THE INFLUENCE OF PRENATAL SSRI USE ON FETAL BRAIN DEVELOPMENT

Bachelor Thesis

Isa Poortman
Studentnumber: S3429768
Education: Biologie; Gedrag- en Neurowetenschappen
Thesis supervisor: Prof. Dr. J.D.A. Olivier

rijksuniversiteit groningen

umcg

TABLE OF CONTENTS

ABSTRACT

Selective serotonin reuptake inhibitor (SSRI) medications are the most common antidepressant treatment used during pregnancy. Up to 2-3% of the pregnant women are prescribed SSRIs. Serotonin plays an important part in brain development, and questions have been raised about the placental transfer of SSRIs and the effects of preventing reuptake of presynaptic serotonin on fetal brain development. Preclinical studies report structural deformations of dendritic structures and a repulsive response from the thalamic axons to netrin-1, both caused by premature SSRI exposure. Elevated 5-HT concentrations also cause malformations in immature oligodendrocytes and in myelin sheaths. Furthermore, SSRI exposure during development affects plasticity which influences behaviour. It is important to understand that a lot of studies are done in healthy rodents and not in rodents showing depression-like behaviours. In addition, some studies give SSRI treatment during a time of rodent neural development that is corresponding to SSRI exposure of foetuses during third trimester. At this point, the primary construction of the brain is already completed. In humans, developmental outcomes of SSRI exposure after gestation remain largely unstudied. Therefore, the effects of SSRI use during whole pregnancy needs to be further investigated to identify the potential risks on fetal brain development.

INTRODUCTION

The neurotransmitter serotonin (5-HT) plays a central role in behavioural and cognitive functions such as brain development and regulation of mood, but it also has its key role in psychiatric disorders, for instance in depression (Simpson et al., 2011). Serotonin was discovered by Rapport et al. (1948) in the mid-1900s and it is thanking its name for its presence in serum (sero) and its vasoactive properties (tonin) (Rapport et al., 1948). Soon after its chemical identification, the structural similarities between 5-HT and LSD led to the logical speculation that substances related to serotonin might cause mental aberrations (Owens & Nemeroff, 1994). The research on 5-HT elucidated its moderating role on behaviour and mental health. Disturbances in the 5-HT system may lead to the development of depression (Meltzer, 1990). Depression is a devastating mood disorder that indiscriminately affects individuals of all backgrounds and ages, and is common in women during gestation.

As disturbed levels of 5-HT were hypothesized to increase the vulnerability to develop depression, Selective Serotonin Reuptake Inhibitors (SSRIs) were developed to restore the serotonergic levels. These antidepressants were introduced to the market in 1987, where they almost became instantly popular because they were much safer than the other antidepressant options at that time. SSRI antidepressants are still widely the most prescribed medication for the treatment of depression (Källén, 2004). SSRIs block the serotonin transporter (SERT), preventing reuptake of 5-HT in the presynaptic neuron, causing an increase in the extracellular serotonin availability in the brain. SSRIs are proven to be safe when used by adults, common side effects of SSRIs can include metabolic side effects such as weight gain, but also induce sexual dysfunction such as decreasing desire, arousal, and orgasm in men and women (Tschoner et al., 2007; Montejo et al., 2015). However, these drugs are also used for maternal depression during pregnancy, and safety for the unborn child may be harmed by these drugs. The prevalence of depression symptoms during pregnancy is about 10-16%. However, the percentage of pregnant women using SSRIs is much lower, around 2 to 3% (Olivier, 2015; 'SSRI-use and pregnancy', n.d.; Benett et al., 2004).

Prenatal use of SSRIs may cause some problems because these antidepressants readily cross the placental and blood-brain barriers. When this happens, the SSRIs can reach the unborn baby where they can cause an increase of the central serotonergic tone in the fetus. These disturbances can influence the development of the fetal brain (Velasquez., 2013). 5-HT plays a key role in the neurodevelopment in the fetus, innervating essentially the entire central nervous system (Sundstrom et al., 1993). Serotonergic neurons are first evident as early as 5 weeks of gestation (Sundstrom et al., 1993).

In the mature brain, 5-HT acts mainly as a modulatory neurotransmitter, regulating aspects throughout the whole central nervous system like cognition, attention and learning (Chaouloff et al., 1999). During neurodevelopment, 5-HT also acts as a trophic factor that supports both developing and maturing of neurons (Simpson et al., 2011). Thus, long before birth, 5-HT is already setting developmental pathways that contribute to learning, thinking, and stress reactivity. Consequences of blocking SERT during development have been investigated using pharmacological approaches in various species, including mice and rats, in which the first 10 postnatal days correspond to the third trimester of human fetal development (Oberlander et al., 2009).

Some negative effects of SSRIs on the neurodevelopment of a fetus have been reported (Lee et al., 2009). Therefore, some concerns are rising about the placental transfer of SSRIs and the effects of preventing the reuptake of extracellular serotonin. To date, no gross SSRI-related neuroteratogenic outcomes have been identified. Yet reports of subtle neurological disturbances in early childhood associated with fetal SSRI exposure are beginning to emerge. In this paper, I will review the existing evidence about the influence of prenatal exposure to SSRIs on the fetal brain development.

Chapter 1: Understanding the Basics

1.1 Serotonin

The biogenic amine serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter. Serotonin is synthesized in the serotonergic neurons of the central nervous system (CNS) and the enterochromaffin cells of the gastrointestinal tract in the peripheral nervous system (PNS). The serotonergic system is one of the longest known transmitter systems in the brain. Serotonin is diversely present through the whole animal kingdom and can be found in the neuronal systems of all organisms ranging from Drosophila to humans (Jonnakuty et al., 2008). Traditionally it has been known as an important signalling molecule that has numerous important roles in health and disease. It is especially important in regulating and modulating the physiological, behavioural and cognitive functions such as mood, emotions and sleep (Hall, 2013). Since its discovery in 1948 and its connection with its key role in the human body, 5-HT has been the subject of intense research.

Serotonin is a monoamine neurotransmitter, which means that 5-HT consist of an amino group connected to an aromatic ring by a two-carbon chain. Its chemical structure can be seen in fig. 1. Examples of other monoamines are dopamine and epinephrine. All monoamines are derived from aromatic amino acids, and in the case of 5-HT, this amino acid is tryptophan. The production of 5-HT occurs in the human body, we cannot make tryptophan on our own so it is extremely important that we get our tryptophan from our diet. After ingestion, tryptophan is converted into 5-HT via a series of reactions (Jonnakuty et al., 2008).

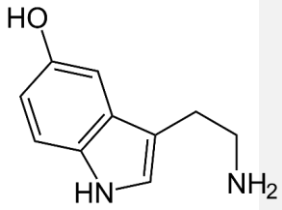

Fig. 1 Chemical structure

As said earlier, 5-HT is made in the peripheral nervous system and in the central nervous system. About 95% of the 5-HT is made peripherally with only a small fraction of the total body serotonin produced by the central nervous system (Maurer-Spurej et al., 2004). Serotonin controls the growth and maturation of its own serotonergic system, a process known as autoregulation of development.

For the production of 5-HT tryptophan is needed. The availability of tryptophan is the rate limiting step in the synthesis of serotonin (Owens & Nemeroff, 1998). The blood-brain barrier (BBB) is impermeable to peripheral 5-HT and tryptophan itself cannot easily cross the BBB (Yuwiler et al., 1977). Therefore, tryptophan is carried across the BBB by the large neutral amino acids (LNAA) transport system. However, tryptophan is not the only amino acid which uses the LNAA transport system to get across the BBB. It must compete with 5 other amino acids, namely valine, leucine, isoleucine, tyrosine, phenylalanine and methionine for access to the carrier-binding site. If plasma tryptophan levels change while the other 5 LNAA remain constant, the rate of tryptophan transport into the brain will also change (Pardridge, 1983). Therefore, the amount of 5-HT that is produced centrally is dependent on the amount of available tryptophan which can bind to the LNAA transport system peripherally (Pardridge, 1983; Yuwiler et al., 1977).

In the central nervous system, 5-HT is stored in secretory granules and released from the serotonergic neurons into the synapse. Then, the ligand 5-HT can bind to its 5-HT receptors which activates multiple responses.

1.2 Signalling Pathway

The neurotransmitter 5-HT acts through fourteen membrane receptors which are distributed throughout the nervous system and the peripheral organs. Based on structural, transductional and operational features, the 5-HT receptors are divided into seven classes (5-HT_1 to 5-HT_7). Many of these receptor classes have multiple subtypes. These classes are a combination of two different kinds of receptors, namely the G-protein coupled receptor family and ligand-gated ion channels. The 5-HT_3 receptor is the only ligand-gated ion channel, all of the other 5-HT receptors are members of the G-protein coupled receptor (GPCR) family (Hannon et al., 2008).

