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Review

Serotonin: A never-ending story

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ABSTRACT

The neurotransmitter serotonin is an evolutionary ancient molecule that has remarkable modulatory effects in almost all central nervous system integrative functions, such as mood, anxiety, stress, aggression, feeding, cognition and sexual behavior. After giving a short outline of the serotonergic system (anatomy, receptors, transporter) the author's contributions over the last 40 years in the role of serotonin in depression, aggression, anxiety, stress and sexual behavior is outlined. Each area delineates the work performed on animal model development, drug discovery and development. Most of the research work described has started from an industrial perspective, aimed at developing animals models for psychiatric diseases and leading to putative new innovative psychotropic drugs, like in the cases of the SSRI fluvoxamine, the serenic eltoprazine and the anxiolytic flesinoxan. Later this research work mainly focused on developing translational animal models for psychiatric diseases and implicating them in the search for mechanisms involved in normal and diseased brains and finding new concepts for appropriate drugs.

Contents

1. Serotonin, history, drugs

My career in serotonin research started in Groningen at the Biological Psychiatry laboratory of Professor Herman van Praag in 1973 where I contributed to the PhD research of Netty Bouhuys (Bouhuys, 1976). At that time the involvement of serotonin (5-hydroxytryptamine; 5-HT) in mental functions was only emerging after the first suggestions in the 1950s (Gaddum, 1954; Brodie et al., 1955). Initially much research was performed into the synthesis and degradation of this neurotransmitter. In the 1960s serotonin containing neurons were visualized using histochemical fluorescence techniques and via lesion experiments (Dahlström and Fuxe, 1964; 1965). It appeared that the 5-HT containing cell bodies were localized in groups of cells (called B groups) together creating the raphé system in the central nervous system (CNS). The neurons in these systems project both frontally and caudally. The frontally projecting neurons originate in the most rostral localized cell bodies; B5 and B8 (the medial raphé

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nucleus), B6 and B8 (the dorsal raphé nucleus) and B9, more diffusely localized around the lemniscus medialis. The caudally projecting neurons originate from cell bodies in B1-B3. Lesioning of the dorsal (DR) and medial raphé (MR) nuclei reduces the synthesis of 5-HT (Kuhar et al., 1971). There was some evidence at that time (Lorens and Guldberg, 1974) that the medial and dorsal raphé nuclei project to different areas in the forebrain; DR lesions led to decreases in serotonin turnover in the striatum, whereas MR lesions reduced it in the hippocampus. Bouhuys (1976) was interested in the role of the MR and DR in behavior. She found that simultaneous lesioning of the MR and DR in rats led to enhanced locomotor behavior in a new environment. The question of my research in this experiment was whether the enhanced locomotion was due to inactivation of the MR, the DR or both. It appeared that MR lesions led to enhanced locomotion, but not after DR lesions (Olivier, 1976). Both type of lesions led to reduction of 5-HT turnover in the forebrain. Others also found this differential effect of the DR and MR on locomotion (Jacobs et al., 1974). Although a direct correlation between a decrease in 5-HT activity and locomotion is not evident, it appears that different parts of the serotonergic system, projecting to different projection areas contribute differentially to behavior. The experiment described still used quite simple methods to study the relationship between behavior and serotonergic activity, but it already indicated that serotonin contributes in a rather subtle way to various behavioral systems.

Serotonin, a phylogenetically ancient signaling molecule (Hay-Schmidt, 2000), is the most widely distributed neurotransmitter in the brain (Dahlström and Fuxe, 1964; Steinbush, 1981), although its CNS content is less than 5% of the whole bodies content (Jacobs and Azmitia, 1992). In the following decades it has become clear that 5-HT signaling pathways are involved in essential brain functions including sensory processing, cognitive control, emotion regulation, autonomic control and motor activity. At the same time it is a target of many physiological regulatory mechanisms and modulators like gene transcription, neurotrophic peptides, steroids but also psychotropic drugs (Lesch and Waider, 2012). The surprising fact about the 5-HT system in the CNS is that, whereas in the entire mammalian CNS billions of neurons exist, serotonergic cells number only in the ten thousands in rats and cats and in the hundred thousands in man, representing a very tiny amount of all CNS neurons. However, their influence on their target sites goes far beyond these numbers. In the rat brain estimations suggest 6×10^6 serotonergic varicosities/mm^3 of cortical tissue. By extrapolation, this means that each serotonergic neuron projecting to the cortex may be responsible for 5×10^5 serotonergic varicosities, that each of their cortical target neurons receives ~200 varicosities, and that serotonergic terminals may account for as many as 1/500 of all axon terminals in rat cortex (Jacobs and Azmitia, 1992).

The present paper will particularly deal with the rostral serotonergic system (B5-B9) because these serotonergic systems seem to be involved in the modulatory role in higher mental functions, including mood, anxiety and fear, cognition and other functions, whereas the caudal system (B1-B3) mainly projects to the spinal cord and cerebellum and is involved in motor activity, pain control and regulation of autonomic processes (Jacobs and Azmitia, 1992).

Rostral serotonergic projections project to the forebrain innervating virtually all regions (Calizo et al., 2011). Two classes of fine and beaded fibers (D and M fibers, resp.) exist, originating from the dorsal and median raphé nuclei, resp. (Törk, 1990). The M fibers, also called the basket axon system, contain thick fibers without varicosities and originate in the MR. These M fibers form extensive (classic) synaptic connections and project heavily e.g. to cortical areas, and hippocampus. The other system, the varicose axon system (D fibers) originates in the DR, has thin fibers with lots of spindle-like varicosities. These fibers ramify extensively and are extremely diffuse. The small spindle-like boutons are present along the fibers and it is still doubtful whether real synaptic

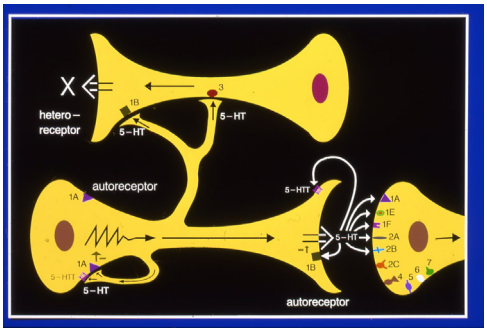

Fig. 1. Cartoon of a serotonergic neuron with a schematic depiction of the localization of 14 serotonergic receptors and the serotonergic transporter.

contacts are present. They mainly innervate the dorsal striatum, amygdala and prefrontal cortex. Although the functional role of both serotonergic systems is badly understood, there is evidence that they display a differential sensitivity for certain substituted amphetamines (including e.g. methylenedioxymethamphetamine (MDMA-ecstacy). The latter has been suggested to induce degeneration of the fine, varicose system, leaving the thick M system unaffected (Biezonski and Meyer, 2011; Parrott, 2013).

Although the raphé nuclei are generally described as 'serotonergic', large quantities of non-serotonergic cells emerge in these structures, including neurons transmitting glutamate, GABA, dopamine, nitric oxide and various neuropeptides (Adell et al., 2002). Numerous projections from different brain regions reach the raphé nuclei including glutamatergic, cholinergic, GABA-ergic, noradrenergic and various neuropeptidergic transmitters (Adell et al., 2002; Artigas, 2013a,b).

The serotonergic system contains at least 14 different serotonin classes of receptors (5-HT$_{1A, 1B, 1D, 1E, 1F; 2A, 2B, 2C, 3, 4, 5A, 5B, 6 \text{ and } 7}$) and a serotonin transporter (SERT)(Fig. 1). Except the 5-HT$_3$ receptor, gating a cation-permeable ion channel, all 5-HT receptors are G-protein coupled. The signaling via G-protein-coupled serotonin receptors is extremely diverse and we are still in the dark how these brain 5-HT receptors operate in real life (for an extensive review Millan et al. (2008)), but they generate many possibilities to modulate other systems in the brain, like the glutamatergic and GABA-ergic ones (Artigas, 2013b; Fink and Göthert, 2007). This diversity coupled to a complex and differential distribution of the various 5-HT receptor classes in the CNS brings 5-HT into a position where it may modulate various core functions in the brain. It can also easily be assumed that disruption of various aspects of serotonergic neurotransmission may lead to vulnerability or even pathology, including depression, anxiety disorders, schizophrenia, food disorders, chronic pain and others.

Some 5-HT receptors function as autoreceptors, some as heteroreceptors and some as both (5-HT$_{1A,1B}$). Although it is unclear whether every single 5-HT neuron in the brain is equipped with similar autoreceptors, for the DR and MR it is known that they have somatodendritically localized 5-HT$_{1A}$ autoreceptors, presynaptically localized 5-HT$_{1B}$ autoreceptors and 5-HT transporters, both localized at the cell body and the synapse (see Fig. 1). All 5-HT receptors are also localized postsynaptically as heteroreceptors, e.g. on glutaminergic and GABA-ergic cells (Adell et al., 2002; Artigas, 2013a,b).

If a serotonergic neuron fires, 5-HT is released into the synaptic cleft where it exerts its action on nearby pre- and postsynaptic serotonergic receptors. In order to terminate its action, 5-HT activity has to be terminated which is effectuated via reuptake of 5-HT by the serotonin transporter molecule (SERT), a complex molecule with 13 transmembrane loops located at the presynaptic and somatodendritic membranes of most (if not all) serotonergic neurons. After this uptake over the cell membrane via the SERT from the synapse, 5-HT is subsequently taken up by the vesicular

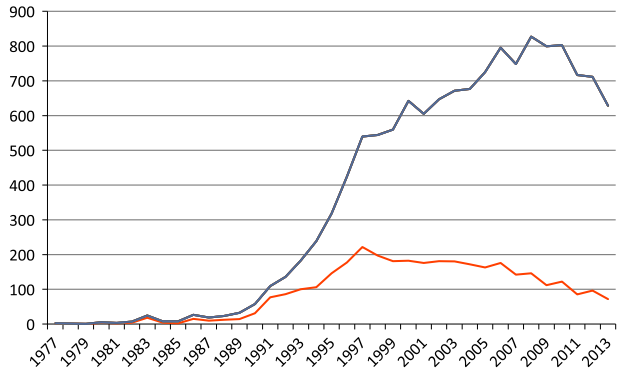

Fig. 2. The number of articles published on fluvoxamine (red) and the number of citations on fluvoxamine (blue) portrayed per year from 1977 till 2014.

monoamine transporter (VMAT2) and stored in the synaptic vesicles for reuse. Another major route to end serotonergic activity is the process whereby 5-HT is taken up by surrounding glial cells and degraded by the enzyme monoamineoxidase-A (MAO-A) (Youdim et al., 2006) to its metabolite 5-hydroxyindole acetic acid (5-HIAA).

