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Sex, aggression and pair-bond : a study on the serotonergic regulation of female sexual function in the marmoset monkey

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SUMMARY

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Sexual dysfunctions in women are highly prevalent. Hypoactive sexual desire disorder (HSDD), as the most frequently diagnosed condition, is found in approximately 1 out of 10 women, causing distress and interpersonal difficulty that arise from unwanted, persistent or recurrent low sexual desire. Etiology and underlying neurobiological mechanisms of HSDD are not well understood, but neurotransmitter dysfunction has been proposed involving the excitatory regulators dopamine and norepinephrine, as well as inhibitory serotonin (5-HT). The objective of the research described in this thesis was to investigate the serotonergic regulation of female sexual behavior in a nonhuman primate model of female sexual function. Due to its pairmate social setting comparable to humans, the common marmoset monkey (*Callithrix jacchus*) was the animal model of choice. Marmosets form stable male-female relationships that are strengthened by partner-directed social interactions, including grooming behavior. Females can signal interest for sexual interactions (proceptivity) and control the occurrence of sexual intercourse by accepting (receptivity) or rejecting the pairmate's sexual advances.

A behavioral testing paradigm was developed to quantitatively assess the behavioral expression of proceptive and receptive sexual states of the female marmoset, as well as other parameters of pairmate interactions that give information about the pair-bond quality between the sexual partners. This paradigm was used to test the chronic effects of the serotonergically active substances flibanserin, a 5-HT_{1A} agonist and 5-HT_{2A} antagonist, and 8-OH-DPAT, a 5-HT_{1A} agonist. Flibanserin is currently being developed as a potential pharmacotherapeutic treatment of HSDD in women.

Chapter 1 introduced female sexuality and provided an overview of the classification of female sexual dysfunctions. The current knowledge of the neurobiology of female sexual function and dysfunction in both humans and animal models was presented. A concept was introduced that sexual behavior is centrally regulated by both excitatory and inhibitory factors. Such factors include steroid hormones, neuropeptides and neurotransmitters. On the excitatory side, estradiol, testosterone, oxytocin, melanocortin, dopamine and norepinephrine play an important role, while on the inhibitory side, opioids, cannabinoids and serotonin exert a suppressive function. The central serotonin neurotransmitter system was introduced in more detail, and the stimulatory or inhibitory function of different serotonin receptor subtypes was highlighted. The effects of flibanserin and 8-OH-DPAT on female sexual behavior in rodents (8-OH-DPAT) and in humans (flibanserin, 8-OH-DPAT) were described in this context. Chapter 1 also outlined how female sexuality can be modeled in animals, and why the common marmoset monkey is a

particularly suitable model species to explore female sexual behavior and to test the chronic effects of flibanserin and 8-OH-DPAT on behavior and its underlying neurobiological substrate.

The main objective of **Chapter 2** was to quantify the effects of chronic flibanserin and 8-OH-DPAT on sexual and social interactions between treated females and their untreated male pairmates. Repeated observations of the pairmates' interactions at reunion following a 90 minute separation revealed remarkable and contrasting differences between flibanserin and 8-OH-DPAT on marmoset pair behavior, despite the drugs' shared 5-HT_{1A} agonist properties. While pairmates with flibanserin-treated females were more frequently engaged in sexually-related and affiliative, pro-social interactions, the pair-bond between 8-OH-DPAT-treated females and their male partners was impaired as evidenced by increased female rejection of male sexual advances, a tendency for diminished female sexual attractiveness, reduced affiliative and increased agonistic interactions. We proposed that the drugs' effects on social interactions and pair-bond between the pairmates play an important role in determining female sexual behavior in the marmoset. Chapter 2 furthermore described the pharmacokinetic 24-hour profiles of circulating flibanserin and 8-OH-DPAT levels during chronic administration. The results showed that blood levels of flibanserin and 8-OH-DPAT were low or absent during the time of behavioral tests, as well as during endocrine, brain imaging and gene expression experiments described below.

In **Chapter 3**, the impact of chronic flibanserin and 8-OH-DPAT on HPA axis function was investigated to delineate the possibility of an HPA axis-mediated mechanism in the serotonergic regulation of female sexual behavior. Chronic flibanserin and 8-OH-DPAT neither altered circulating morning basal cortisol levels nor ACTH and cortisol responses to an acute 5-HT_{1A} agonist challenge. Activation of hypothalamic 5-HT_{1A} receptors stimulates the HPA axis, and a neuroendocrine 5-HT_{1A} challenge can thus serve as peripheral indicator of central 5-HT_{1A} receptor function. In response to 30 minute restraint stressor, the ACTH response was enhanced by flibanserin and 8-OH-DPAT, suggesting sensitized HPA axis reactivity to stress after chronic flibanserin and 8-OH-DPAT treatment. Cortisol was elevated after 8-OH-DPAT, but not after flibanserin treatment. Enhanced ACTH responses to restraint were correlated with increased aggression and reduced sexual receptivity in 8-OH-DPAT treated female marmosets, supporting the hypothesis that increased stress reactivity may have contributed to inhibition of sexual behavior. Such correlations were absent in flibanserin treated females, suggesting no inhibitory effect of HPA axis reactivity on female sexual function after flibanserin.