The vast majority of central nervous system serotonergic nerve terminals originate in neuronal cell bodies of the raphe nuclei in the brainstem. The raphe nuclei is a cluster of nuclei located in the brainstem. Many but not all of these neurons in the nuclei are serotonergic. They interact with numerous areas of the brain where they play a role in altering the serotonin levels (Azmitia et al., 1996). In fig. 2 the 5-HT projections of the raphe nuclei are shown. In the central nervous system, 5-HT is believed to act predominantly as an inhibitory neurotransmitter although the intracellular effects on second messenger systems, like GPCRs, varies with the receptor subtype (Owens & Nemeroff, 1998).

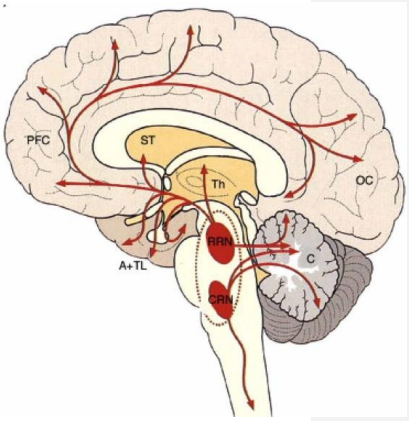

Fig. 2 Shown are 5-HT projections from the caudal raphe nuclei (CRN) and one of the rostal raphe nuclei (RRN), i.e. the dorsal raphe nucleus. C, cerebellum; Th, thalamus; A, amygdala; TL, temporal lobe; ST, striatum; PFC, prefrontal cortex; OC, occipital cortex (Sambeth, 2010).

Serotonin is believed to regulate the production of neurotrophic factors in the cerebrospinal fluid (Sodhi & Sanders-Bush, 2004). These are small proteins that support the growth, survival and differentiation of both developing and mature neurons (Malenka et al., 2009). Serotonin exerts its neurotrophic actions through 5-HT receptors and their downstream signalling pathways (Sodhi & Sanders-Bush, 2004). All these different types of receptors have a different kind of functional property. For example, 5-HT_{1A} receptors are located on presynaptic serotonergic neurons as well as postsynaptic non-serotonergic neurons in several corticolimbic areas such as the hippocampus and the raphe nuclei. In the raphe nuclei, 5-HT is extended to almost all forebrain areas involved in learning and memory (Meneses & Perez-Garcia, 2007). The 5-HT_{2B} receptors are predominantly located in the heart, and inactivation of the gene coding for this receptor caused fatal cardiac morphogenetic defects in mice (Nebigil et al., 2000). As a final example, 5-HT_{5A} receptors are expressed only in the central nervous system, mainly in the cortex, hippocampus and cerebellum (Grailhe et al., 2001). This shows that the serotonin receptors are distributed all over the body and have a key role in the brain.

1.3 Serotonin Transporter

As with all neurotransmitters, 5-HT needs to be regulated. The primary mechanism by which the action of serotonergic neurons is terminated is via the serotonin transporter (5-HTT), also known as SERT. After 5-HT is released from the nerve terminals, it acts on the pre- or post-synaptic receptors. Then 5-HT is transported out of the synaptic cleft back into the nerve terminal via the action of the membrane uptake carrier, SERT. This effect of SERT reduces concentrations of 5-HT near the synapse to levels not capable of maintaining pre- or post- synaptic receptor activation. In the nerve terminal, serotonin is either metabolized by monoamine oxidase or repackaged into secretory vesicles by the vesicular transporter (fig. 3) (Owens & Nemeroff, 1998).

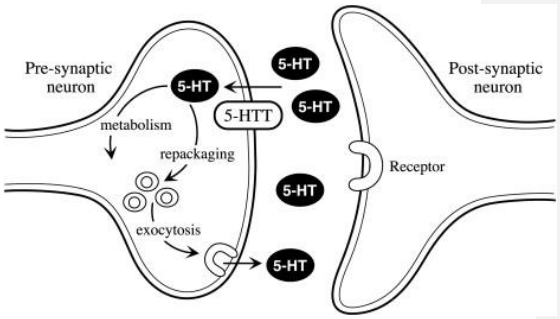

Fig. 3 5-HT is synthesized by presynaptic neurons and stored in vesicles. 5-HT is released into the synaptic space, activates the post-synaptic receptors and stimulates the post-synaptic neuron. The reuptake mechanism via 5-HTT (SERT) controls the duration of 5-HT effects and recycle or degrade 5-HT. Inhibition of SERT prevents uptake of 5-HT resulting in its accumulation within the synaptic cleft and the prolonging of receptor activation (Elizabeth, 2013).

A number of biochemical and pharmacological studies have shown that the

actual reuptake process involves the binding of 5-HT to a recognition site within the SERT protein and is transported across the membrane together with a Na+ and a Cl- ion. A second step involves the translocation of a K+ ion across the membrane to the outside of the cell, which is thought to alter the conformation of the SERT so that it is able to transport a subsequent molecule of 5-HT (Owens & Nemeroff, 1998). The SERT protein is one of the critical elements in determining synaptic concentrations of serotonin (Jonnakuty et al., 2008).

In depression, the levels of serotonin are reduced. To re-establish the 5-HT concentration, SERT is blocked. By doing this, the effects of 5-HT will last longer because the reuptake of 5-HT is prevented.

1.4 Selective Serotonin Reuptake Inhibitors

The first effective antidepressants, monoamine oxidase inhibitors (MAOIs) and the tricyclic antidepressants (TCAs), did not become available until the late 1950s. The disadvantages of these antidepressants are the major side effects and difficulties in achieving adequate dosing (Lochmann & Richardson, 2019). Some examples of these side effects include constipation, decreased vision and insomnia (Remick, 1988; Remick et al., 1989). The need of a new class of antidepressants was high and in the past decades one has come available. The Selective Serotonin Reuptake Inhibitors (SSRIs), which are as effective as TCAs but with less side effects (Lochmann & Richardson, 2019).

The goal in development of the SSRIs was to design an antidepressant that would both potently and selectively inhibit SERT, which causes the re-uptake of 5-HT. As explained before, re-uptake is a major inactivating mechanism for 5-HT after its release into the synaptic cleft. Thus, SERT inhibition by SSRIs lead to an accumulation of 5-HT in the extracellular space. As a result, the magnitude and duration of the activity of serotonin on pre- and postsynaptic serotonin receptors increases (fig. 4). Therefore, SSRIs can be used as a solution to relieve the symptoms related to depression (Lochmann & Richardson, 2019).

All SSRIs have a similar mechanism of action, selectively blocking the serotonin transporter which is responsible for the high affinity reuptake of 5-HT at the plasma membrane. The selectivity of the SSRIs allows them to achieve the desired effect, which is inhibition of SERT, while having no effects on other transporter proteins. This is not the case with TCAs. While both TCAs and SSRIs inhibit the SERT protein, the SSRIs do not affect other receptors or sodium channels in the way that TCAs do. TCA's do affect other receptors or channels resulting in the associated adverse effects of these drugs (Lochmann & Richardson, 2019).

In addition, SSRIs were initially developed for the treatment of depression where they appear to be as effective as TCAs (Benfield et al., 1986). But they may also have additional effects in treating anxiety disorders. Many patients with depression suffer from anxiety as well, and thus having one effective treatment for both is desirable. Additionally, SSRIs are also effective in the relief of sleep disturbance within depression, without causing daytime psychomotor impairment (Lapierre, 1991). Thus, the SSRI antidepressants appear to have a broad spectrum of therapeutic activity by not only relieving depression but also other depression related disorders.

A disadvantage of SSRIs is its onset of action, they will take 4-6 weeks to produce a clinically meaningful response in depressed patients. The SSRI fluoxetine even has a slower onset of action due to its long half-life. It may take up to 75 days to reach a steady-state condition in a healthy adult patient and up to 105 days in a healthy patient over the age of 65. Because of this, other options are typically preferable in elderly patients (Lochmann & Richardson, 2019). Vilazodone was marketed to have a more rapid onset of action though this has not been proven in subsequent studies (Deardorff & Grossberg, 2014).