Simultaneously, the released 5-HT activates 5-HT_{1B} autoreceptors leading to inhibition of further 5-HT release from the vesicles, and activates also the somatodendritical 5-HT_{1A} autoreceptors, leading to inhibition of cell firing (Artigas, 2013a,b). The interplay between these three mechanisms (5-HT reuptake, inhibition of release via activation of 5-HT_{1B} autoreceptors and inhibition of cell firing via activation of somatodendritic 5-HT_{1A} autoreceptors) reduces the activity of the serotonergic neurons after activity, preparing the neuron for a new discharge (Artigas, 2013a,b). Of course many non-serotonergic input is acting on serotonergic cells in the raphé nuclei; a nice schematic overview is shown in Fig. 2 of Adell et al. (2002).

2. Serotonin and depression

A neuronal reuptake mechanism for terminating action of neurotransmitters released from nerve terminals was first found for noradrenaline (Hertting and Axelrod, 1961), followed for other neurotransmitters, including serotonin (Blackburn et al., 1967; Ross and Renyi, 1967). Soon it was realized that this mechanism had an important regulatory function in the activity of serotonergic neurons. Moreover, due to the then existing theories about the relationship between (low) activity of catecholaminergic systems and the occurrence of depression (Bunney and Davis, 1965; Schildkraut, 1965), pharmaceutical companies tried to design and synthetize drugs that block the reuptake of catecholamines from the synaptic cleft into the presynaptic terminals of the neurons. However, others claimed that 5-HT played an important role in the pathogenesis of depression, formulated as the 'serotonin hypothesis of depression' (Coppen, 1969). This hypothesis suggested that enhancing synaptic 5-HT might lead to antidepressant activity. Although this mechanistic principle (5-HT reuptake inhibition) generated many new antidepressants, the selective serotonin reuptake inhibitors (SSRIs: zimeldine, fluoxetine, fluvoxamine, sertraline, (es)citalopram, paroxetine), it became pretty fast clear that this mechanism is only the beginning of a very complex sequel of largely unknown processes in the brain leading to antidepressant efficacy.

When I started my career in Philips-Duphar (later Solvay Pharmaceuticals) in the mid-1970s I became immediately involved in programs to find new antidepressants. Initially, the search was for monoamine oxidase inhibitors, but in a series of 2-aminoethyloximethers of arylketones, compounds were detected with serotonin and noradrenaline uptake inhibiting properties. The relative activity with respect to NA reuptake and 5-HT reuptake processes appeared quite structure specific and as a result a selective serotonin reuptake blocker, fluvoxamine (Claassen et al., 1977; Claassen, 1983) and a mixed 5-HT/NA reuptake blocker, clovoxamine (Claassen et al., 1978) were developed. Interestingly, fluvoxamine was taken into development for depression based only on biochemical and pharmacological evidence that it had a selective profile for 5-HT reuptake both in vitro and in vivo. This evidence, of course with acceptable safety and toxicity profiles, was sufficient to run the clinical and safety programs with fluvoxamine, leading to a launch of fluvoxamine in Switzerland in 1984. In the mean time, Astra had developed another SSRI, zimeldine that was launched in 1982 but had to be withdrawn from the market in 1983 because of some severe cases of polyneuropathy (Guillain-Barré syndrome) in Sweden (Fagius et al., 1985). Successively, various other SSRIs were introduced into the antidepressant market, like fluoxetine (1987), citalopram (1989), sertraline (1990) and paroxetine (1991). Since the introduction of the SSRIs they have become the first choice treatment of depressive disorders but appeared also effective in panic disorders, obsessive–compulsive disorders (OCD), social phobias and generalized anxiety disorder. Fluvoxamine, for example, has been registered for OCD in the USA in 1995. Fig. 2 shows the number of publications on fluvoxamine in the Web of Knowledge database (Thomson Reuters) from 1977 till 2014. The number of publications is rather low till the late 1980s but then a rather steep increase happens, leveling around 1996 till 2005. Since that time a gradual decrease is observed. This curve probably reflects and parallels the interest in a new chemical entity throughout its life span. The number of citations on fluvoxamine (again from Web of Knowledge during the same time period) reflects the normal lag between publication time and citations.

2.1. Preclinical acute studies

Fluvoxamine, like all the other SSRIs, has been used as preclinical tool in many studies either to find whether presumed animal models of depression are able to detect antidepressant activity or as a tool to challenge the serotonergic system in the brain. When we found and developed fluvoxamine, specific animal behavioral models of depression were hardly available although the development of the SSRIs boosted the search for new and better models. At the end of the 1970s and 1980s several interesting tests to screen for potential antidepressant efficacy in rats and mice were developed, notably the Forced Swim test (FST), sometimes called the Porsolt test (Porsolt et al., 1977) and the Tail Suspension test (TST) (Stéru et al., 1985). The 1980s and 1990s of last century saw an incredulous search for new and better animal models of depression, characterized by, if possible, face, predictive and construct validity (Willner, 1985, 1991). Initially, the antidepressant efficacy of SSRIs was difficult to detect, particularly in the FST (Borsini, 1995; Porsolt et al., 1991) but intensive search for modifications (strain, design of test, drug application schedules, behavioral scoring, etc.) led to reliable detection of antidepressant activity in the FST (Cryan et al., 2005a; Detke et al., 1995; Van der Heyden et al., 1991; Carr and Lucki, 2011). The TST was initially developed as a 'dry' swim test as it does not use water, is far simpler and faster to use and immediately detected the SSRI-induced antidepressant activity (Cryan et al., 2005b; Stéru et al., 1985; Van der Heyden et al., 1987). Several other 'acute' tests have been used to detect antidepressant activity of the SSRIs including fluvoxamine, e.g. differential reinforcement of low rate-72s (DRL72s) behavior and drug discrimination. The DRL 72s is a very specific and sensitive tool to screen for drugs with antidepressant-like

efficacy after acute administration (O'Donnell et al., 2005). The DRL72s contingency reinforces responses when separated by at least 72s. Rats trained on this DRL72s schedule show a temporal pattern of lever pressing best illustrated by portraying interresponse time (IRT) data. The relative frequency distribution of IRT length shows a peak at IRT length less than 72s; during such a short interval response the timer is reset to zero and a new trial starts. Under these conditions animals earn relative few reinforcers. Antidepressants with different mechanisms of action show a very typical profile in this paradigm (O'Donnell et al., 2005): the response rate decreases, the reinforcement rate increases and the peak in the relative frequency distribution shifts toward the right, i.e. toward longer IRTs. Although different mechanisms contribute to the typical profile of antidepressants, the brain serotonergic systems are clearly involved. Lesioning of the serotonergic system by intracerebroventricular injection of the neurotoxin 5,7-dihydroxytryptamine destroying 5-HT neurons, to rats trained to criterium in the DRL72 s schedule led to long term depletion of brain 5-HT and reduction of DRL performance (Jolly et al., 1999). In the lesioned rats, the 5-HT_{1A} receptor agonist buspirone still had a right-shifting effect on the IRT peak, suggesting that postsynaptic 5-HT_{1A} receptors contribute to the antidepressant-like effects of the serotonergic antidepressants including fluvoxamine (Van Hest et al., 1992; Olivier et al., 1993; Paterson et al., 2011). The suggestion that SSRI-induced improvement of DRL behavior could be mediated by activation of postsynaptic 5-HT_{1A} receptors (Jolly et al., 1999) would be in line with the antidepressant-like profile of selective 5-HT_{1A} receptor agonists like flesinoxan (Van Hest et al., 1992) and 8-OH-DPAT (Marek et al., 1989) in the DRL-72s paradigm.

Another paradigm of use to study the potential 'in vivo' mechanism of action of SSRIs (and other antidepressants) is drug discrimination (DD). The DD-paradigm is a powerful and specific procedure to determine the distinctive physiological or interoceptive alterations (state, cue) induced by the psychoactive drug under study compared to its vehicle (which is assumed to have no associated cue). The animal must attend to, or discriminate, its own drug-induced altered state to receive an award. In the DD procedure subjects are trained to perform one response (e.g. pressing the left lever) in the presence of a drug, and another response (pressing the right lever) in an operant chamber (Skinner box). Once trained (e.g. performing above 90% right choice on subsequent training days with random drug or vehicle sessions), animals can be subjected to generalization or antagonism tests. In generalization tests it is investigated whether another drug induces a similar cue (substitutes) as the training drug, in antagonism studies the training drug is combined with a putative antagonist. The DD-procedure is thus an 'in vivo' mechanism of action paradigm extremely helpful in elucidating important characteristics of drugs.

SSRIs appear notoriously difficult to induce stable discriminative stimuli (DS), certainly in comparison to many 5-HT receptor agonists like e.g. 5-HT_{1A} receptor (Ybema et al., 1990, 1993, 1994a, b; Gommans et al., 1995) and 5-HT_2 receptor agonists (Gommans et al., 1998b). Citalopram generated a robust and specific discriminative stimulus in rats (Dekeyne and Millan, 2003; Marona-Lewicka and Nichols, 1998). A low dose of citalopram generated a much faster acquisition of the DS than a high dose, which may reflect that citalopram is acting much more specific on the serotonergic system than at higher doses where noradrenergic effects may blur the DS. Other SSRIs, including sertraline (Marona-Lewicka and Nichols, 1998), fluvoxamine (Gommans et al., 1998a; Olivier et al., 1993) and LY233708 (Wolff and Leander, 1999) also generated discriminative control (in rats and pigeons, resp.). We (Olivier et al., 1993) found the discrimination of fluvoxamine from vehicle extremely difficult; it took approximately 100 sessions to establish adequate discrimination at a dose of 15 mg/kg i.p. However, at higher doses the DS got lost because most animals stopped responding. The group of Millan at Servier (Dekeyne and Millan, 2003) did extensive substitution studies in citalopram trained rats and found that other SSRIs (paroxetine, sertraline) substituted for citalopram. The complex cue induced by citalopram could be substituted by the 5-HT_{2C} receptor agonist RO60,0175 and antagonized by the selective 5-HT_{2C} receptor antagonist SB242,084 (Millan et al., 1999) but not by the selective 5-HT_{1A} receptor antagonist WAY100,635 (Dekeyne and Millan, 2003) in contrast to studies using LY233708 in pigeons (Wolff and Leander, 1999) and fluoxetine in rats (using a conditioned taste aversion procedure Berendsen and Broekkamp (1994)) that showed involvement of the 5-HT_{1A} receptor in the DS. Selective antagonists of 5-HT_{2A} and 5-HT_{2B} receptors were ineffective against the citalopram cue (Millan et al., 1999).

