol-O-methyltransferase activity in the brain and blood pressure regulation. *Biol Pharm Bull* 29, 202-205.

Meldrum BS (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr* 130, 1007S-1015S.

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, & Grant SG (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396, 433-439.

Miller EM, Pomerleau F, Huettl P, Russell VA, Gerhardt GA, & Glaser PE (2012). The spontaneously hypertensive and Wistar Kyoto rat models of ADHD exhibit sub-regional differences in dopamine release and uptake in the striatum and nucleus accumbens. *Neuropharmacology* 63, 1327-1334.

Missale C, Nash SR, Robinson SW, Jaber M, & Caron MG (1998). Dopamine receptors: from structure to function. *Physiol Rev* 78, 189-225.

Morales M & Root DH (2014). Glutamate neurons within the midbrain dopamine regions. *Neuroscience* 282C, 60-68.

Muskiet FAJ (2010). Pathophysiology and Evolutionary Aspects of Dietary Fats and Long-Chain Polyunsaturated Fatty Acids across the Life Cycle. Frontiers in Neuroscience, chapter 2.

Narendran R, Frankle WG, Mason NS, Muldoon MF, & Moghaddam B (2012). Improved working memory but no effect on striatal vesicular monoamine transporter type 2 after omega-3 polyunsaturated fatty acid supplementation. *PLoS One* 7, e46832.

Niemi WD, Audi J, Bush B, & Carpenter DO (1998). PCBs reduce long-term potentiation in the CA1 region of rat hippocampus. *Exp Neurol* 151, 26-34.

Nieoullon A (2002). Dopamine and the regulation of cognition and attention. *Prog Neurobiol* 67, 53-83.

Nissim I (1999). Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am J Physiol* 277, F493-F497.

Norenberg MD, Huo Z, Neary JT, & Roig-Cantesano A (1997). The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. *Glia* 21, 124-133.

Nyffeler M, Zhang WN, Feldon J, & Knuesel I (2007). Differential expression of PSD proteins in age-related spatial learning impairments. *Neurobiol Aging* 28, 143-155.

Peet M & Stokes C (2005). Omega-3 fatty acids in the treatment of psychiatric disorders. *Drugs* 65, 1051-1059.

Pessah IN, Hansen LG, Albertson TE, Garner CE, Ta TA, Do Z, Kim KH, & Wong PW (2006). Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca2+ channel complex type 1 (RyR1). *Chem Res Toxicol* 19, 92-101.

Petrovic S, Takic M, Arsic A, Vucic V, Drakulic D, Milosevic M, & Glibetic M (2014). Effect of sex hormones on plasma phospholipid fatty acid composition in intact rats and rats with bilaterally occluded carotid arteries. *Physiol Res* 63, 331-339.

Pezzulo G, van der Meer MA, Lansink CS, & Pennartz CM (2014). Internally generated sequences in learning and executing goal-directed behavior. *Trends Cogn Sci*, doi: 10.1016/j.tics.2014.06.011.

Polanczyk G, de Lima MS, Horta BL, Biederman J, & Rohde LA (2007). The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry* 164, 942-948.

Porterfield SP (2000). Thyroidal dysfunction and environmental chemicals--potential impact on brain development. *Environ Health Perspect* 108 Suppl 3, 433-438.

Racine RJ, Milgram NW, & Hafner S (1983). Long-term potentiation phenomena in the rat limbic forebrain. *Brain Res* 260, 217-231.

Rao A, Kim E, Sheng M, & Craig AM (1998). Heterogeneity in the molecular composition of excitatory postsynaptic sites during development of hippocampal neurons in culture. *J Neurosci* 18, 1217-1229.

Reinhardt MC & Reinhardt CA (2013). Attention deficit-hyperactivity disorder, comorbidities, and risk situations. *J Pediatr (Rio J)* 89, 124-130.

Richardson AJ (2006). Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *Int Rev Psychiatry* 18, 155-172.

Richardson AJ, Burton JR, Sewell RP, Spreckelsen TF, & Montgomery P (2012). Docosahexaenoic acid for reading, cognition and behavior in children aged 7-9 years: a randomized, controlled trial (the DOLAB Study). *PLoS One* 7, e43909.

Roberts E & Rankel S (1950). gamma-Aminobutyric acid in brain: its formation from glutamic acid. *J Biol Chem* 187, 55-63.