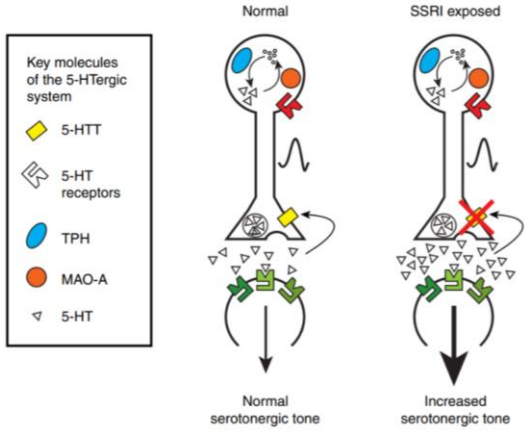

Fig. 4 Molecular serotonin system components, effects of selective serotonin reuptake inhibitor (SSRI) exposure, and outcomes related to antenatal SSRI exposure. Pre- and postsynaptic components of the serotonin system with key molecules that regulate serotonin synthesis, release, reuptake, and degradation are shown under normal conditions (left) and under SSRI exposure (right). Blockade of the serotonin transporter leads to an increase in serotonergic tone. 5-HT, serotonin; 5-HTT, serotonin transporter; MAO-A, monoamine oxidase A; TPH, tryptophan hydroxylase (Oberlander et al., 2009).

1.5 Selective Serotonin Reuptake Inhibitors and Pregnancy

The management of mood disturbances during pregnancy frequently requires mothers and their clinicians to weigh the potential consequences of the mental illness against the consequences of the psychopharmacotherapy. Therefore, the benefits of the SSRI use must overcome the risks since untreated depression has a negative impact on child development. The popularity of SSRIs relative to other antidepressants is because of their proven safety in adults and because they are considered to be safe for antenatal use. This has led to an increase in their use for optimization of maternal health during pregnancy by improving maternal mood and relieving symptoms of depression (Oberlander et al., 2009). As a result, SSRIs are the drug of choice for treating depressed pregnant and postpartum women. Exposure of the fetus to maternal depression during gestation occurs in 10-16% of all pregnancies (Benett et al., 2004). Although SSRIs are considered to be safe for premature use, literature about safety and/or efficacy of antidepressants during pregnancy are still controversial and a matter of debate. There is an unclear safety profile and a lack of well-controlled safety studies.

It is important to do research on all medication during pregnancy to see if it causes any teratogenic side effects. The majority of teratogenic studies regarding SSRI use in pregnancy have focused on the SSRI fluoxetine. This medication will persist in the body for weeks after discontinuation due to its long half-life. Chambers et al. (1996) found more than a twofold increase in the incidence of three or more minor structural anomalies. Minor anomalies are unusual external physical features which do not inhibit a major function of the body. But there was no increase found in major structural anomalies which do inhibit an important function of the body (Chambers et al.,

1996). Pastuszak et al. (1993) did not find any difference in the rate of major structural anomalies but did find the miscarriage rate doubled. Also, postnatal complications in 13% of the neonates were found (Goldstein, 1995). Therefore, the use of antidepressants in pregnancy should be first-line only if the depression is severe or the likelihood of relapse in the patient is high (Cooper et al., 2007).

Despite the possible side effects of SSRI use during pregnancy, an estimated 2-3% of pregnant women are prescribed SSRI antidepressant during all or part of their pregnancy (Olivier, 2015). As said before, SSRIs do not only have an effect on the mother, but also on the fetus. This is the consequence of this medication which can transfer through the placenta and the BBB, affecting the unborn child. Because of this, a significant number of unborn children are exposed to these antidepressants during critical phases of neurodevelopment (Homberg et al., 2010).

Long before birth, during gestation, developmental pathways that influence a child's capacity to learn, think and respond to stress are already taking shape. The neurotransmitter 5-HT plays a critical role in this process, because it is widely distributed throughout the whole brain. Serotonin signalling is regulated by a complex network of genes in our DNA. These genes code for transcription factors, transporters, receptors and synthetic metabolic enzymes resulting in the serotonergic system. These serotonergic neurons are formed early during fetal development, forming one of the most ubiquitous circuits in the mammalian brain (Velasquez et al., 2013). Serotonin plays two key roles in the developing brain: in the early developmental period, 5-HT acts as a growth factor, regulating the development of its own and related neural system (Whitaker-Azmitia et al., 1996). It also has its role as a neurotrophic factor, these factor molecules allow neuronal development and help maintaining the connections between them. As a neurotrophic factor, 5-HT regulates diverse and developmentally critical processes, such as cell division, differentiation, migration, myelination, synaptogenesis and dendritic pruning (Gaspar et al., 2003). Then, in the mature brain, 5-HT acts as a modulatory neurotransmitter influencing cognition, attention, emotion, learning, sleep, arousal and stress responsivity (Chaouloff et al., 1999).

With this perspective that 5-HT has its broad function in shaping the human brain and consequently behaviour, it is not unthinkable that altering the 5-HT levels during developmentally sensitive periods would have critical implications on the brain. This has been even more highlighted by the increased use of SSRI antidepressants to treat maternal depression during pregnancy, which has raised critical questions about the impact of altering 5-HT levels *in utero* and the implications on the fetal brain. Most research on serotonergic disturbances during development has been done in mice and rats. In addition, mice with genetically disrupted SERT function *(5-htt$^{-/-}$)* serve as an extreme model for lifelong SSRI exposure and is a widely used model to study the consequences of early SERT blockade on development. 5-HTT$^{-/-}$ mice display behavioural, neurophysiological, neuroanatomical and molecular alterations (Oberlander et al., 2009). This is caused by elevated extracellular 5-HT concentrations which is also seen in SSRI use. In the next chapter the possible implications of premature SSRI use on the fetal brain development will be discussed.

CHAPTER 2: THE EFFECTS OF PRENATAL SSRI USE ON FETAL BRAIN DEVELOPMENT

2.1 DENDRITES

Dendrites are the major receiving elements of neurons and represent the targets for synaptic input from thousands of surrounding neurons. They extend from the cell body of the neuron and are specialized for processing synaptic information. Dendritic branches often look like tree branches and can have diverse forms in different animals, which can be seen in fig. 5. This branching occurs in characteristic spatial domains where they receive specific synaptic inputs. The shape and composition of dendrites and their synaptic specializations are influenced throughout life by genes, environment, learning, memory and neuropathological conditions (Dharani, 2014). Both the geometry and the density of the dendritic branching, or arbors, are important for understanding connectivity in the nervous system. The structure of a dendrite arbor determines the presynaptic inputs to the neuron, the integration of input information and the output from the cell. Therefore, dendrites do more than simply collect and funnel this input to the soma and axon. Consequently, mechanisms regulating the development and maintenance of the structure of dendrites, play a critical role in circuit function (Stuart et al., 2016). Since 5-HT plays important roles during brain development, SSRI exposure might disorganize the shaping of dendritic structures leading to altered behavioural performances.

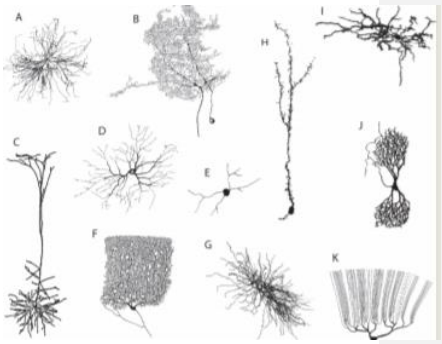

Fig. 5 Dendritic trees come in different shapes and sizes. **(A)** Cat motoneuron. **(B)** Locust mesothoracic ganglion spiking neuron. **(C)** Rat neocortical layer 5 pyramidal neuron. **(D)** Cat retinal ganglion neuron. **(E)** Salamander retinal amacrine neuron. **(F)** Human cerebellar Purkinje neuron. **(G)** Rat thalamic relay neuron. **(H)** Mouse olfactory granule neuron. **(I)** Rat striatal spiny projection neuron. **(J)** Human nucleus of Burdach neuron. **(K)** Fish Purkinje neuron (Dharani, 2014).

This hypothesis was tested by Lee (2009) by examining structural alterations in the barrel cortex of neonatal fluoxetine-treated rat pups and its behavioural effects. The rat pups received physiological saline or the SSRI, fluoxetine subcutaneously in the first postnatal week. When the drugs are given within an early period, in the first two postnatal weeks, it corresponds to the third trimester of human fetal stage (Romijn et al., 1991).

To test if postnatal SSRI exposure in rat pups lead to the malformation of dendritic structures, Lee (2009) has looked at the rodent whisker-to-somatosensory cortex pathway. This is an excellent model system for examining the mechanisms in the cortical development. This neuronal pathway is specialized for processing information about the special coordinates of objects and their identity. The rodent receives somatosensory input from their whiskers which travels from the brainstem to the thalamus and ends in the cortical layer IV in the primary somatosensory cortex. Patches of thalamocortical afferent (TCA) arbors and layer IV neurons, form discrete modules known as barrels (Diamond et al., 2008). Each barrel represents one specific whisker, the layout of the barrels in the somatosensory cortex replicates the layout of the whisker on the snout (fig. 6) (Woolsey & Van der Loos, 1970). Therefore, the barrel cortex, with its cellular structure, organisation and functional significance, is a useful tool to understand cortical processing in rodents.