Acute administration of an SSRI apparently generates a complex cue in animals and probably also in man, presumably activating a number of serotonergic receptors that in a completely not understood manner create a discriminative stimulus. Although SSRIs are generally believed to represent one class of therapeutic drugs, the different individual SSRI molecules probably also generate a 'characteristic' DS which may vary more or less from other SSRIs. Complex issues in the DS of SSRIs are e.g. pharmacokinetic aspects like active metabolites (fluoxetine, citalopram) that might generate a differential profile than the mother molecule alone. Substitution experiments where SSRIs are tested in animals trained on a specific cue (e.g. a 5-HT_{1A} receptor agonist) might help to untangle the subtle differences between the various SSRIs. Fluvoxamine, e.g., generalized for about 40% to flesinoxan-trained rats and 30% to flesinoxan-trained pigeons (unpublished data), whereas fluvoxamine did not generalize to metachlorophenylpiperazine (mCPP), a 5-HT_{2C} receptor agonist (unpublished data). It is unclear however whether the discriminative stimulus properties of SSRIs are related directly to their antidepressant quality; the latter only occurs after chronic administration whereas discriminatory stimuli are acutely induced phenomena.

2.2. Preclinical chronic tests

The preclinical tests were mainly designed to detect acute (i.e. after one or a limited number of injections in a short time) efficacy, whereas in clinical therapy antidepressants only start to work after weeks, if not months of treatment. SSRIs selectively block 5-HT transporters and increase extracellular concentrations of 5-HT at all pre-and postsynaptic 5-HT receptors (Sanchez and Hyttel, 1999). Chronically, SSRIs continue to increase extracellular levels of 5-HT (Blier and El Mansari, 2013) which in some not yet understood way leads to a diversity of secondary changes probably needed in the antidepressant action, including desensitization of 5-HT autoreceptors and serotonin transporters (Piñeyro and Blier, 1999), downregulation of neurotransmitter receptors, changes in signal transduction (Price and Drevets, 2012), mobilization of neurotrophic factors (Pittinger and Duman, 2008; Duman and Li, 2012) and enhanced hippocampal neurogenesis (Sahay and Hen, 2007).

Several animal depression models reflecting antidepressant activity after chronic treatment have been developed including chronic mild stress, olfactory bulbectomy, novelty-suppression of feeding, chronic social defeat and learned helplessness.

In the present context, I only focus on the olfactory bulbectomy (OBX) paradigm to detect putative antidepressant activity of new and existing drugs. Bilateral removal of the olfactory bulbs in rodents induces a complex behavioral, neurological and physiological phenotype (Song and Leonard, 2005). One of the most marked behavioral changes after OBX is enhanced activity in an

open field (Kelly et al., 1997; Song and Leonard, 2005). This OBX-induced hyperactivity is very robust, highly stable and reproducible. In our lab, we have since more than 10 years regularly performed OBX studies and have always found hyperactivity. Besides hyperactivity several other behavioral disturbances occur after OBX including among many cognitive impairments (Borre et al., 2012a,b). Moreover, OBX results in disturbances in monoaminergic and GABA neurotransmission (Jancsar and Leonard, 1984; Prins et al., 2010; van der Stelt et al., 2005). This whole constellation of changes has led to the use of the OBX paradigm as model for human depression (Song and Leonard, 2005). In particular hyperactivity after OBX has been used as a readout parameter to judge the effects of psychoactive drugs. Hyperactivity emerges relatively fast after OBX and is significantly enhanced in a home-cage situation approx. 3 days post-bulbectomy particularly during the dark period (Vinkers et al., 2009a). In general drug experiments are performed on animals after at least two weeks after bulbectomy when the hyperactivity is stable. This stability in hyperactivity is probably lifelong present: 8 months after OBX animals still display hyperactivity (Van der Stelt et al., 2005).

One of the most attractive characteristics of the OBX paradigm is that antidepressants of all classes normalize hyperactivity but only after (sub) chronic treatment, whereas they have no effect acutely (Song and Leonard, 2005; Breuer et al., 2007). This enables the use of the OBX as a potential model for studying onset-of-action of antidepressant activity, one of the main issues in the search for better antidepressants. SSRIs, like other antidepressants (Song and Leonard, 2005) are effective in reducing hyperactivity but these effects do not start before 5 days of treatment (Opal et al., 2014; Saitoh et al., 2007) in rats that have undergone bilateral OBX at least two weeks earlier. We have extensive experience with imipramine in the OBX paradigm as we have used it over the last decade as our positive antidepressant reference compound (Breuer et al., 2007). Imipramine (10 or 20 mg/kg, i.p. or p.o.) always inhibited hyperactivity after 7 and 14 days pretreatment (Breuer et al., 2007, 2008, 2009a,b). In an unpublished study we investigated whether 3, 7 and 14 days of imipramine (10 mg/kg i.p.) reduced OBX-induced hyperactivity and found that all three treatments led to inhibitory activity. In a previous study (Breuer et al., 2007) we found that chronic (14-days) treatment with either imipramine (20 mg/kg i.p.) or escitalopram (5 or 10 mg/kg i.p.) led to long-term behavioral changes after cessation of treatment. In order to check whether the animals had normalized their behavior we checked if after one-week washout (which is more than sufficient to remove all drugs and metabolites from the body) animals had returned to their initial phenotype (i.e. hyperactivity for the OBX animals). We observed that the hyperactivity remained absent, depending on the pretreatment dose, for 6 (escitalopram) or 10 weeks (imipramine). In the previously mentioned unpublished study we also measured up to six weeks after cessation of treatment and found that the inhibitory effects on hyperactivity after 3 days of pretreatment with imipramine (10 mg/kg i.p.), was lost one week after cessation of treatment. In the 7-days, but particularly in the 14-days pretreatment group the hyperactivity in the pretreated OBX-group slowly returned to the original levels. Confirming the earlier profile (Breuer et al., 2007) we hypothetized that chronic, but not acute treatment with antidepressants led to plastic changes in the brain of OBX-rats. These changes appear quite resilient in that they, dependent on the dose of the antidepressant and presumably also the total time of treatment, are present a long time after the drugs have left the body. This makes the OBX model attractive as it not only can measure the onset of action of an antidepressant, but also the optimal dose and treatment duration for long-term therapeutic effects after cessation of treatment. We postulate that a drug does not have a true 'antidepressant effect' in the OBX model unless it not only normalizes hyperactivity during treatment, but also keeps this effect for some time after cessation of treatment. An illustration of this theory is shown in the case of pramipexole, a dopamine D_3/D_2 receptor agonist used in the treatment of Parkinson symptoms. Pramipexole reduces depressive symptoms in Parkinson's patients suffering from depression (Lemke et al., 2005; 2006). A low dose of pramipexole (0.3 mg/kg i.p.) reduced the OBX-induced hyperactivity after 7 and 14 days of pretreatment. The high dose tested (1 mg/kg i.p.) had no effect on hyperactivity or even increased it. Remarkably, one week after cessation of treatment the high pramipexole group showed an antidepressant-like profile, in that the OBX-induced hyperactivity was not present. This indicated that, notwithstanding the psychomotor stimulation of pramipexole during treatment that precluded the antidepressant profile, the underlying plasticity changes leading to an 'antidepressant-like' behavioral profile still had occurred (Breuer et al., 2009b).

This constellation of pharmacological findings indicating that antidepressants induce neuroplastic changes in the brain of olfactory bulbectomized animals makes this a very attractive model to use in further investigations of processes underlying mood disorders. Olfactory bulbectomy leads to a sequella of neuronal processes involved in neuronal degeneration, which is followed by neuronal regeneration (Kelly et al., 1997). The olfactory bulbs projects to and receives input from a large number of different brain areas and removal of the bulbs results in a complex cascade of retrograde degeneration of neurons innervating the bulbs and anterograde degeneration of neurons leaving the bulbs innervating many brain areas (Song and Leonard, 2005). Although many areas are involved, in particular the limbic system seems most affected and many changes induced by OBX have been related to limbic disturbances (Song and Leonard, 2005). What changes in the brain of OBX animals lead to a 'depression-like' phenotype are largely unknown, neither do we know what changes due to antidepressant treatment constitute recovery. Besides the overwhelming data on neurochemical and behavioral changes in the OBX animal (Song and Leonard, 2005), several physiological, immunological (Leonard, 2014; Song and Leonard, 2005) and autonomic parameters are also affected whereas recently also disturbances in gut functions were found in OBX mice (Park et al., 2013) likely due to activation of the HPA-axis. In light of autonomic dysfunction in depression (Carney et al., 2005), an important aspect of OBX animals appears an inability to adapt to stress, which might be due to amygdaloid disinhibition (Kelly et al., 1997; Wrynn et al., 2000). Vinkers et al. (2009a) found indeed stable and persistent changes in nocturnal locomotor activity, body temperature, heart rate and heart rate variability. Circadian rhythmicity in these parameters was not affected, but mainly amplitudes of the responses were changed; moreover, stress responsivity in OBX rats appear to depend on the stress intensity. All these changes are also observed in human depressed patients underlining the quality of the OBX model as an animal model of depression.