Rothman SM & Olney JW (1995). Excitotoxicity and the NMDA receptor--still lethal after eight years. *Trends Neurosci* 18, 57-58.

Safe S (1984). Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *Crit Rev Toxicol* 13, 319-395.

Sagvolden T (2006). The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behav Brain Funct* 2, 41.

Sagvolden T, Johansen EB, Aase H, & Russell VA (2005a). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behav Brain Sci* 28, 397-419.

Sagvolden T, Johansen EB, Woien G, Walaas SI, Storm-Mathisen J, Bergersen LH, Hvalby O, Jensen V, Aase H, Russell VA, Killeen PR, Dasbanerjee T, Middleton FA, & Faraone SV (2009). The spontaneously hypertensive rat model of ADHD--the importance of selecting the appropriate reference strain. *Neuropharmacology* 57, 619-626.

Sagvolden T, Russell VA, Aase H, Johansen EB, & Farshbaf M (2005b). Rodent models of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57, 1239-1247.

Sans N, Petralia RS, Wang YX, Blahos J, Hell JW, & Wenthold RJ (2000). A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J Neurosci* 20, 1260-1271.

Sazonova NA, Dasbanerjee T, Middleton FA, Gowtham S, Schuckers S, & Faraone SV (2011). Transcriptome-wide gene expression in a rat model of attention deficit hyperactivity disorder symptoms: rats developmentally exposed to polychlorinated biphenyls. *Am J Med Genet B Neuropsychiatr Genet* 156B, 898-912.

Schantz SL, Widholm JJ, & Rice DC (2003). Effects of PCB exposure on neuropsychological function in children. *Environ Health Perspect* 111, 357-576.

Schmitt KC, Rothman RB, & Reith ME (2013). Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. *J Pharmacol Exp Ther* 346, 2-10.

Schousboe A, Svenneby G, & Hertz L (1977). Uptake and metabolism of glutamate in astrocytes cultured from dissociated mouse brain hemispheres. *J Neurochem* 29, 999-1005.

Schultz W (2007). Multiple dopamine functions at different time courses. *Annu Rev Neurosci* 30, 259-288.

Seegal RF, Fitzgerald EF, Hills EA, Wolff MS, Haase RF, Todd AC, Parsons P, Molho ES, Higgins DS, Factor SA, Marek KL, Seibyl JP, Jennings DL, & McCaffrey RJ (2011). Estimating the half-lives of PCB congeners in former

capacitor workers measured over a 28-year interval. *J Expo Sci Environ Epidemiol* 21, 234-246.

Shohamy D & Adcock RA (2010). Dopamine and adaptive memory. *Trends Cogn Sci* 14, 464-472.

Skaare JU, Bernhoft A, Derocher A, Gabrielsen GW, Goksoyr A, Henriksen E, Larsen HJ, Lie E, & Wiig (2000). Organochlorines in top predators at Svalbard--occurrence, levels and effects. *Toxicol Lett* 112-113, 103-109.

Snyder GL, Fienberg AA, Huganir RL, & Greengard P (1998). A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J Neurosci* 18, 10297-10303.

Sonnewald U, Westergaard N, & Schousboe A (1997). Glutamate transport and metabolism in astrocytes. *Glia* 21, 56-63.

Su HM (2010). Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. *J Nutr Biochem* 21, 364-373.

Sudhof TC (2004). The synaptic vesicle cycle. *Annu Rev Neurosci* 27, 509-547.

Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HH, & Niznik HB (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* 350, 614-619.

Takesian AE & Hensch TK (2013). Balancing plasticity/stability across brain development. *Prog Brain Res* 207, 3-34.

Thapar A, Cooper M, Eyre O, & Langley K (2013). What have we learnt about the causes of ADHD? *J Child Psychol Psychiatry* 54, 3-16.

Tilson HA & Kodavanti PR (1998). The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology* 19, 517-525.

Timmermans W, Xiong H, Hoogenraad CC, & Krugers HJ (2013). Stress and excitatory synapses: from health to disease. *Neuroscience* 248, 626-636.

Unni VK, Zakharenko SS, Zablow L, DeCostanzo AJ, & Siegelbaum SA (2004). Calcium release from presynaptic ryanodine-sensitive stores is required for long-term depression at hippocampal CA3-CA3 pyramidal neuron synapses. *J Neurosci* 24, 9612-9622.