In the study by Lee (2009) newborn (postnatal day 0, P0) Wistar rat pups of both sexes were used. The rat pups received physiological saline or fluoxetine hydrochloride (10 mg/kg/day in physiological saline) subcutaneously from P0 to P6. This period was chosen because the thalamocortical afferents achieve adult-like pattern by the end of the first postnatal week (Rebsam et al., 2002). Some rats were sacrificed for TCA labelling at P7. Adolescent rats (P30-P35) of control group and fluoxetine-treated group were used in multiple behavioural examinations namely the hotplate test, gap-crossing test and open field test. These examinations were done, to see if

fluoxetine treatment has an effect on the sensory information processing and thus behaviour. After these tests, all rats were sacrificed and the brains were processed for Golgi-Cox impregnation.

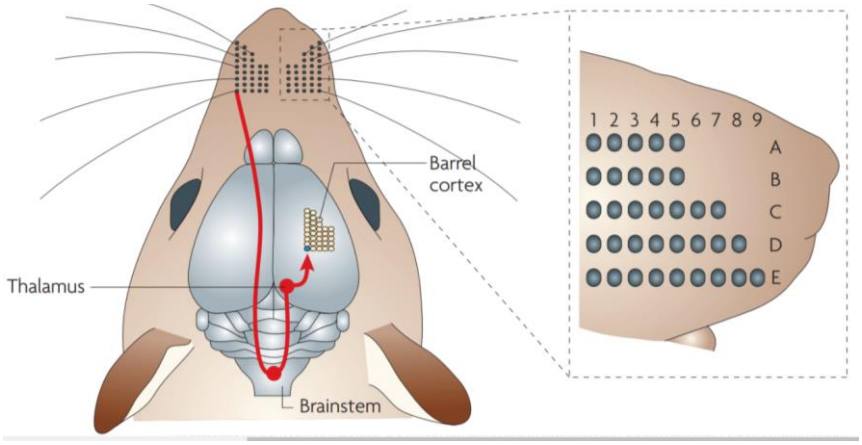

Fig. 6 The vibrissae form a two-dimensional grid of five rows on each side of the snout, each row containing five to nine whiskers ranging between 15 and 50 mm in length (see inset). After a synapse in the brainstem, axons of the second-order neurons cross the midline and travel to the thalamic somatosensory nuclei; thalamic neurons project to the barrels in the primary somatosensory cortex (Diamond et al., 2008)

The results showed that TCA cells had fewer branches, indicating structural deformation (Lee, 2009). Also, in fluoxetine-treated rats, layer IV cells in the rat barrel cortex had a smaller dendritic field and a reduced total dendritic length compared with the control group. Fluoxetine-treated rats also had reduced dendritic complexity and spine density compared with controls. The reduced spine density can be seen in fig. 7. These results suggest that sensory information processing may be disturbed in neonatal fluoxetine-treated animals due to structural deformation of TCA cells and dendritic structures of the cells in layer IV (Lee, 2009).

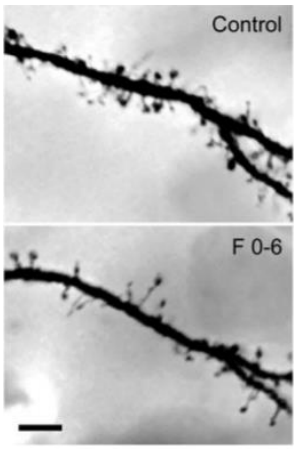

Fig. 7 Density of the dendritic spines. The dendritic spines of layer IV spiny stellate cells in the somatosensory cortex were revealed by Golgi-Cox impregnation method. Spine density was determined at each dendritic order (1st to 4th). The density of total spine was significantly reduced in fluoxetine-treated (F 0–6) rats compared with controls, especially in the 2nd, 3rd, and 4th dendritic orders.

The study of Lee (2009) showed that the structure of the somatosensory cortex is altered, but does this affect the somatosensory-related behavioural performances? Rats are tactile animals and many behaviours are reliant on whisker-specific perception therefore, many behaviours are reliant on whisker functions (Diamond et al., 2008). To test if somatosensory-related behaviour performances are affected by postnatal fluoxetine exposure, Lee (2009) also conducted some behavioural examinations in adolescent rats.

The function of the pain- and thermal information pathway (spinothalamic pathway) was examined by the hotplate test for determining the threshold for thermal pain. Fluoxetine-treated rats have a longer latency to the first licking on the hotplate compared with the control animals, implying a higher thermal threshold (Lee, 2009). This blunted pain reactivity was also seen in prenatal SSRI-treated infants and suggests that early fluoxetine exposure may cause long-term perceptual effects on the pain system (Oberlander et al., 2005).

The gap-crossing test was used to examine the function of medial lemniscal sensory pathway (carrying tactile information). Rats are tactile animals and many behaviours are reliant on whisker-specific perception (Lee, 2009). Rats need their whiskers when they have to cross a gap. Normally, the maximum crossable gap distance of adolescent rats is between 6 and 8 cm. However, fluoxetine-exposed adolescent rats had significantly shorter maximum crossable gap distance (4.54 ± 0.37 cm). The whisker function-dependent gap-crossing performance was altered in fluoxetine-treated rats implying that the whisker-specific tactile function is impaired (Lee, 2009).

While the perceptive function of the adolescent rats is affected by neonatal fluoxetine treatment, their explorative activity may also be affected. To test this possibility, an open field test was conducted. The results suggested that neonatal fluoxetine treatment altered the explorative behaviour (Lee, 2009). Fluoxetine-treated rats showed altered explorative behaviour as well as blunt tactile perceptions.

In conclusion, neonatal disturbances in 5-HT tone by fluoxetine in the first postnatal week, corresponding to the third trimester in human pregnancy, has long-lasting effects on the function and structure of the somatosensory system and also on its associated behaviour.

2.2 AXONS

An axon leaves the nerve cell body at the axon hillock and gives rise to a number of branches. These branches end in sets of terminal branches with presynaptic complexes. Generally, axons in the CNS are highly branched and contact several hundreds of target neurons, locally or distally. The axon effects transmission of electrically information. But, the function of the axon is not purely limited to the conduction of the action potential from the cell body to the terminal. It also plays a role in the transmission of chemically coded information and transports cytoplasmic materials (Waxman, 1995). The axon follows the conduction of information from the cell body to the nerve terminal, functioning as the output structure of the neuron. The coded information comes from an impulse originating from in- or outside the body. Propagation of electrically coded information is unidirectional and is passed on by neurons. Their branching patterns are fundamental for the integration of activities between receptors, neurons and effectors (Waxman, 1995). A major investigated topic in this area are the thalamocortical axons (TCA). These axons express the guidance receptor Deleted in Colorectal Carcinoma (DCC) and Unc-5 Netrin Receptor C (Unc5c) as well as 5-HT receptors (Stone, 2020). Therefore, questions can be raised about the influence of these 5-HT receptors on the axon guidance.

TCAs are established in the dorsal thalamus which can be further subdivided into anterior and posterior regions. Netrin-1 is a protein and an important guidance cue for axons, which binds to the DCC and Unc5c receptor. Bonnin et al. (2007) found *in vitro* that the axons in the posterior half of the dorsal thalamus were attracted toward the source of netrin-1. In contrast, axons growing out of the explants isolated from the anterior half of the dorsal thalamus are repelled from the source of netrin-1. This means that axons in the posterior half are guided to the source of netrin-1 and axons from the anterior half are not. These different responses may be an important mechanism for the initial sorting of dorsal thalamus axons. Axons in the dorsal thalamus express different types of serotonin receptors, namely the 5-HT_{1B} and 5-HT_{1D} receptor. Bonnin et al. (2007) tested whether increasing concentrations of 5-HT modulates the response of the posterior dorsal axons to netrin-1. This was done by exposing explants from the posterior dorsal thalamus to increasing concentrations of 5-HT with doses ranging between 3nM and 30µM.

The results showed that when explants from the posterior dorsal thalamus were treated with 30µM 5-HT, axons were repelled from, instead of being attracted to, the source of netrin-1. Dose responsive analysis showed that nanomolar concentrations of 5-HT were sufficient to alter the responsiveness of posterior axons to netrin-1, with the optimal response at 30 µM. Thus, elevated 5-HT concentrations inhibit axonal outgrowth in the posterior half of the dorsal thalamus (Bonnin et al., 2007). As mentioned earlier, 5-HT_1 receptors are GPCRs, specifically the $G_{i/o}$ receptor kinds. Their

activation leads to an intracellular decrease of cAMP which is responsible for the switch in response of TCAs to netrin-1 from attraction to repulsion (Adham et al., 1992).