2.3. Novel developments

There is a serious drought in the finding and development of new antidepressants (holds also for the rest of drugs for CNS diseases) over the last decades. Recent new antidepressants that have reached the market acting via serotonergic mechanisms (at least partly) are agomelatine (de Bodinat et al. 2010), vilazodone (Dawson, 2013) and vortioxetine (Gibb and Deeks, 2014). Agomelatine is a melatonin-MT_1 and MT_2 receptor agonist and 5-HT_{2C} receptor antagonist with antidepressant and anxiolytic effects. According to the Cochrane collaboration (Gupta et al., 2013) agomelatine does not provide advantage over other

antidepressants, although it might have a somewhat better side effect profile. Vilazodone is a combined SSRI plus partial 5-HT_{1A} receptor agonist that was admitted to the US market in 2011 (Dawson, 2013). Vilazodone is clearly antidepressant and seems to have some advantages over existing SSRIs (onset of action; sexual side effects) although much more research is needed to prove this (Dawson, 2013). Vortioxetine has been approved for major depressive disorder by the FDA in 2013. Vortioxetine is a selective SSRI combined with agonistic activity at 5-HT_{1A} and 5-HT_{1B} receptors whereas it exerts antagonistic properties at 5-HT_{1D}, 5-HT_3 and 5-HT_7 receptors (Gibb and Deeks, 2014). Although it is clearly too early to judge the real qualities of vortioxetine, the expectations that this drug might contribute some promising extra properties compared to plain SSRIs are high, particularly on side effects or higher efficacy. Because these three new drugs all act in some way or the other via the serotonergic system, one might argue that this does not really constitute a breakthrough in the treatment of MDD. On the other hand small improvements in our arsenal of antidepressant therapeutics are very welcome. Recently, triple monoaminergic reuptake inhibitors (TRIs) have been suggested as a welcome addition to the arsenal of existing uptake inhibitors (Millan, 2009; Prins et al., 2011; Shao et al., 2014). The additional activity of dopamine reuptake inhibition in combination with 5-HT/NA reuptake inhibition might be worthwhile in major depression, particularly in melancholic depression. Based on a balanced monoaminergic regulation in a healthy brain, it can be suggested that in situations where this balance is disturbed certain kind of depressions (melancholic and atypical) prevail; Prins et al. (2011) suggest that TRIs might be particularly effective in these latter kind of depression as, depending on the magnitude and the direction of the disturbed balance, they may be able to restore the underlying monoaminergic up- or down regulations. Different TRIs are described and in development (see review in Prins et al. (2011); Caldarone et al. (2010) and Shao et al. (2014_) but none has yet reached the market.

3. Serotonin and aggression

My story on aggression started at Groningen University, where I did an undergraduate study in neurobiology on the role of the ventromedial hypothalamus in social behavior in male mice. Electrolytic lesions of the median hypothalamus led to complex behavioral changes in feeding, aggression and sexual behavior (Olivier and Wiepkema, 1974). Subsequently I continued my graduate studies on the role of the hypothalamus in social and aggressive behavior in the rat receiving my PhD in 1977 (Olivier 1977a,b, Olivier et al., 1983). In 1977 I moved to Philips-Duphar (later taken over by Solvay Pharmaceuticals) where research into the area of aggression had started in the previous years. Drugs used clinically at those times (and still used nowadays) were not at all specific for aggression but induced sedation, motor disturbances or other unwanted side effects (e.g. neuroleptics, barbiturates). Our rationale for new drugs in this area was that there was a huge, unmet need for psychotropic drugs that specifically inhibited aggression without interfering with other important modalities. This concept was based on the assumption that specific neural systems are present in the brain of mammals that can be manipulated in such a way that aggression is lowered without interfering with other important neural and behavioral systems (Olivier et al., 1986; Olivier and Mos, 1986). At that time it was completely unknown whether this concept would realistically lead to therapeutically active drugs and certainly not that we would end up in the serotonergic system.

At that time an in vivo behavioral screening was performed using the isolation-induced aggression test in male mice as primary screen (Olivier and van Dalen, 1982). Over the years thousands of drugs were synthetized and tested on their anti-aggressive qualities in this test. Initially we randomly searched for hits with an effective dose inhibiting 50% (ED_{50}) of lower than 100 mg/kg p.o., but after finding some hits in new, patentable chemical structures, compounds were detected with ED_{50} $<$ 50 mg/kg p.o. Soon, driven by extensive structure-activity based chemical synthesis some very interesting anti-aggressive properties (ED_{50} $<$ 2 mg/kg p.o.) were found in a series of chemical compounds of the phenylpiperazine class (Olivier et al., 1986). Because only specific anti-aggressive effects of drugs were of interest, a number of more natural animal aggression paradigms were developed enabling to measure how specific the anti-aggressive activity was. In these ethologically derived models (territorial aggression, maternal aggression, defensive behavior, brain-stimulation induced behaviors) the whole behavioral repertoire was scored (frequencies, durations, sequence of behavior: Olivier et al., 1990a,b,c). These kind of analyses provided detailed insight how drugs influence behavior. The early anti-aggressive drugs (DU27725 (Olivier, 1981), DU28412 (Bradford et al., 1984), DU27716 (fluprazine; Olivier and van Dalen, 1982) affected offensive aggression in a very typical way. Only the consummatory parts of aggression (the last elements in the sequence) were decreased, but social and explorative activities (introductory elements in the sequence) were not affected or even enhanced (Olivier et al., 1986; Olivier and Mos, 1986). This profile of anti-aggressive action has been labeled as 'serenic' and was not present in any other drug class tested (antipsychotics, benzodiazepines, alcohol, psychostimulants, antidepressants, anticholinergics, antihistaminergic, etc.). Unfortunately, these early serenics appeared teratogenic and further synthesis and testing of new serenics was needed to exclude this serious side effect. This led to the discovery and development of eltoprazine (DU28853), a highly potent and selective anti-aggressive drug (Olivier et al., 1986, 1990a,b,c, 1994). From basic neurochemical and pharmacological studies it already had become clear that serenics had serotonergic agonistic properties (Bradford et al., 1984) and along with the discovery of all the 14 serotonergic receptors in the 1980s and 1990s, it became clear that serenics were partial 5-$HT_{1A/1B}$ receptor agonists (Schipper et al., 1990; Olivier and Mos, 1990; Olivier et al., 1989, 1994). Extensive pharmacological studies strongly suggested that in particular activation of 5-HT_{1B} receptors underlie the specific antiaggressive effects of serenics (Olivier and van Oorschot, 2005). These 5-HT_{1B} receptors are located as presynaptic autoreceptors in the raphé nuclei and as postsynaptic heteroreceptors in many different brain areas. Lesioning the raphé nuclei with the serotonergic neurotoxin 5,7- dihydroxytryptamine (5,7-DHT) which destroys the presynaptic autoreceptors but leaves the postsynaptic heteroreceptors (relatively) unaffected, had an aggression-reducing effect on itself, but in these animals eltoprazine still had an anti-aggressive effect, strongly suggesting that the serenic activity is due to postsynaptic mediated activation of 5-HT_{1B} heteroreceptors (Sijbesma et al., 1991). Similarly, eltoprazine injection in the ventricles, aiming for postsynaptic 5-HT_{1B} receptors had an anti-aggressive effect, whereas direct raphé injections, aimed at presynaptic 5-HT_{1B} autoreceptors were ineffective (Mos et al., 1992, 1993).

3.1. Serotonin and aggression: an inhibitory relationship?

A big dogma in the relationship between serotonin and aggression is still that 5-HT inhibits aggression (Yanowich and Coccaro, 2011). Studies in which serotonin activity in the brain was decreased by para-chlorophenylalanine (pCPA), irreversibly inhibiting tryptophan hydroxylase (TPH2) or the neurotoxic 5,7-DHT, both depleting serotonin from serotonergic neurons, show that

low serotonergic activity leads to enhanced aggression as already suggested in the 1970s (Brown et al., 1979). Such an inverse relationship between 5-HT activity and aggression has been found in animals and humans, although in humans 5-HT activity measurements were based on lumbar cerebrospinal fluid (CSF) levels of 5-hydoxyindoleacetic acid (5-HIAA), the main metabolite of serotonin. For many years the latter was the only measure in humans reflecting (indirectly) the functional status of the 5-HT system. As a consequence much of the evidence relating low serotonin to aggression was correlational, without causal evidence. More recently, acute tryptophan depletion studies indicate that low serotonin can lower mood and increase aggression, whereas tryptophan supplementation may decrease aggression (Duke et al., 2013; Young, 2013). In animals, 5-HT and 5-HIAA can be measured directly in the brain and an inverse relationship between functional serotonergic activity and aggression could be investigated more directly. However, several contradictory results have been found and even reports of a positive relationship between 5-HT and aggression occur (review Duke et al. (2013)). In humans aggression is associated with suicidal behavior and both seem to be associated with low serotonergic function, although it is possible that both phenomena are independently regulated (see for an extensive discussion on the neurobiology of suicidal behavior Mann (2003)). Measurement of 5-HT and 5-HIAA in postmortem brain tissue and determining a turnover rate from these two parameters, the latter was found to be lower in aggressive than in non-aggressive mice (Giacalone et al., 1968; Miczek et al., 1989). Such a serotonergic hypofunction was also found in humans using CSF samples (Brown et al., 1979; Kruesi et al., 1990; Linnoila et al., 1983). However, this 5-HT hypofunction or deficiency trait has been associated with impulsivity and risk-taking behavior rather than aggression per sé (Mann, 2003). A causal relation between 5-HT activity and aggression or impulsivity cannot be derived from static measurements of 5-HT or 5-HIAA measurements in brain tissue or CSF-fluid. A functional role of serotonergic neurons in the initiation, execution and stopping of aggression (Coccaro, 1989; Miczek and Fish, 2002) still has to be established although some progress has been made using in vivo microdialysis techniques in freely moving (aggressive) animals. This technique however, still lacks sufficient resolution because sample time (minutes) is still on a different time scale than actual behavior (seconds). Van Erp and Miczek (2000) measured extracellular serotonin (and dopamine) release in 10 min samples in the nucleus accumbens and prefrontal cortex in rats, before, during and after a 10 min aggressive interaction with a male conspecific. During agonistic interactions no change in 5-HT release was found in the nucleus accumbens, but 5-HT levels were decreased during fighting in the prefrontal cortex. After the confrontation 5-HT levels in the prefrontal cortex remained lowered (compared to pre-confrontation baseline) for at least 1 h, whereas 5-HT in the nucleus accumbens was unaffected. Dopamine levels were enhanced in both brain areas after (but not during) the agonistic confrontation. However, 5-HT levels were decreased in the nucleus accumbens of rats that have been conditioned to fight at a specific time each day over a 10-day period (Ferrari et al., 2003). In the latter experiments heart rate and dopamine release were concurrently measured and both were raised in anticipation of the fight. Apparently, the actual performance of aggression can be dissociated from the anticipation of a fight, where dopamine plays an important role in the physiological and behavioral sequels around the performance and anticipation of aggression, whereas serotonin seems to be particularly related to termination of aggression. Measuring electrophysiological events happening in the serotonergic neurons during the performance of aggressive behavior would be very helpful in unraveling the precise role of the serotonergic system but this seems technically not yet feasible. Moreover, the serotonergic system is not made up of one homogeneous mass of cells but is electrophysiologically (Beck et al., 2004), anatomically and functionally differentiated. The dorsal and median raphé nuclei partly project to different areas of the forebrain and partly to the same areas (Kusjlic et al., 2003) and are the most prominent sources of serotonergic neurons innervating areas involved in the initiation, performance and termination of aggressive behavior. Interestingly, no systematic studies are performed thus far trying to delineate the role of the different serotonergic cell groups in various aspects of aggression, although local lesions or local application of drugs in the dorsal or median raphé nuclei have been performed. It is highly unlikely that all serotonergic cell groups are involved and selective blockade or activation of individual cell groups in determining its role in aggression would be very fruitful. A recent approach to unraveling the role of the 5-HT system in aggression is studying the differences between highly aggressive and low-aggressive individuals as has been pursued by the group of Koolhaas (de Boer et al., 2003). They argued, based on the assumption that the individual level of aggression of a rat (offensive aggression) is part of an individual coping strategy of the animal and thus an important indicator of a trait-like behavioral and physiological response pattern. In their extensive studies on the endophenotypes of high-aggressive and non-aggressive rodents, serotonergic activity was also studied. In contrast to the existing theory of an inverse relationship between 5-HT activity and aggression, a positive correlation was found between the level of trait-like aggression (high or low) and basal CSF concentrations of 5-HT and 5-HIAA (Van der Vegt et al., 2003). Moreover, levels of 5- HT and 5-HIAA after in vivo microdialysis in the frontal cortex did not differ between endophenotypes. Apparently, normal offensive aggression is positively related to serotonergic neuronal activity, whereas an inverse relationship probably exists between 5-HT activity and impulse-like violent aggression (Coccaro, 1989). Thus a general pattern emerges where trait and state aggression are probably differentially regulated by the 5-HT system (and also other systems) although much more research is needed to substantiate this hypothesis. Recently, an interesting pharmacogenetic technique in mice was used to study the question whether decreased serotonin activity is sufficient to increase aggression and whether decreased 5-HT activity during development has influence on adult aggression (Audero et al., 2013). Conditional overexpression of the serotonin 1A receptor (Htr1a) in serotonergic neurons, associated with chronic 5-HT neuron firing, was associated with enhanced aggression. Overexpression of Htr1a in adulthood, but not during development induced enhanced aggression. In mice that only expressed Htr1a in serotonergic neurons (no postsynaptic receptors) administration of 8-OH-DPAT rapidly decreased serotonin neuron firing and enhanced aggression. Audero et al. (2013) conclude that serotonin plays a role of serotonin in setting thresholds for aggression. Moreover, they conclude strong support for a direct association between low levels of 5-HT homeostasis and enhanced aggression.