Varoqui H & Erickson JD (1998). Functional identification of vesicular monoamine and acetylcholine transporters. *Methods Enzymol* 296, 84-99.

Viluksela M, Heikkinen P, van der Ven LT, Rendel F, Roos R, Esteban J, Korkalainen M, Lensu S, Miettinen HM, Savolainen K, Sankari S, Lilienthal H, Adamsson A, Toppari J, Herlin M, Finnila M, Tuukkanen J, Leslie HA, Hamers T, Hamscher G, Al-Anati L, Stenius U, Dervola KS, Bogen IL, Fonnum F, Andersson PL, Schrenk D, Halldin K, & Hakansson H (2014). Toxicological Profile of Ultrapure 2,2',3,4,4',5,5'-Heptachlorbiphenyl (PCB 180) in Adult Rats. *PLoS One* 9, e104639.

Waagepetersen HS, Qu H, Sonnewald U, Shimamoto K, & Schousboe A (2005). Role of glutamine and neuronal glutamate uptake in glutamate homeostasis and synthesis during vesicular release in cultured glutamatergic neurons. *Neurochem Int* 47, 92-102.

Wang GJ, Volkow ND, Wigal T, Kollins SH, Newcorn JH, Telang F, Logan J, Jayne M, Wong CT, Han H, Fowler JS, Zhu W, & Swanson JM (2013). Long-term stimulant treatment affects brain dopamine transporter level in patients with attention deficit hyperactive disorder. *PLoS One* 8, e63023.

Weihe E, Schafer MK, Erickson JD, & Eiden LE (1994). Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. *J Mol Neurosci* 5, 149-164.

Weiss M, Worling D, & Wasdell M (2003). A chart review study of the inattentive and combined types of ADHD. *J Atten Disord* 7, 1-9.

Wheal HV, Chen Y, Mitchell J, Schachner M, Maerz W, Wieland H, Van RD, & Kirsch J (1998). Molecular mechanisms that underlie structural and functional changes at the postsynaptic membrane during synaptic plasticity. *Prog Neurobiol* 55, 611-640.

Wigal SB (2009). Efficacy and safety limitations of attention-deficit hyperactivity disorder pharmacotherapy in children and adults. *CNS Drugs* 23 Suppl 1, 21-31.

Wigestrand MB, Stenberg M, Walaas SI, Fonnum F, & Andersson PL (2013). Non-dioxin-like PCBs inhibit [(3)H]WIN-35,428 binding to the dopamine transporter: a structure-activity relationship study. *Neurotoxicology* 39, 18-24.

Willatts P, Forsyth JS, DiModugno MK, Varma S, & Colvin M (1998). Influence of long-chain polyunsaturated fatty acids on infant cognitive function. *Lipids* 33, 973-980.

Willcutt EG, Pennington BF, Olson RK, & DeFries JC (2007). Understanding comorbidity: a twin study of reading disability and attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 144B, 709-714.

Williams JM, Guevremont D, Mason-Parker SE, Luxmanan C, Tate WP, & Abraham WC (2007). Differential trafficking of AMPA and NMDA receptors during long-term potentiation in awake adult animals. *J Neurosci* 27, 14171-14178.

Winneke G (2011). Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. *J Neurol Sci* 308, 9-15.

Yang P, Cai G, Cai Y, Fei J, & Liu G (2013). Gamma aminobutyric acid transporter subtype 1 gene knockout mice: a new model for attention deficit/hyperactivity disorder. *Acta Biochim Biophys Sin (Shanghai)* 45, 578-585.

Yoshizawa K, Heatherly A, Malarkey DE, Walker NJ, & Nyska A (2007). A critical comparison of murine pathology and epidemiological data of TCDD, PCB126, and PeCDF. *Toxicol Pathol* 35, 865-879.

Zainuddin MS & Thuret S (2012). Nutrition, adult hippocampal neurogenesis and mental health. *Br Med Bull* 103, 89-114.

Zhou Y & Danbolt NC (2014). Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm* 121, 799-817.

Zigmond MJ, Hastings TG, & Perez RG (2002). Increased dopamine turnover after partial loss of dopaminergic neurons: compensation or toxicity? *Parkinsonism Relat Disord* 8, 389-393.