During embryonic and early postnatal development, thalamocortical axons transiently express SERT. However, during the early phase of fetal thalamocortical axon growth, the role of SERT is not known. In another study, Bonnin et al. (2012) looked further into which effect SSRIs have on the minimal 5-HT concentration needed to switch axonal responses to netrin-1 from attraction to repulsion. They found that in the presence of the SSRI citalopram, the lowest concentration of extracellular 5-HT (3nM) was able to affect thalamic axon responses to netrin-1. Unexpectedly, even in the absence of extracellular 5-HT, citalopram alone was capable of switching the response of thalamic axons to netrin-1 from attraction to repulsion. This data suggests that extracellular 5-HT is not needed to affect the thalamic response, but blockage of SERT is. Alternatively, citalopram could also bind to other receptors which could affect axons behaviour. An alternative target of citalopram is the σ1 receptor. Like several other SSRIs, citalopram can act as an agonist of σ1 receptors. Results showed that σ1 receptor agonists switch thalamic axons response to netrin-1 from attraction to repulsion (Bonnin et al., 2012).

To summarise, elevated serotonin concentrations causes a switch in the response of TCAs to netrin-1 from attraction to repulsion. Citalopram has an effect on SERT, causing an elevation of extracellular 5-HT concentrations which results in TCA switching. Moreover, citalopram and many other SSRIs can bind to the σ1 receptor which also induces switching of thalamic axons response to netrin-1. Although an effect of citalopram on axon guidance *in vivo* through σ1 receptors must now be demonstrated, it is clear that SSRIs could affect the neural development in the fetus.

2.3 Oligodendrocytes and Myelination

In the peripheral nervous system, the axon is wrapped by multiple layers of myelin. Schwann cells mainly contribute to the production and maintenance of these myelin sheaths. This process is called myelination. In the central nervous system these myelinating glial cells are oligodendrocytes (OLs) instead of Schwann cells. Myelination is essential for rapid conduction of action potentials and for appropriate neuronal communications supporting higher brain functions. Therefore, myelinated fibres participate in fine temporal regulation of neuronal activities. Moreover, functional changes in myelinated fibres could underlie the concept of white matter plasticity, which is critical for learning and higher-level cognitive functions (Fields, 2010). Myelination is dependent on the developmental stage and is controlled by neuronal axon-oligodendrocyte signalling. For the initial myelination production, OLs need to generate a wide variety of processes and need to locate suitable axons. After location, each OL is capable of myelinating 10-30 axons (Sango et al., 2019). Serotonin plays a critical role in early brain development, and manipulation of 5-HT levels during this period could elicit a disruption of normal neuronal connections and interactions. A question that can be raised relates to whether perinatal exposure to antidepressants, such as SSRIs, can affect this development. Which might be a contributing factor for neurobehavioral abnormalities.

An experiment by Fan et al. (2015) tried to give an answer to this question by performing an *in vitro* experiment. In this study immature OLs were used which were differentiated from OL progenitor cells obtained from a P1 rat forebrain. In addition, a myelination co-culture from the spinal cord of an E16 rat were also used. By treating the immature OL cells and the neuron-OL myelination culture with manipulated 5-HT levels, Fan et al. (2015) could investigate the effect of 5-HT exposure on the development and/or myelination of OLs. The elevated 5-HT concentration is similar to SSRIs use, where elevated serotonin levels are also seen.

Very little, if any, information is available regarding whether 5-HT receptors are expressed by OL cells. Therefore, the first experiment was to investigate the expression of certain 5-HT receptors on OL cells. A strong positive staining of 5-HT_{1A} and 5-HT_{2A} receptors, two widely studied serotonin receptor subtypes, was observed on OL cells. After this, Fan et al (2015) looked further into the effect of manipulated 5-HT levels on the OL and myelination development. This was done by treating immature OLs with 10 and 100 µM of 5-HT for 5 days. Evaluation of the effect of 5-HT exposure on

OL development was based on expression patterns of developmental markers as well as morphological criteria. The experiment showed that the morphology of immature OLs was altered by 5-HT exposure compared with control. The cells had fewer and distorted branches and simpler morphology, which was in contrast to the control cells showing an extensive, elaborating process network. In addition to these signs of developmental disturbance, a few of 5-HT-treated immature OLs also showed degenerative characteristics.

Furthermore, at higher 5-HT concentrations (100 µM), damaging effects of 5-HT on the morphology of immature OLs was further increased, which was concomitant with increased cell death. These data suggest that manipulating 5-HT levels *in vitro* affects OL development and even triggers cell death at higher concentrations. Besides malformation of immature OL cells, elevated serotonin levels also caused a reduced expression of myelin proteins in OLs. Two major myelin proteins found in the myelin sheath, MBP and PLP, were markedly decreased in immature OL cells after 5-HT exposure.

Finally, the effect of 5-HT on myelination was assessed in myelination cell cultures. This was done by exposing the cell culture to a medium with 5-HT (10 and 100 µM). By quantifying the number of myelinated internodes, the results showed that 5-HT exposure significantly reduced the myelination in both concentrations. The control group showed regularly spaced myelinated internodes. This was in contrast to the 5-HT-treated cultures, these cultures showed scattered, irregular patterns of the paranodal domain protein contactin associated protein 1 (Caspr).

Fan et al. (2015) reports that manipulating 5-HT concentrations *in vitro* affects OL development and myelination, and even triggers cell death at higher concentrations. These findings might suggest that elevated serotonin levels following neonatal SSRI exposure may directly target developing OLs leading to myelin malformation. Because the processing of neural information requires proper integrity of axons, myelin sheath and the nodal structures, myelin malformation might contribute to neurobehavioral deficits in SSRI-exposed rats (Fan et al., 2015).

These findings were also found in an *in vivo* experiment by Simpson et al. (2011). In this experiment, OL cell cultures derived from the optic nerve and/or forebrain of rats were used. To examine the effects of 5-HT on OLs, the cell cultures were exposed to 5-HT concentrations in the range of 10-100 µM. The data of the study done by Simpson et al. (2011), indicate that treatment with these concentrations of 5-HT induces OL pathology. The processes of OL progenitor cells were shortened, distorted, and/or polarized compared with those grown in the absence of 5-HT. The same was observed in cultures of immature OLs; however ∼20–30% of OLs in this stage of development also exhibited evidence of apoptosis. Thus, certain aspects of OL pathology are specific to the developmental stage of the OL, with immature OLs being more vulnerable to the effects of elevated 5-HT levels (Simpson et al., 2011).

Taken together, these results indicate that early exposure to elevated 5-HT concentrations may interfere with the immature OL cells, resulting in OL and myelin malformation. Because neural information processing requires myelin sheaths and therefore the proper function of oligodendrocytes, which might contribute to neurobehavioral deficits (Fan et al., 2015).

2.5 Neuronal Plasticity

Neuronal plasticity is generally defined as the ability of the brain to change its structure and/or function in response to internal and external constraints or goals. You can also say that it is the responsiveness of a neuron to its environment. This process can occur at various levels of brain organization, for example its involvement in the efficiency of transmission at existing synapses. Neuronal plasticity is also involved in changes in the number of connections between the synapses (Zimmerman & Hummel, 2014).

Serotonin has an important role as a neurotransmitter in the central nervous system. Serotonergic neurons, arising from the raphe nuclei, project to the hippocampal formation and the prefrontal cortex (PFC), suggesting their participation in many psychological functions. These regions are involved in information processing and working memory in rat behavioural tests (Floresco et al.,

1997). Different forms of synaptic plasticity exist in the pathway between the hippocampus and the PFC, also known as the hippocampo-mPFC pathway. These forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) (Burette et al., 1997). LTP increases synaptic strength where LTD leads to the opposite result.

Research has begun to investigate key factors and biomarkers locating in peripheral fluids and its relation to the central nervous system and neuronal plasticity. Two factors that have been investigated are brain-derived neurotrophic factor BDNF and S100B. SSRIs may affect neurodevelopment and plasticity by alterations in BDNF- and S100B-levels.

2.5.1 BDNF

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. It is located in specific neuronal populations at low levels peripherally and at much higher levels centrally. BDNF is most highly expressed in the developing brain but is still expressed at lower levels in the adult brain. In addition, BDNF expression is rapidly and potently regulated by synaptic activity where it is critically involved in the regulation of neuronal plasticity. It acts through binding to two receptors. One of these receptors is TrkB, which mediates most of the neuronal effects of BDNF. The other receptor is p75, which can influence cell death and can modulate other neurotrophins (McAllister, 2002).