The foregoing discussion ignores more or less the existence of 14 different 5-HT receptors, each with its own function and localization in the brain. But the neurotransmitter serotonin can only exert anti-aggressive activity by stimulating or inhibiting one (or more) of these receptors. As already outlined in the description of the development of the serenics, the postsynaptically localized 5-HT_{1B} heteroreceptor seems an important player in the anti-aggressive effects of serotonin. Manipulation of the activity of a certain pool of serotonergic neurons (e.g. in the dorsal raphé nucleus), leading to changed 5-HT release in all the areas where these neurons project to, may affect postsynaptic 5-HT_{1B} receptors thereby influencing the level of aggression. The same manipulation has similar effects on other 5-HT receptors thereby leading to differential effects on many other functions and behaviors.

This is probably the cause of the nonspecific effects of many manipulations used in the studies of serotonergic influence on physiology and behavior. For instance, treatment of animals or humans with SSRIs, drugs that acutely but also chronically lead to enhanced serotonergic neurotransmission and consequently stimulation of all 5-HT receptors, have some anti-aggressive activity but have simultaneously pleiotropic central and peripheral effects (e.g. cognition, nausea, dizziness, fear and anxiety, sleep) that may heavily interfere with the intentional therapeutic application (aggression in this case). Genetic knockout of the SERT in mice leads to an extreme number of changes on all levels in the body and CNS (biochemical, behavioral, anatomical, physiological and pharmacological) including decreased aggression (Murphy and Lesch, 2008). Similarly, genetic disruption of tryptophan hydroxylase-2, the key enzyme in the synthesis of central serotonin, catalyzing the hydroxylation of l-tryptophan to 5-hydroxytryptophan (5-HTP), the precursor of 5-hydroxytryptamine (5-HT; serotonine) also leads to a plethora of effects including hyper aggressiveness (Lesch and Waider, 2012). In humans, polymorphisms in and downstream of the transcriptional control region of the TPH2 gene are also associated with various personal traits including antisocial, borderline and narcissistic personality disorders, comparable to the phenotypic changes in mice (Gutknecht et al., 2007).

All this evidence points to the hypothesis that changes in the serotonergic tone has, amongst a plethora of other effects, also an effect on aggression. Based on our pharmacological studies with a variety of serotonergic ligands, lesions and local brain administration (Olivier et al., 1995; Olivier and van Oorschot, 2005), postsynaptic 5-HT_{1B} receptors seem a very strong candidate in fulfilling an important role in the modulation of aggressive mechanisms. The important role of 5-HT_{1B} receptors has also been found in many studies of the Miczek group (review Takahashi et al. (2011)). It is also clear that 5-HT_{1A} receptors play a role in aggression. The last decade most researchers in this field make a distinction between normal functional species-specific aggression and (abnormal) escalated aggression and violence (e.g. Miczek et al., 2007a) that clearly exceeds the species-normative boundaries and can be considered as pathological (e.g. de Boer and Koolhaas, 2005). There is evidence that experiencing repeated victorious experience (leading to escalated or violent aggression) is associated with lowered tonic CNS 5-HT levels (de Boer et al., 2003; van Erp and Miczek and Fish, 2002; Ferrari et al., 2003) whereas in normal functional aggression the basal level is positively related to basal CSF 5-HT concentrations (Van der Vegt et al., 2003). This might indicate differential control by serotonin activity in adaptive vs. maladaptive aggression. In rats showing this maladaptive or violent aggression, 5-HT_{1B} receptor agonists have a specific aggression-reducing effect whereas certain (full) 5-HT_{1A} receptor agonists (repinotan, 8-OH-DPAT) caused non-specific decreases in aggression (at similar doses also increased inactivity), whereas some other (partial) 5-HT_{1A} receptor agonists (alnespirone, S-15535, buspirone, ipsapirone) specifically decreased aggression. de Boer and Koolhaas (2005) postulate that alnespirone and S-15535 exert full agonistic activity at somatodendritic 5-HT_{1A} autoreceptors and at the same time are full/partial agonist (alnespirone) or antagonist (S-15535) at postsynaptic 5-HT_{1A} receptors (although not clear where this pool of receptors is localized). In our group we have never found specific anti-aggressive effects of 5-HT_{1A} receptor agonists, being full (8-OH-DPAT, flesinoxan) or partial (buspirone, ipsapirone). We tested 5-HT_{1A} receptor agonists also in a rat model of extreme offensive aggression, electrical brain stimulation-induced aggression, where rats are stimulated in the hypothalamic attack area (Kruk, 1991). During stimulation, rats attack without provocation any opponent, be a bigger or dominant male, a female, or even a dead conspecific. Under stimulation, rats skip introductory and threat behaviors, which make their attacks unpredictable for opponents. Moreover, stimulated rats do not attack inanimate objects (e.g. rat models), and show no behavioral changes in the absence of attackable opponents; thus, their behavior cannot be considered as an automatic reflex. In addition to aggression, hypothalamic stimulation in this hypothalamic attack area also induced locomotory behavior and teeth chattering (autonomic response associated with aggression) when an opponent is not present (Kruk et al., 1984a,b; Van der Poel et al., 1982). Drug effects can be measured by changes in the current thresholds required to evoke the respective behaviors (Van der Poel et al., 1982). Eltoprazine (partial 5-$HT_{1A/1B}$ receptor agonist) and 5-HT_{1B} receptor agonists enhance the threshold current for aggression (anti-aggressive effect) and slightly for teeth chattering, whereas the threshold for locomotion was even decreased. 8-OH-DPAT had no influence on thresholds for aggression and teeth chattering similar to buspirone (Olivier et al., 1994). The absence of any anti-aggressive activity of 5-HT_{1A} receptor agonists in this model representing a pathological form of aggression makes the respective roles of 5-HT_{1A} and 5-HT_{1B} receptors in aggression and violence rather complicated. Genetic knockout of (all) 5-HT_{1B} receptors in mice (Saudou et al., 1994) makes male mice more aggressive and impulsive (Bouwknecht et al., 2001) suggesting that under normal conditions activation of 5-HT_{1B} receptors inhibits aggression in line with the pharmacological findings. In contrast, 5-HT_{1A} receptor knockout mice are less aggressive and more fearful (Zhuang et al., 1999) suggesting that under normal conditions 5-HT_{1A} receptors might have a stimulatory role in aggression which contrasts the pharmacological findings somewhat. In humans, functional polymorphisms in either the 5-HT_{1B} or 5-HT_{1A} gene or corresponding promoter areas are known (Arango et al., 2003; Benko et al., 2010; Sari, 2004; Zouk et al., 2007). Although many groups failed to find associations between suicide and the 5-HT_{1B} receptor gene (see Zouk et al. (2007)), some polymorphisms (notably the G(861)C polymorphism) have been found associated with suicide attempts (see Zouk et al. (2007)). Zouk et al. (2007) found that the A-161T locus of the HTR1B gene leading to reduced transcriptional activity and reduced serotonergic turnover, may increase suicide susceptibility possibly via an association with impulsive aggressive behaviors. Although these kinds of association studies do not reveal where and what kind of 5-HT_{1B} receptors contribute it indicates the putative involvement of 5-HT_{1B} receptors in aggressive behaviors. Witte et al. (2009) found higher aggression scores in healthy human subjects showing higher density or affinity of inhibitory postsynaptic 5-HT_{1A} receptors in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) using positron emission tomography (PET) and the radioligand [^{11}C]WAY-100635. This might suggest (but remind that this is measured in healthy subjects) that activation of these postsynaptic 5-HT_{1A} receptors may lead to enhanced aggression due to increased inhibition or hypofunction of prefrontal areas. A further interesting finding was that higher aggression scores were associated with lower sex hormone-binding globuline (SHBG) levels in the plasma, whereas higher SHBG plasma levels were associated with lower postsynaptic 5-HT_{1A} receptor affinity or density in the PFC, the ACC, posterior cingulate cortex (PCC) and amygdala. In women (compared to males low testosterone levels), but not in males, a strong positive correlation was found between testosterone levels and frontal areas. Although contrasting findings about the role of postsynaptic 5-HT_{1A} receptors in the PFC with regard to aggression have been reported (Parsey et al., 2002; Rabiner et al., 2002), recent findings that direct injection of 5-HT_{1A} receptor agonists into the PFC of mice and rats (Centenaro et al., 2008; Stein et al., 2013) seem in line with the findings of Witte et al. (2009). Also, mice selected for short latency attacks have higher 5-HT_{1A} receptor expression and sensitivity than the long latency animals (Korte et al., 1996; Van der Vegt et al., 2001; van Riel et al., 2002; Veenema et al., 2005), while rats selected for extreme defensive reactions had lowered 5-HT_{1A} receptor

expression in various brain areas (Popova et al., 1998). Possibly, polymorphisms directly or indirectly affecting 5-HT_{1A} receptor function may be associated with either aggressive or defensive responses.