Chapter 4 described the results of a PET/MRI functional brain imaging experiment that was designed to measure cerebral glucose metabolism, an indicator of neural activity, in chronic 8-OH-DPAT or vehicle treated female marmosets during sexual and social interactions with their male pairmates. Radiolabeled [^{18}F]fluorodeoxyglucose (FDG) was infused immediately prior to a 30 minute pair test, and the females were subsequently imaged by PET under isoflurane anesthesia. Structural MRI scans were recorded and overlaid on the PET images to improve the visualization of anatomical structures. In predefined regions of interest that were chosen for their involvement in mediating female sexual behavior and their high 5-HT_{1A} density (mPFC, mPOA, VMH, CA1 and DRN), chronic 8-OH-DPAT was without effect on FDG uptake. Whole brain voxel-wise mapping, however, showed significantly reduced neural activity in a cluster located in the medial occipital cortex (mOCC), overlapping with a cluster derived from correlations of behavioral scores of female rejection behavior with FDG signals. This finding suggests that reduced neural activity in the mOCC may underlie the 8-OH-DPAT induced reduction in female sexual receptivity.

Chapter 5 described a large-scale gene expression experiment using the marmoset-specific EUMAMA microarray, as well as a candidate gene approach using real-time quantitative PCR that targeted mRNA expression of the 5-HT_{1A}, 5-HT_{2A}, 5-HT₇ and 5-HTT genes. The same brain regions of interest were targeted as those studied in the PET imaging experiment (Chapter 4), with exception of the VMH, which was not analyzed.

Functional annotation clustering of microarray data showed that chronic 8-OH-DPAT specifically altered genes associated with neurotransmission in the mPOA, while gene clusters associated to ion channel activity and learning and memory were affected in the CA1. Genes involved in intracellular signal transduction were affected in the DRN. Gene clusters involved in neural development were altered in the mPFC, mPOA and DRN, energy production was affected in the mPFC and mPOA, mitochondrial function in the CA1 and DRN, and protein transport in the mPOA and DRN. We thus proposed that transcriptomic changes related to neural plasticity, energy production and learning and memory processes in cortical, hippocampal and hypothalamic brain areas may have contributed to sexual impairment in response to chronic 8-OH-DPAT administration. The microarray analysis also revealed a greater than 10-fold increased expression of oxytocin (OXT) in the mPOA of 8-OH-DPAT treated females. This finding is of particular interest as the function of hypothalamic OXT has been closely associated with social and sexual behavior in rodents, nonhuman primates and humans. Serotonergic regulation of marmoset sexual and social behavior might thus be mediated by hypothalamic

OXT. However, increased hypothalamic oxytocin is normally attributed to pro-sexual and pro-social behavior, contrasting the finding reported in Chapter 5. Assessment of oxytocin receptor expression and signaling will be essential to shed more light into this apparent contradiction.

The candidate gene approach revealed that the serotonin transporter gene (5-HTT) was strongly upregulated in the DRN. The expression of 5-HT_{1A} autoreceptors in the DRN was not altered by chronic 8-OH-DPAT, but there was a trend to increased 5-HT_{1A} expression in the mPFC. Expression of 5-HT_{2A} was not affected by 8-OH-DPAT in any of the investigated brain regions, while there was a trend for decreased 5-HT₇ expression in the CA1 area of the hippocampus. We proposed that activation of 5-HT_{1A} autoreceptors in the DRN by 8-OH-DPAT likely suppressed serotonergic activity, triggering a compensatory upregulation of 5-HTT in the DRN and of 5-HT_{1A} in the mPFC to restore the serotonergic tone.

In **Chapter 6**, a synthesis of the experimental findings described in Chapters 2-5 was proposed. Contrasting effects of flibanserin and 8-OH-DPAT on female sexual and social behavior (Chapter 2) were discussed in context with enhanced HPA axis responsiveness to stress (Chapter 3), and brain region-specific alterations in glucose metabolism (Chapter 4) and gene transcription (Chapter 5). In light of the discipline-spanning data set presented in this thesis and the multifactorial etiology of female sexual dysfunctions (see Chapter 1), we proposed that flibanserin and 8-OH-DPAT regulate female sexual behavior through four separate, but interactive modules.

In a monoamine regulatory module (i), flibanserin and 8-OH-DPAT alter serotonergic, dopaminergic and noradrenergic neurotransmission, causing altered excitatory or inhibitory input on female sexual behavior. Through this module, flibanserin was proposed to exert both an excitatory and dis-inhibitory influence on female sexual behavior. An HPA axis module (ii) accounts for sexually inhibitory effects due to enhanced activation of the endocrine stress system. This module could play a role in increased sexual rejection behavior of chronic 8-OH-DPAT treated female marmosets. A pair-bond, experience and memory module (iii) was proposed as impactful mechanism through which flibanserin and 8-OH-DPAT may exert their pro-sexual and pro-social (flibanserin), or anti-sexual and anti-social (8-OH-DPAT) actions. Oxytocin might play a key role in linking this module to a regulatory module of female sexual and social behavior (iv), which integrates the inputs from modules (i)-(iii) and ultimately determines the expression of female sexual and social behavior.

In conclusion, the synthesis of experimental data within the proposed theoretical framework highlighted the importance of pair-bond quality on the

expression of female sexual behavior and called for further investigation of the role of oxytocin in the serotonergic regulation of female sexual function. The thesis was concluded by suggesting that flibanserin's therapeutic effect in women with HSDD may be rooted on improvements in sexual, social and emotional bonding between partners. In translating the marmoset findings to humans, the thesis also suggested that future HSDD trials should consider the inclusion of parameters that characterize the intimacy of a relationship and emphasize on the role of the partner as clinical end-points.