The molecular pathways underlying the effects of early-life SSRI exposure on neuronal plasticity are still poorly understood. Boulle et al. (2016) did more research in this field by exploring the effects of postnatal fluoxetine application during a time of rodent neural development that is analogous to SSRI exposure to human neonates. In this way, the results obtained from this research would be analogous to SSRI exposure to human neonates beginning during the third trimester. Since perinatal maternal stress and depression itself can have marked effects on the neuronal development, it is important to investigate the effects of SSRIs using a model of maternal depression. Using a model of maternal depression with SSRI exposure also more closely mimics the clinical situation and allows for further understanding of the effects of maternal depression and SSRIs on development. Maternal depression was obtained by exposing the pregnant rats to stress during late pregnancy. This can result in depressive-like behaviour and thus as a maternal depression model (Leuner et al., 2014; O'Mahony et al., 2006). After birth, the mother was administered the SSRI fluoxetine or vehicle via an osmotic minipump. Fluoxetine, and its active metabolite norfluoxetine, will be present in breast milk and can pass to offspring through lactation resulting in detectable levels of fluoxetine and norfluoxetine in serum of pups (knaepen et al., 2013). In this way, we can observe the possible effects of maternal depression and fluoxetine exposure in offspring. On P140, offspring was decapitated and hippocampi were extracted and histology research was performed.

The results showed a significant reduction of BDNF- and TrkB mRNA levels in hippocampal regions due to the premature fluoxetine treatment. This means that gene expression of BDNF itself and its receptor, TrkB, is lowered. Lowered hippocampal BDNF and TrkB levels cannot only give problems during development, but also during adulthood. In this period, BDNF is involved in the regulation of stress responses and thus behaviour (Boulle et al., 2016).

Furthermore, Molteni et al. (2010) did a research with SERT mutant rat. The SERT knockout rat can be used as a model of lifelong SSRI treatment because the SERT protein is not operable. Molteni and colleagues investigated the influence of the SERT deletion on the BDNF expression compared to wild-type animals. Results showed that the total BDNF mRNA levels both in the hippocampus and the prefrontal cortex were reduced significantly, in SERT knockout rats. However, this experiment did not look into the effects on offspring neurodevelopment. Though it can be used as a comparable model because the same mechanism is happening in the fetal brain reducing BDNF levels in the brain (Molteni et al., 2010).

Lastly, similar results were also found by Karpova et al. (2009). Karpova and colleagues examined the effects of early postnatal fluoxetine treatment on depression- and anxiety related behaviours as well as exploratory locomotion in adult males. Fluoxetine treatment was done via

injection between postnatal days P4 and P21. Exploratory locomotion, depression- and anxiety-related behaviour were assessed in the light-dark, open field and forced swim test. The results showed that postnatal fluoxetine administration resulted in an increase of depression- and anxiety-like behaviour and long-lasting inhibition of exploratory activity in adulthood. These results were associated with changes in hippocampal BDNS and TrkB mRNAs expression levels (Karpova et al. (2009).

To summarise, SSRI exposure during development can have an effect on the BDNF-pathways implicated in neuronal plasticity but also on its associated behaviour.

2.5.2 S100B

Not only BDNF plays a critical role in neurodevelopment but also S100B. Serotonin regulates the development of key neuronal tissues such as the hippocampus and the PFC, in combination with the calcium-binding protein S100B. This protein is released in response to 5-HT_{1A} receptor activation and then binds to calcium. After the binding to calcium, S100B has a conformational change which is responsible for the biological activity (Gazzolo & Michetti, 2003). When released in nanomolar concentrations, S100B stimulates glial cell proliferation, neuronal survival and can induce neurite outgrowth (Gazzolo & Michetti, 2003). Therefore, it mediates the positive outgrowth and survival of neurons. However, in micromolar concentrations S100B can be cytotoxic. Its level in human biological fluids such as cerebrospinal fluid, urine and serum have been used as a biomarker and may be useful for determining brain maturation (Gazzolo & Michetti, 2003). However, little is known about the impact of premature SSRI use on the levels of this serotonin-related biomarker in the fetus. Pawluski et al. (2009) sought answers to this matter by trying to determine whether prenatal SSRI exposure altered neonatal serum S100B levels. This was done by comparing neonatal S100B levels between renatally SSRI-exposed and nonexposed groups. The results showed that S100B levels among prenatally SSRI-exposed neonates were significantly lower than those among nonexposed neonates. They also looked at different SSRIs and which SSRI lowers the S100B levels the most. The data showed that neonatal S100B levels were lower after exposure to paroxetine and sertraline, compared with fluoxetine, venlafaxine and citalopram exposures. This suggests pharmacological differences regarding the effects on the S100B levels. These findings can also be found in other prenatal exposures that alter central serotonin levels and decrease S100B levels, such as alcohol and cocaine.

Prenatal exposures to different SSRIs and its related S100B deficits are associated with altered central serotonin levels. This supports the possibility that increased extracellular serotonin levels in the developing brain may delay the outgrowth of serotonergic neurons. The delay in serotonergic neuronal outgrowth delay the release of S100B, resulting in a loss of neuronal maturation. This is associated with functional deficits in learning and memory which can also be seen in prenatal alcohol and cocaine exposure (Pawluski et al., 2009).

2.5.3 Other

The rodent barrel field cortex had been used as an excellent model to study the roles of various intrinsic and extrinsic factors on the development and plasticity of the neocortex because of its topographic whisker representation. Research has shown that the barrel field in the rodent somatosensory cortex can change its structure due to altered 5-HT levels caused by an SSRI. This was seen in paroxetine exposed rat pups during the first postnatal week (Xu et al., 2004). These rats showed an altered organization of the cortical barrels. The organisation between the barrels, which normally can be clearly seen, was regionally disrupted. The brain is very plastic, especially when its young. This shows that the brain, due to its plasticity, can alters its structure as a result of manipulation of the extracellular serotonin concentration (Xu et al., 2004).

Recent work has been investigating the role of SSRIs on the neuronal plasticity in the cerebellum. Research on this field has been done by Zusso et al. (2008). Cerebellar granule cell (CGC) cultures obtained from rats demonstrated concentration-dependence of the effects of fluoxetine on

cell proliferation. The CGC cultures were exposed to 1-2 µM or 20 µM fluoxetine. Whereas 1-2 µM fluoxetine stimulated the cell proliferation, 20 µM fluoxetine produced an opposite effect, it inhibited cell proliferation. It is reported that SSRIs bind to the 5-HT_{1A} receptors in the cerebellum, which causes the negative effect of the SSRIs on cerebellar neuroplasticity. However, further research *in vivo* has to be done to determine how SSRIs affect plasticity and development in the cerebellum (Zusso et al., 2008).

In addition, brain development and neural plasticity can directly be influenced by changes in the serotonergic functions. Neonatal exposure to SSRIs significantly reduces the expression of SERT in fibres of the hippocampus. Furthermore, this effect appears to be dose-dependent where higher doses of the SSRI citalopram induces a more pronounced decrease of SERT expression. Taken together, neonatal exposure of SSRI can alter the dynamics of the serotonin transporter within the cortex impairing higher cortical functions and cognitive processes. These findings suggest that abnormal development of the 5-HT system could lead to altered wiring of neural circuits and inappropriate behaviour.

Discussion

SSRIs are widely the most used medication to treat depressive disorder. Although they are proven to be safe used by adults, this is not so clear using these antidepressants antenatally. SSRIs improve maternal mood and relieve depression symptoms improving maternal health, but not so much is known on how SSRI use alter fetal brain development.

In rodents, fluoxetine treatment caused structural deformations of TCA cells as a result of fewer dendritic branches, smaller dendritic fields and reduced total dendritic length. This disturbance in sensory information processing also affects its behavioural related performances. Not only dendrites are affected by SSRIs but also axons. Axon growth is inhibited by elevated 5-HT concentrations by switching the response of TCAs to netrin-1 from attraction to repulsion. SSRIs cause 5-HT elevation, but they also bind to the σ1 receptor which also induces switching of thalamic axons response to netrin-1. Thus, axon growth is inhibited in two ways by SSRIs. Furthermore, oligodendrocyte morphology alters due to 5-HT exposure. Elevated 5-HT levels cause immature oligodendrocytes to be degenerative which increases as the 5-HT levels increases. But oligodendrocytes are also the myelinating glial cells which contribute to the production and maintenance of myelin sheaths. The interference of the elevated serotonin levels with immature OL cells also results in myelin malformation. Two major myelin proteins markedly decreased in immature OL cells leading to myelin malformation. Additionally, SSRI exposure during development can also have reducing effects on the BDNF- and S100B-pathways in the brain. Both pathways are implicated in neuronal plasticity and maturation leading to functional altered wiring of neural circuits and deficits in learning, memory and behaviour.