The big questions about the involvement of the serotonergic system in aggression are however still out. Does the serotonergic system play a role under normal conditions when functional activity in aggressive neural systems is needed or does it only play a role under extreme, pathological conditions? These questions are important for the possibility to develop adequate drugs to treat violence (serenics). One of the problems of the pharmacological studies performed thus far is a lack of chronic treatments. Practically all studies are aimed at acutely influencing the mechanisms modulating aggression. As it is a well-known phenomenon, chronic stimulating or blocking receptors may have sincere influence on the latter´s functional capacity (e.g. up or down regulation). In our animal research into the development of eltoprazine we showed that (sub) chronic treatment in rats and mice (Mos et al., 1996; Olivier et al., 1990a) did not lead to lowered anti-aggressive activity. However, human studies in severely aggressive patients (Mak et al., 1995) found some indication of decreased anti-aggressive activity after 8 weeks of eltoprazine treatment. There is quite some evidence that chronic treatment with presynaptic acting agonists for 5-HT_{1A} and 5-HT_{1B} receptors might lead to rapid desensitization with all the consequences for the activity of the serotonergic system (Albert and François, 2010).

4. Serotonin and anxiety/stress

In the serenics program performed in the 1970s and early 1980s, a number of compounds synthesized exerted blood pressure lowering effects (Bevan et al., 1986; Wouters et al., 1988). The serenic line appeared devoid of this activity, but directed synthesis of compounds to optimize the blood pressure lowering effects led to the discovery and development of flesinoxan (DU29373). Upon testing in humans the drug was completely devoid of antihypertensive activity and consequently this line of research was abolished. In the meantime it had become clear that flesinoxan exerted agonistic activity at 5-HT_{1A} receptors. Moreover, there was emerging evidence that 5-HT_{1A} receptor agonists might be effective anxiolytics, at least in animals (Barrett et al., 1989; Olivier et al., 1998; Taylor, 1988). An extensive body of research around the 5-HT_{1A} receptor and anxiety/stress has developed over the last 25 years but has not led to successful development of selective 5-HT_{1A} receptor ligands for therapeutic anxiolytic (or antidepressivant) use. Apart from the partial 5-HT_{1A} receptor antagonist/dopamine D_2 receptor antagonist buspirone, which is the only nonselective 5-HT_{1A} anxiolytic in the market, no other selective 5-HT_{1A} receptor agonists have reached it. Although in double-blind placebo-controlled phase 2 studies flesinoxan showed superiority over placebo (similar to the positive control alprazolam), when subsequently tested in two big phase 3 studies in generalized anxiety, flesinoxan did not differ from the placebo groups, upon which the further development was halted. Only tandospirone was registered but only in Japan for generalized anxiety disorder.

Notwithstanding the lack of effective anxiolytics acting via 5-HT_{1A} receptor modulation, considerable evidence has accumulated showing the involvement of 5-HT_{1A} receptors in anxiety (and depression) both in preclinical and clinical studies (Akimova et al., 2009; Altieri et al., 2013; Savitz et al., 2009; Garcia-Garcia et al., 2014). As already mentioned, 5-HT_{1A} receptors represent as two main populations in the CNS, autoreceptors and heteroreceptors that function by coupling to G_i/G_0 proteins controlling different intracellular signaling cascades like inhibition of cAMP formation, inactivation of calcium channels and activation of potassium channels (Barnes and Sharp, 1999). There is considerable evidence that 5-HT_{1A} autoreceptors in the raphé nuclei are different from postsynaptic 5-HT_{1A} heteroreceptors; autoreceptors preferentially use Gαi3 G-protein subunit coupling in signaling, heteroreceptors primarily mediate Gα0 subunit coupling (Garcia-Garcia et al., 2014). Because of their anatomical localization in areas involved in anxiety and their differential ability to influence postsynaptic processes (e.g. via GABAergic interneurons and pyramidal cortical neurons (Artigas et al., 2006), 5-HT_{1A} receptors when activated may play an important role in modulating essential emotional processes. Moreover, overall evidence suggest that 5-HT_{1A} receptor functions, both of pre- and postsynaptic populations, are altered in anxiety disordered and depressed patients compared to healthy controls. This may indicate that 5-HT_{1A} receptor functioning reflects some vulnerability factor for emotional psychopathology (cf. Garcia-Garcia et al., 2014; Neumeister et al., 2004).

Animal models of anxiety or stress are abundantly available (see review Haller et al. (2013)). In our lab we developed a simple stress model in mice and rats, the Stress-induced Hyperthermia (SIH), based on the original studies of Borsini et al. (1989) (Van der Heyden et al., 1997; Vinkers et al., 2008; Zethof et al., 1994, 1995). The procedure we developed is based on repeated temperature measurements in one subject (Groenink et al., 2009; Vinkers et al., 2009b). Temperature of animals was rectally measured twice with a 10 min interval. The first rectal procedure evokes a stress response that can easily be seen in enhanced core body temperature, heart rate and corticosterone levels (Groenink et al., 1996, 1997; Bouwknecht et al., 2007). In order to test drugs in the SIH paradigm, an injection-test interval of 60 min is needed because the injection procedure on itself induced a temperature rise of approximately 45 min. whereas most drugs need some time to establish sufficient brain levels of the active constituent of the injected drug (PKPD factors). Sixty (T_1) and 70 min (T_2) after the injection two rectal temperature measurements are taken and $T_2-T_1=\Delta T=$ SIH. The intensity of SIH (amplitude of the rise in temperature) depends on the intensity of the stressor; in rats entering the experimental room leads to a smaller and a shorter lasting SIH than putting an animal in a novel cage (Vinkers et al., 2008). In a series of experiments manipulating the intensity of the stressor (handling, injection, novel cage, novelty, intruder) the intensity was measured as the area under the curve (AUC) of the hyperthermic response (time × height) and showed a nice intensity dependent increase (Van Bogaert et al., 2006a,b). The SIH paradigm appeared broadly employable for screening of many classes of psychotropic drugs (Zethof et al., 1995; Vinkers et al., 2010a,b). Vinkers et al. (2010b) give an extensive overview of serotonergic ligands on SIH in mice and rats. 5-HT_{1A} receptor agonists (including flesinoxan, 8-OH-DPAT, ipsapirone, flibanserin and buspirone) display an axniolytic profile in the SIH, showing a decrease in ΔT without a parallel decrease in basal body temperature (T_2). 5-HT_{1A} receptor agonists have an intrinsic hypothermic effect that could also be shown in the SIH paradigm. It is suggested that this hypothermic effect is induced by activation of presynaptic autoreceptors (Cowen, 2000). This anxiolytic effect but also the hypothermic effect on T_1, could be antagonized by the silent 5-HT_{1A} receptor antagonist WAY100,635, thereby confirming that the effect was mediated via 5-HT_{1A} receptors (Olivier et al., 1998). Based on the hypothesis that 5-HT_{1A} receptor-induced hypothermia is mediated presynaptically, one could suggest that the anxiolytic effects are mediated via activation of postsynaptic receptors, although different pools of the two groups (pre vs. post) might also be a realistic possibility. In 5-HT_{1A} receptor knockout mice of three different strains, buspirone had no effect on either basal temperature (T_1) or ΔT (SIH), while it had clear

effects on both parameters in the corresponding wildtypes (Van Bogaert et al., 2006a). The hypothermic effects of 5-HT_{1A} receptor agonists in mice are comparable to those in humans (Pitchot et al., 2002, 2004) and are probably caused by serotonergic receptors in the medullary rostral raphe pallidus, effectuating cutaneous vasodilatation and reduced thermogenesis in the brown adipose tissue (Dimicco and Zaretsky, 2007; Ootsuka and Blessing, 2006), the generator of the heat needed to produce the temperature increase of SIH in such a short time. Chronic treatment with SSRIs attenuates 5-HT_{1A} receptor agonist-induced hypothermia in healthy subjects (Lerer et al., 1999; Sargent et al., 1997) but also in patients with anxiety disorders and depression (Lesch, 1991; Navinés et al., 2007). This indicates, as already stipulated earlier, that downregulated (desensitized) 5-HT_{1A} autoreceptors are involved in the effects of chronic SSRI administration. In this line, a selective postsynaptically acting 5-HT_{1A} receptor agonist would be extremely helpful in delineating the mechanism of action of the anxiolytic activity of non-selective (pre-and post) acting 5-HT_{1A} receptor agonist. F15599, a highly selective 5-HT_{1A} receptor agonist seems to exert a preferential action for postsynaptic 5-HT_{1A} receptors (Newman-Tancredi et al., 2009) present both under vitro and vivo conditions (Assié et al., 2010). This supports the hypothesis that antidepressant activity of 5-HT_{1A} receptor agonists is mediated by postsynaptic receptors. Whether the difficulty to develop new 5-HT_{1A} receptor agonists for anxiety or depression might have to do with the use of non-selective agonists (working both pre-and postsynaptically), partial agonists or combinations, is not clear. It would be worthwhile to study the effects of F15599 in psychiatric patients to improve our understanding of the contributions of the differential parts of the serotonergic systems.

There is quite some evidence that an individual's sensitivity to stressful events is influenced by its genetic makeup (Hettema et al., 2005). The genetic methodologies used to detect putative genes involved in anxiety disorders have not yielded convincing candidates (Olivier et al., 2013) and is illustrative of the extremely complex underlying mechanisms of anxiety disorders (this holds true for most psychiatric diseases). The picture becomes even more blurred by recent evidence that brain somatic mutations in individual patients may contribute to a psychiatric endophenotype (Insel, 2014). Somatic mutations in progenitor cells during the development might lead to small but sometimes devastating abberations in parts of the brain. Such mutations will not be detected by the present technologies using e.g. blood DNA screening. Somatic mutations require brain tissue for analysis, which constitutes a big problem in living patients. Animal models of somatic brain mutations have to be developed to better understand the (im)possibilities of this new way of considering putative causes of brain diseases.