Many aspects in the brain are influenced by elevated 5-HT concentrations and SSRI use, but more research has to be done. To date, the majority of preclinical research with regards to the effects of perinatal antidepressant medication exposure on brain developmental outcomes has investigated the effects of these antidepressant medications on offspring of healthy mothers. However, the primary action of antidepressant medications is to alleviate depressive symptoms. Therefore, more preclinical research is needed on the impact of these medications in the face of maternal adversity. More work is also needed to better mimic the clinical administration method of SSRIs. In humans, SSRIs are taken orally, usually one time per day. Unfortunately, the typical administration of antidepressant medications in rodent models of depression is invasive and stressful, with administration being done most often via injection, oral gavage, or minipump implant. These administration methods affect the metabolism and effects of the medication which can influence the outcomes. In addition, the majority of the preclinical work on prenatal exposure to SSRI medications on neural development and plasticity has used rodent models. Therefore, it is important to note that developmental stages before and after birth vary between species. A lot of research is done during the first postnatal week of the pups which is corresponding to the third trimester of human pregnancy. However, SSRIs are mostly taken during whole pregnancy where neurodevelopment begins as early 5 weeks of gestation (Sundstrom et al., 1993). When SSRIs are only administered in the third trimester, the primary construction of the brain is already completed (Ploeger, 2017). Therefore, more research has to be done where SSRI exposure takes place in the early neurodevelopment. Lastly, further studies are needed to determine whether the actions mediated by σ1 receptors, participate in the side effects of antidepressant use during gestation.

In conclusion, premature SSRI use can cause neurological disturbances during fetal brain development and not every SSRI causes the same effects. In the end the benefits of SSRI use during pregnancy must outweigh the implications of the SSRI treatment.

Literature

Adham, N. I. K. A., Romanienko, P. E. T. E. R., Hartig, P. A. U. L., Weinshank, R. L., & Branchek, T. H. E. R. E. S. A. (1992). The rat 5-hydroxytryptamine1B receptor is the species homologue of the human 5-hydroxytryptamine1D beta receptor. *Molecular Pharmacology*, *41*(1), 1-7.

Azmitia, E. C., Gannon, P. J., Kheck, N. M., & Whitaker-Azmitia, P. M. (1996). Cellular localization of the 5-HT1A receptor in primate brain neurons and glial cells. *Neuropsychopharmacology*, *14*(1), 35-46.

Benfield, P., Heel, R. C., & Lewis, S. P. (1986). Fluoxetine. *Drugs*, *32*(6), 481-508.

Bennett, H. A., Einarson, A., Taddio, A., Koren, G., & Einarson, T. R. (2004). Prevalence of depression during pregnancy: systematic review. *Obstetrics & Gynecology*, *103*(4), 698-709.

Bonnin, A., Torii, M., Wang, L., Rakic, P., & Levitt, P. (2007). Serotonin modulates the response of embryonic thalamocortical axons to netrin-1. *Nature neuroscience*, *10*(5), 588-597.

Bonnin, A., Zhang, L., Blakely, R. D., & Levitt, P. (2012). The SSRI citalopram affects fetal thalamic axon responsiveness to netrin-1 in vitro independently of SERT antagonism. *Neuropsychopharmacology*, *37*(8), 1879-1884.

Boulle, F., Pawluski, J. L., Homberg, J. R., Machiels, B., Kroeze, Y., Kumar, N., ... & Van den Hove, D. L. (2016). Prenatal stress and early-life exposure to fluoxetine have enduring effects on anxiety and hippocampal BDNF gene expression in adult male offspring. *Developmental psychobiology*, *58*(4), 427-438.

Burette, F., Jay, T. M., & Laroche, S. (1997). Reversal of LTP in the hippocampal afferent fiber system to the prefrontal cortex in vivo with low-frequency patterns of stimulation that do not produce LTD. *Journal of neurophysiology*, *78*(2), 1155-1160.

Chemical structure serotonin. (2017, January 13). [Illustration]. Retrieved from **{** HYPERLINK https://www.simpto.nl/menselijk-lichaam/serotonine/ **}**

Chambers, C. D., Johnson, K. A., Dick, L. M., Felix, R. J., & Jones, K. L. (1996). Birth outcomes in pregnant women taking fluoxetine. *New England Journal of Medicine*, *335*(14), 1010-1015.

Chaouloff, F., Berton, O., & Mormède, P. (1999). Serotonin and stress. *Neuropsychopharmacology*, *21*(1), 28-32.

Cooper, W. O., Willy, M. E., Pont, S. J., and Ray, W. A. (2007). Increasing use of antidepressants in pregnancy. Am. J. Obstet. Gynecol. 196, 544.e1–544.e5.

Daly, E., Tricklebank, M. D., & Wichers, R. (2019). Neurodevelopmental roles and the serotonin hypothesis of autism spectrum disorder. In *The Serotonin System* (pp. 23-44). Academic Press.

Deardorff, W. J., & Grossberg, G. T. (2014). A review of the clinical efficacy, safety and tolerability of the antidepressants vilazodone, levomilnacipran and vortioxetine. *Expert opinion on pharmacotherapy*, *15*(17), 2525-2542.

Dharani, K. (2014). *The biology of thought: A neuronal mechanism in the generation of thought-A new molecular model*. Academic Press.

Diamond, M. E., Von Heimendahl, M., Knutsen, P. M., Kleinfeld, D., & Ahissar, E. (2008). 'Where'and'what'in the whisker sensorimotor system. *Nature Reviews Neuroscience*, *9*(8),601-612.

Elizabeth, M. (2013). *5-HT signaling within the central nervous system* [illustration]. Retrieved from **{** HYPERLINK https://www.sciencedirect.com/topics/neuroscience/serotonin-transporter **}**

Fan, L. W., Bhatt, A., Tien, L. T., Zheng, B., Simpson, K. L., Lin, R. C., ... & Pang, Y. (2015). Exposure to serotonin adversely affects oligodendrocyte development and myelination in vitro. *Journal of neurochemistry*, *133*(4), 532-543.

Fields, R. D. (2010). Change in the brain's white matter. *Science*, *330*(6005), 768-769.

Floresco, S. B., Seamans, J. K., & Phillips, A. G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *Journal of Neuroscience*, *17*(5), 1880-1890.

Gaspar, P., Cases, O., & Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nature Reviews Neuroscience*, *4*(12), 1002-1012.

Gazzolo, D., & Michetti, F. (2003). S100B testing in pregnancy. *Clin Chim Acta*, *335*, 1-7.

Goldstein, D. J. (1995). Effects of third trimester fluoxetine exposure on the newborn. *Journal of clinical psychopharmacology*, *15*(6), 417-420.

Grailhe, R., Grabtree, G. W., & Hen, R. (2001). Human 5-HT5 receptors: the 5-HT5A receptor is functional but the 5-HT5B receptor was lost during mammalian evolution. *European journal of pharmacology*, *418*(3), 157-167.

Grossman, P., Tiefenthaler-Gilmer, U., Raysz, A., & Kesper, U. (2007). Mindfulness training as an intervention for fibromyalgia: evidence of postintervention and 3-year follow-up benefits in well-being. *Psychotherapy and psychosomatics*, *76*(4), 226-233.

Hall, F. S., Drgonova, J., Jain, S., & Uhl, G. R. (2013). Implications of genome wide association studies for addiction: are our a priori assumptions all wrong?. *Pharmacology & therapeutics*, *140*(3), 267-279.

Hannon, J., & Hoyer, D. (2008). Molecular biology of 5-HT receptors. *Behavioural brain research*, *195*(1), 198-213.

Homberg, J. R., Schubert, D., & Gaspar, P. (2010). New perspectives on the neurodevelopmental effects of SSRIs. *Trends in pharmacological sciences*, *31*(2), 60-65.

Källén, B. (2004). Neonate characteristics after maternal use of antidepressants in late pregnancy. *Archives of pediatrics & adolescent medicine*, *158*(4), 312-316.

Karpova, N. N., Lindholm, J., Pruunsild, P., Timmusk, T., & Castrén, E. (2009). Long-lasting behavioural and molecular alterations induced by early postnatal fluoxetine exposure are restored by chronic fluoxetine treatment in adult mice. *European neuropsychopharmacology*, *19*(2), 97-108.

Knaepen, L., Rayen, I., Charlier, T. D., Fillet, M., Houbart, V., Van Kleef, M., ... & Pawluski, J. L. (2013). Developmental fluoxetine exposure normalizes the long-term effects of maternal stress on post-operative pain in Sprague-Dawley rat offspring. *PLoS One*, *8*(2), e57608.

Jonnakuty, C., & Gragnoli, C. (2008). What do we know about serotonin?. *Journal of cellular physiology*, *217*(2), 301-306.

Lapierre, Y. D. (1991). Controlling acute episodes of depression. *International clinical psychopharmacology*.

Lee, L. J. (2009). Neonatal fluoxetine exposure affects the neuronal structure in the somatosensory cortex and somatosensory-related behaviors in adolescent rats. *Neurotoxicity research*, *15*(3), 12-223.

Leuner, B., Fredericks, P. J., Nealer, C., & Albin-Brooks, C. (2014). Chronic gestational stress leads to depressive-like behavior and compromises medial prefrontal cortex structure and function during the postpartum period. *PloS one*, *9*(3), e89912.

Lochmann, D., & Richardson, T. (2019). Selective serotonin reuptake inhibitors. *Antidepressants: From Biogenic Amines to New Mechanisms of Action*, 135-144.

Malenka, R. C., Nestler, E. J., & Hyman, S. E. (2009). Atypical neurotransmitters. *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*, 199-215.

Maurer-Spurej, E., Pittendreigh, C., & Solomons, K. (2004). The influence of selective serotonin reuptake inhibitors on human platelet serotonin. *Thrombosis and haemostasis*, *91*(01), 119-128.

McAllister, A. K. (2002). Bdnf. *Current Biology : Cb*, *12*(9), 310.

Meltzer, H. Y. (1990). Role of Serotonin in Depression a. *Annals of the New York Academy of Sciences*, *600*(1), 486-499.

Meneses, A., & Perez-Garcia, G. (2007). 5-HT1A receptors and memory. *Neuroscience & Biobehavioral Reviews*, *31*(5), 705-727.

Molteni, R., Cattaneo, A., Calabrese, F., Macchi, F., Olivier, J. D., Racagni, G., ... & Riva, M. A. (2010). Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans. *Neurobiology of disease*, *37*(3), 747-755.

Montejo, A. L., Montejo, L., & Navarro-Cremades, F. (2015). Sexual side-effects of antidepressant and antipsychotic drugs. *Current opinion in psychiatry*, *28*(6), 418-423.

Nebigil, C. G., Choi, D. S., Dierich, A., Hickel, P., Le Meur, M., Messaddeq, N., ... & Maroteaux, L. (2000). Serotonin 2B receptor is required for heart development. *Proceedings of the National Academy of Sciences*, *97*(17), 9508-9513.

Oberlander, T., Gingrich, J. A., & Ansorge, M. S. (2009). Sustained neurobehavioral effects of exposure to SSRI antidepressants during development: molecular to clinical evidence. *Clinical Pharmacology & Therapeutics*, *86*(6), 672-677.

Oberlander, T. F., Grunau, R. E., Fitzgerald, C., Papsdorf, M., Rurak, D., & Riggs, W. (2005). Pain reactivity in 2-month-old infants after prenatal and postnatal selective serotonin reuptake inhibitor medication exposure. *Pediatrics*, *115*(2), 411-425.

Oberlander, T. F., Warburton, W., Misri, S., Aghajanian, J., & Hertzman, C. (2006). Neonatal outcomes after prenatal exposure to selective serotonin reuptake inhibitor antidepressants and maternal depression using population-based linked health data. *Archives of general psychiatry*, *63*(8), 898-906.

Olivier, J. (2015). De impact van maternale depressie en antidepressiva tijdens de zwangerschap op de nakomelingen. *Neuropraxis*, *19*(2), 25-30.

Owens, M. J., & Nemeroff, C. B. (1994). Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clinical chemistry*, *40*(2), 288-295.

Owens, M. J., & Nemeroff, C. B. (1998). The serotonin transporter and depression. *Depression and anxiety*, *8*(S1), 5-12.
Pardridge, W. M. (1983). Brain metabolism: a perspective from the blood-brain barrier. *Physiological reviews*, *63*(4), 1481-1535.
Pastuszak, A., Schick-Boschetto, B., Zuber, C., Feldkamp, M., Pinelli, M., Sihn, S., ... & Gardner, A. (1993). Pregnancy outcome following first-trimester exposure to fluoxetine (Prozac). *Jama*, *269*(17), 2246-2248.
Pawluski, J. L., Galea, L. A., Brain, U., Papsdorf, M., & Oberlander, T. F. (2009). Neonatal S100B protein levels after prenatal exposure to selective serotonin reuptake inhibitors. *Pediatrics*, *124*(4), e662-e670.
Rapport, M. M. (1948). Green AA, Page IH. *Serum vasoconstrictor (serotonin) IV. Isolation and characterization. J Biol Chem*, *176*, 1243-1251.
Rebsam, A., Seif, I., & Gaspar, P. (2002). Refinement of thalamocortical arbors and emergence of barrel domains in the primary somatosensory cortex: a study of normal and monoamine oxidase a knock-out mice. *Journal of Neuroscience*, *22*(19), 8541-8552.
Remick, R. A. (1988). Anticholinergic side effects of tricyclic antidepressants and their management. *Progress in neuro-psychopharmacology & biological psychiatry*.
Remick, R. A., Froese, C., & Keller, F. D. (1989). Common side effects associated with monoamine oxidase inhibitors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *13*(3-4), 497-504.
Romijn, H. J., Hofman, M. A., & Gramsbergen, A. (1991). At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby?. *Early human development*, *26*(1), 61-67.
Ploeger, A. (2017). De ontwikkeling van het babybrein. *Bijblijven*, *33*(3), 188-195.
Sambeth, A. (2010, April). 5-HT projections [Illustration]. Retrieved from https://www.researchgate.net/figure/Fig-1-Shown-are-5HT-projections-from-the-caudal-raphe-nuclei-CRN-and-one-of-the_fig1_43048975
Sango, K., Yamauchi, J., Ogata, T., & Susuki, K. (Eds.). (2019). *Myelin: Basic and Clinical Advances* (Vol. 1190). Springer Nature.
Simpson, K. L., Weaver, K. J., de Villers-Sidani, E., Lu, J. Y. F., Cai, Z., Pang, Y., ... & Lin, R. C. (2011). Perinatal antidepressant exposure alters cortical network function in rodents. *Proceedings of the National Academy of Sciences*, *108*(45), 18465-18470.
Sodhi, M. S., & Sanders-Bush, E. (2004). Serotonin and brain development. *Int Rev Neurobiol*, *59*(6), 111-74.
SSRI-gebruik en zwangerschap - Startpagina - Richtlijn - Richtlijnendatabase. (n.d.). Retrieved 6 July 2020, from **{** HYPERLINK "https://richtlijnendatabase.nl/richtlijn/ssri_en_zwangerschap/ssri-gebruik_en_zwangerschap_-startpagina.html" **}**
Stone, T. W. (2020). Dependence and Guidance Receptors—DCC and Neogenin—In Partial EMT and the Actions of Serine Proteases. *Frontiers in Oncology*, *10*, 94.
Sundström, E., Kölare, S., Souverbic, F., Samuelsson, E. B., Pschera, H., Lunell, N. O., & Seiger, Å. (1993). Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. *Developmental brain research*, *75*(1), 1-12.
Tschoner, A., Engl, J., Laimer, M., Kaser, S., Rettenbacher, M., Fleischhacker, W. W., ... & Ebenbichler, C. F. (2007). Metabolic side effects of antipsychotic medication. *International journal of clinical practice*, *61*(8), 1356-1370.
Velasquez, J. C., Goeden, N., & Bonnin, A. (2013). Placental serotonin: implications for the developmental effects of SSRIs and maternal depression. *Frontiers in cellular neuroscience*, *7*, 47.
Waxman, F. (1995). *The axon: structure, function, and pathophysiology*. Oxford University Press, USA.
Whitaker-Azmitia, P. M., Druse, M., Walker, P., & Lauder, J. M. (1995). Serotonin as a developmental signal. *Behavioural brain research*, *73*(1-2), 19-29.
Woolsey, T. A., & Van der Loos, H. (1970). The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. *Brain research*, *17*(2), 205-242.
Xu, Y., Sari, Y., & Zhou, F. C. (2004). Selective serotonin reuptake inhibitor disrupts organization of thalamocortical somatosensory barrels during development. *Developmental brain research*, *150*(2), 151-161.
Yuwiler, A., Oldendorf, W. H., Geller, E., & Braun, L. (1977). EFFECT OF ALBUMIN BINDING AND AMINO ACID COMPETITION ON TRYPTOPHAN UPTAKE INTO BRAIN 1. *Journal of neurochemistry*, *28*(5), 1015-1023.
Zimerman, M., & Hummel, F. C. (2014). Brain stimulation and its role in neurological diseases. In *The Stimulated Brain* (pp. 333-369). Academic Press.

Zusso, M., Debetto, P., Guidolin, D., Barbierato, M., Manev, H., & Giusti, P. (2008). Fluoxetine-induced proliferation and differentiation of neural progenitor cells isolated from rat postnatal cerebellum. *Biochemical pharmacology*, *76*(3), 391-403.