5. Serotonin and sexual behavior

During my PhD studies I examined the behavioral role of the medial hypothalamus in social, including sexual behavior in the rat (Olivier, 1977a,b). Before and after specific hypothalamic lesions (anterior or posterior lesions in the medial aspects of the hypothalamus) among other behaviors the sexual behavior of male rats was studied. If bilateral lesions were positioned in the preoptic area/anterior hypothalamus sexual behavior was severely disrupted whereas posterior hypothalamic lesions (including mammilary bodies) did not interfere with sexual behavior. These studies founded my interest in the behavioral, neurochemical and pharmacological aspects of male sexual behavior, and for the next 35 years has been an important aspect of my research. In the pharmaceutical industry, influence of psychoactive drugs on sexual behavior was for a long time not a big topic but became more important when prominent sexual side effects were reported upon the introduction of SSRIs into the depression market. Although in the first decade of SSRI use hardly any sexual complaint was noted, in the following decades sexual side effects appeared an important complaint often leading to stopping of treatment. All SSRIs seem to be inhibitory on male and female sexual functions although good statistics are lacking, probably due to the fact that major depression itself already depresses sexual functions and behavior.

In our research we tried to develop animal models of sexual behavior in order to investigate the possible causes and mechanisms behind these SSRI-induced sexual dysfunctions. Starting with outbred male Wistar rats we first studied the stability of sexual behavior over time because for determining pharmacological effects intra-animal data is very useful and enables chronic studies. We found in large cohorts of male Wistar rats that individual rats display a stable sexual phenotype that already stabilizes after a number of sexual training tests (Chan et al., 2008, 2010; Pattij et al., 2005). In 30 min tests with a hormonally-induced estrus female that fully deploys proceptive and receptive behaviors under these conditions, male rats show from 0 till 5 ejaculations per test. In more than 2000 male rats tested thus far, we find an inverted U-shaped distribution with relatively few animals with low or high numbers of ejaculations and high number of animals with 2–3 ejaculations/test. We postulated that animals with low ejaculations might model human delayed or retarded sexual function, whereas the high ejaculators might model premature ejaculation in men (Pattij et al., 2005). The intermediate (normal) ejaculators (2–3 ejaculations) were used to study the effects of antidepressants (Chan et al., 2010) because these animals are both sensitive to drugs that either stimulate or inhibit sexual behavior. Our standard way of testing is that we train a large cohort of rats (around 100 animals) for 4 successive weekly tests of 30 min upon which each individual rat has developed a stable sexual phenotype. Animals with 2–3 ejaculations/test are randomized over groups of approximately 12–15 animals per treatment group and these animals are then during 14 days treated with a drug. Depending on the halflife time of the drug under study once or twice daily drug treatment (oral or i.p.) is applied and sexual behavior is observed and scored on day 1 of treatment, i.e. after acute administration, after 7 days (subchronic) and after 14 days of treatment (chronic dosing). Seven days after stopping treatment (Day 21) the sexual behavior of the rats is again recorded to judge the putative residual effects of the 14 days drug treatment. SSRIs (paroxetine, escitalopram, fluvoxamine) inhibit the sexual behavior of male rats but only after subchronic and chronic dosing, and not after an acute challenge. One week after stopping treatment (washout) no residual effects of SSRIs are present. This inhibitory profile of SSRIs matches the human profile where sexual complaints gradually emerge during treatment, do not dissipate during treatment and disappear after stopping treatment. Our initial research into effects of psychoactive drugs on male sexual behavior in the rat applied acute treatment, e.g. after 30–60 min pretreatment time (Mos et al., 1999). We tested a series of SSRIs (clomipramine, fluoxetine, fluoxetine (all in a range of 0, 3, 10 and 30 mg/kg p.o.) and sertraline and paroxetine (0, 1, 3 and 10 mg/kg p.o.)) on sexually experienced and selected rats. Tests run for maximally 25 min or stopped immediately after the first postejaculatory behavior (mount or intromission), whichever came first. Although some changes were found at some doses of some SSRIs (sertraline and fluoxetine), they were never dose-dependent and did not systematically show a certain pattern. Our main conclusion was that after acute treatment with SSRIs, no inhibitory effects were present. In our later experiments in chronic studies (cf. Olivier et al., 2011) this pattern was reproduced. In

these studies inhibitory effects of (all) SSRIs were always found after (sub)chronic dosing and not after acute dosing although incidentally some acute inhibitory action was noted (Bijlsma et al., 2014). Because we hypothesized that animals with less stable sexual behavior were more sensitive to inhibitory effects of drugs, we studied SSRIs after acute administration in sexually naïve rats. Again, SSRIs had no inhibitory actions on sexual behavior. Finally, testing the effects of SSRIs on sexually exhausted experienced male rats, again hypothesizing that those rats are more vulnerable to inhibitory drugs, again no effects of SSRIs could be discerned (Mos et al., 1999). We found this rather remarkable because acute SSRI treatment has effects on serotonergic neurotransmission, notably enhancement of synaptic serotonin measured in various parts of the brain (Piñeyro and Blier, 1999). There is however dispute about the acute effects of SSRIs on serotonin release because the synaptic release of 5-HT that theoretically should be enhanced after acute blockade of the transporter is opposed to activation of 5-HT_{1A} autoreceptors in the raphé nuclei which inhibit the neuronal firing needed to release synaptic 5-HT (Bel and Artigas, 1992; Blier et al., 1987; Cremers et al., 2000). It is conceivable that acute administration of SSRIs does not lead to sufficient enhanced levels of 5-HT at the presumed site of action. Addition of a 5-HT_{1A} receptor antagonist might eradicate the autoreceptor activation by 5-HT and thus lead to strongly enhanced synaptic 5-HT release and consequently to inhibition of sexual behavior. This appears indeed the case (Olivier et al., 2011). Concomitant administration of an SSRI (paroxetine at 10 mg/kg) that alone did not inhibit sexual behavior, with behaviorally inactive doses of WAY100,635 (0.01, 0.03 or 0.3 mg/kg) led to strong (at the highest dose even 100%) inhibition of sexual behavior. Acute SSRI+5-HT_{1A} receptor administration might be considered an acute analog of chronic SSRI administration, where it is assumed that 5-HT_{1A} autoreceptors are desensitized, leading to less inhibition on the SSRI-induced synaptic release (Blier et al., 1987) and also leading to inhibition of sexual behavior.

In humans, there is considerable evidence that polymorphisms in the SERT gene, notably in the promoter region influence the biological activity of the SERT and the two length alleles (S and L) have consequences for the serotonergic system functionality (Murphy and Lesch, 2008). Rats do not have such promoter length polymorphisms but genetic knockout of the SERT gene possibly generates rat models of the S-allele versions of the human SERT. We compared $SERT^{-/-}$ and $SERT^{+/-}$ rats with the corresponding wild-type ($SERT^{+/+}$) in male sexual behavior (Chan et al., 2011). In analogy with chronic-SSRI treated males we expected a lower sexual behavior of the knockout rats compared to wild-types (WT). After extensive training leading to very stable levels of sexual behavior, the homozygote SERT-KO had significantly lower sexual behavior than WT, whereas the heterozygotes did not differ from WT (Olivier et al., 2011). This indicates that the 50% remaining activity of the SERT system (disrupting one allele with the remaining allele producing approximately 50% SERT activity (Murphy et al., 2004a,b) appears sufficient to keep the male sexual behavior intact under the experimental conditions used. It can also be concluded that the SERT is important for male sexual behavior in the rat. Apparently, the lifelong absence of the SERT, leading to an aberrant serotonergic system (Homberg et al., 2007; Kalueff et al., 2010), does not completely wipe out sexual behavior but drastically reduces it by approximately 50%. Whether this is due to developmental disturbances or disturbances in the adult serotonergic homeostatic regulation, is not clear. Genetic knockout or rescue experiments at different stages in the development of an animal would be necessary to unravel this kind of questions.

The lower sexual behavior of SERTKO rats mimics the effects of chronic SSRI treatment in men and rats. Chronic SSRI treatment induces adaptations in 5-HT_{1A} receptors, particularly in somatodendritic autoreceptors (decreased binding: Gray et al., 2013; desensitization: Bouaziz et al., 2014). 5-HT_{1A} receptor agonists stimulate sexual behavior in male rats (Haensel et al., 1991; Mos et al. 1991; Snoeren et al., 2014). 8-OH-DPAT, a potent and full 5-HT_{1A} receptor agonist, was able to stimulate sexual behavior in all three SERT genotypes and in a similar way, indicating that 5-HT_{1A} receptors mediating this effect have not changed in sensitivity (Chan et al., 2011). The 5-HT_{1A} receptor antagonist WAY100,635 had no sexual behavioral effects in WT and heterozygous SERTKO rats. However, WAY100,635 dose-dependently inhibited sexual behavior in the homozygous SERTKO suggesting that a different pool of 5-HT_{1A} receptors is involved in this action and that these receptors appear sensitized in the SERTKO animals (Chan et al., 2011). Remarkably, heterozygous SERTKO rats with approximately 50% SERTs left were not sensitized at all. It has been suggested (Kugaya et al., 2003) that SERTs need to be occupied for at least 80% before any antidepressant activity can be found, indicating that a 50% remaining SERT function in the $SERT^{+/-}$ rat does not seriously affect the serotonergic system and does therefore not interfere with male sexual functioning. These studies suggest that complete absence of SERT alters 5-HT_{1A} receptor functioning. The sensitivity of certain receptor pools mediating the prosexual effects are not affected by the genetic null mutation, whereas another population, mediating the inhibitory effects of 5-HT_{1A} receptor antagonists, seems sensitized. The finding of two differentially regulated pools of 5-HT_{1A} receptors in SERT-KO animals was also found in autonomic regulation of body temperature and stress (Olivier et al., 2008). Although the decrease in male sexual behavior in chronically SSRI-treated rats (with $>80\%$ SERT occupancy) and SERTKO rats might be mediated via the same mechanism is likely, but it is as yet unresolved. Whether this mechanism is mediated by 5-HT_{1A} receptors is unclear. The studies using null mutation rats has at least unraveled that different 5-HT_{1A} receptor pools contribute differentially to the performance of sexual behavior. Whether under normal conditions 5-HT_{1A} receptors are of vital importance for adequate sexual behavior needs further studies.

The sexual inhibitory effects of SSRIs are often considered serious side effects (e.g. when treating depression or anxiety disorders), but also may be used for therapeutic properties, e.g. in premature ejaculation (PE). Premature ejaculation is defined as a lifelong intravaginal ejaculation latency time (IELT) lasting less than one minute (Waldinger et al., 2005; Waldinger, 2014; McMahon et al., 2008). SSRIs are the drugs of choice for treatment of PE; in particular paroxetine exerts efficient elongation of the IELT whereas fluvoxamine was remarkably inactive (Waldinger et al., 1994, 1998b). SSRIs have a somewhat delayed onset of action in PE, although faster compared to the onset of the antidepressant action (Waldinger et al., 1998b). This suggests that SSRI effects on underlying mechanisms in sex and mood might be mediated by different mechanisms. Although the classic SSRIs are not used for 'on demand' treatment of PE (Waldinger et al., 2004; Waldinger, 2007), such an option would be very attractive. This has led to the development of dapoxetine (McMahon, 2012), an SSRI that initially was investigated as an antidepressant but upon failure was repurposed for PE. Dapoxetine is a SERT inhibitor of the aryloxypropanamine type where also fluoxetine and paroxetine belong too (Olivier et al., 2000). Dapoxetine is, like the other SSRIs selective for the SERT (pKi=8.0 nM) but has a slightly lower affinity than fluoxetine (pKi=8.5) and paroxetine (pKi=8.7). Although not proven, investigators try to explain the rapid onset of action of dapoxetine, compared to other SSRIs, to its pharmacokinetic profile pointing at the rapid absorption and elimination of the drug compared to classic SSRIs (Andersson et al., 2006; Giuliano and Clément, 2012) having a differential effect on the level of synaptic 5-HT after acute administration although there is no

convincing evidence for proof. This would imply that dapoxetine would not lead to 5-HT_{1A} autoreceptor activation like the other SSRIs do (Artigas, 2013a, b). There is no evidence for this theory and although dapoxetine has been allowed as an on demand therapeutic in some countries, it does not seem a very effective drug (cf. Mondaini et al., 2013). In our hands (Bijlsma et al., 2014; Olivier et al., 2011) in our rat model of sexual behavior (Pattij et al., 2005) SSRIs do not have acute inhibitory effects on ejaculation.

We studied dapoxetine (11 mg/kg p.o. two hours before testing) along our standard SSRI paroxetine (10 mg/kg) and did not observe any inhibitory effects after acute administration on male sexual behavior in phenotypical 'normal' rats (sexually experienced with stable levels of approx. 2 ejaculation/test of 30 min: unpublished data) similar to paroxetine. Gengo et al. (2006) report a similar finding in phenotypically non-selected rats (data only available in abstract cited in Clément et al., 2012), but in a study using 'rapid ejaculating rats (Clément et al., 2012) a high dose of dapoxetine (300 mg/kg p.o.; 3 h before testing) reduced sexual behavior after acute administration. Remarkably, only the total number of ejaculations in a 30 min test decreased and the ejaculation latency in the first ejaculation series was lengthened. No other effects were observed. The results are difficult to interpret, certainly because of the low number of animals used ($N=8$) and the high dapoxetine dose. A dose-response study is certainly needed to further substantiate these findings. For the time being my hypothesis is that dapoxetine is not different from other SSRIs in their property to inhibit the ejaculation latency either in man or in rat.

There is some evidence that premature ejaculation is influenced by genetic factors (Jern et al., 2007; Waldinger et al., 1998a). In the latter study there was a strong suggestion for familial occurrence of PE, although the number of contributing males was extremely low. In the twin studies of Jern et al. (2007) some genetic factor could be discerned although a large environmental/developmental influence was clearly present.

The 5-HTT is a specific protein transporter— localized in the cell membrane—that facilitates serotonin reuptake from the synapse, and is the target of SSRIs that are known to delay ejaculation (Qian et al., 1995). Having a high affinity for serotonin, 5-HTT controls the duration, availability, and signaling capacity of 5-HT in the synapse. If short IELTs in men with lifelong PE—but also in men without lifelong PE—are associated with lowered central 5-HT neurotransmission, an increased function of the 5-HTT might be related to the occurrence of PE. Such an increased function may depend on genetic polymorphisms in the 5-HTT that have been found in the SERT gene (Heils et al., 1996). 5-HTT functioning is moderated by a polymorphism in the 5-HTT promoter region of the SERT gene (SCL6A4), which encodes for the SERT (5-hydroxytryptamine transporter-linked promoter region [5-HTTLPR]) (Murphy et al., 2004a,b; Kunugi et al., 1997; Smith et al., 2004; Smits et al., 2004). The 5-HTTLPR gene has two variant alleles: a short (S) and a long (L) allele. The short allele has 44 base pairs (bps) less than the L allele (Heils et al., 1996). The transcriptional activity of the L allele has been reported to be twice as high as the S allele (Lesch, 2004). The genotypes composed by these alleles are called LL, SS, and SL. If expressed in cell lines, the short (S) allele of the 5-HTT genotype reduces transcriptional efficiency of the 5-HTT gene promoter, resulting in reduced 5-HTT expression and serotonin uptake compared with the long (L) allele (Lesch et al., 1996). Notably, the S allele has been associated with a nearly 50% reduction in expression of the SERT protein, vulnerability for mood disorders, inadequate response to SSRIs, and side effects (Murphy et al., 2004a,b; Hariri and Brown, 2006). In Caucasians, the genotype frequencies are approximately 25% SS, 47% SL, and 28% LL (Smits et al., 2004). Theoretically, men with one or more S alleles for the 5-HTT have fewer functioning transporters and could therefore lead to a higher serotonergic neurotransmission. Consequently, it is postulated that men with SS genotype have longer IELT durations than men with LL genotype. This was confirmed (Janssen et al., 2009). The LL genotypes had a significantly shorter IELT than both SS and SL genotypes. This is a remarkable finding because in a population with already extreme short ejaculation latencies, a functional polymorphism (LL) was associated with a further reduction of the IELT with 50%. Although no data are known in the normal male population with regard to the effects of these polymorphisms on ejaculation latencies, one might speculate on a comparable pattern. There is no evidence however, that polymorphisms in the SERT gene form the genetic basis for lifelong PE (Janssen et al., 2009; Zuccarello et al., 2012). It also seems clear that the ejaculation process on itself is not critically dependent on the serotonergic system; it is expected that many neurotransmitter systems are involved and that polymorphisms in these systems might also contribute to the ejaculation process. The contribution of the serotonergic system has also been investigated with regard to polymorphisms in the genes for the 5-HT_{1A} (Janssen et al., 2014a) and 5-HT_{2C} receptors (Janssen et al., 2014b). In men with lifelong PE, the C(-1019)G polymorphism in the 5-HT_{1A} receptor gene is associated with the IELT. Men with CC genotypes have shorter IELTs than men with either CG or GG genotypes (Janssen et al., 2014a). Another polymorphism in the 5-HT_{2C} receptor gene (Cys23Ser) is also associated with the IELT duration in lifelong PE men. Cys/Cys genotype men have shorter IELTs than men with Ser/Ser genotypes (Janssen et al., 2014b). It is remarkable that in a population of already extremely fast ejaculating men, polymorphisms in three serotonergic genes (SERT, 5-HT_{1A} and 5-HT_{2C} receptors) all have influence on the ejaculation speed. The SERT polymorphism (5HTTLPR) basically determines the amount of serotonin available for action at 5-HT receptors. The 5-HT_{1A} and 5-HT_{2C} receptors seem to be involved in the process of ejaculation, at least under conditions of ejaculation within 1 min. In how far 'normal' ejaculating men (Waldinger et al., 2009) show a comparable pattern is unknown but is certainly worth investigating.

6. Future

It has become evident over the last decades that the serotonergic system in the CNS is extremely important in practically all areas where subtle modulation is needed in processes involved in regulation of amongst others, mood, anxiety, appetite regulation, aggression, sexual behavior and cognition. It is astonishing that one neurotransmitter is able to play such a role. The distribution of the serotonergic innervation in the CNS, the 14 different receptors, the localizations and the intrinsic regulation of each individual serotonergic neuron are rudimentary known, which makes speculation about function rather precarious. The discovery of the serotonin transporter and its function in the 1960s and the subsequent synthesis and development of the SSRIs was a giant leap into the psychiatric treatment field that has not yet been rivaled by new findings let alone by new drugs. The availability of SSRIs has enabled scientists and clinicians to explore the role of the SERT and serotonin neurotransmission in psychiatric diseases and has shown the broad array of applications of SSRIs in e.g. anxiety, depression, bipolar disorder, premature ejaculation and aggression. SSRIs have also been widely used as tools to study the functional activity of the serotonergic system and as tool in animal models of psychiatric diseases. The discovery of the 14 different 5-HT receptors in the last decades of the 20th century, has led to intense research into the function of each individual receptor and the synthesis of compounds ((inverse) agonists, antagonists) to modulate these receptors. Notwithstanding intense preclinical and clinical efforts, results are meager. Selective agonists for any 5-HT receptor have largely failed to reach the market for any psychiatric disease. Only buspirone, a weak partial 5-HT_{1A} receptor agonist and dopamine D_2 receptor antagonist is in the market for anxiety disorders, but no other 5-HT_{1A} receptor agonist has made it. This might be caused by the complexity of the regulation of the disturbances in the neural pathways involved in mental

disorders. In contrast, effects of SSRIs are not limited to one receptor subtype but lead via enhanced serotonin release (certainly after chronic administration) to activation of all serotonin receptors (with associated inhibitory and excitatory effects) that might be needed to normalize disturbed neural networks. Remarkably, adding a noradrenergic and even a dopaminergic component to an SSRI (in SNRIs and TRIs, resp.) does not necessarily lead to a more efficacious antidepressant effect. This holds also for adding additional 5-HT receptor agonistic (1A, 1B/D) and antagonistic (3,7) properties like in vortioxetine; this drug seems to exert comparable antidepressant efficacy as SSRIs although the side effect profile might be better.

It is therefore doubtful whether a real improved antidepressant (higher efficacy, faster onset of action) can be realized by selectively maneuvering the serotonergic system. Adding some non-monoaminergic activity into a molecule with a serotonergic profile (e.g. an SSRI) like a fast onset induced by NMDA-antagonism (as in ketamine) might lead to new therapeutic avenues. The present research into the involvement of the glutaminergic system in depression (Krystal et al., 2013) might lead to some helpful principles that supports improved antidepressant activity.

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