Effects of acute systemic and intra-cerebral administration of serotonin$_{2A/C}$-receptor ligands in animal models of impulsive behavior

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Erklärung

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1 ZUSAMMENFASSUNG

Impulsivität oder mangelnde Hemmung von Verhaltenskontrolle haben wahrscheinlich einen evolutionären Vorteil, um Individuen zu erlauben, sich erfolgreich an Ungewissheit, Schwierigkeiten oder schnell verändernde Umgebungen anzupassen. In "geringem Maße" kann Impulsivität somit als Eigenschaft für normales Verhalten oder Persönlichkeit in unserem Alltag betrachtet werden, wobei das Maß an Impulsivität individuell unterschiedlich ist. Ein zu hohes Level an Impulsivität wird mit einer Vielzahl neuropsychiatrischer Erkrankungen in Verbindung gebracht, z.B. die antisoziale Persönlichkeitsstörung, die Borderline Persönlichkeitsstörung (BPS) oder das Aufmerksamkeitsdefizit-Hyperaktivitätssyndrom (ADHS) bei Kindern.

Studien zeigten, dass Impulsivität nicht als ein einheitliches Konstrukt anzusehen ist, sondern vielmehr aus eine Gruppe unterschiedlicher und komplexer Verhaltensweisen besteht, welche sich sowohl auf ihrer neuroanatomischen als auch auf ihrer neuropharmakologischen Ebenen unterscheiden. Die äußerst heterogene Natur von Impulsivität wird somit oft in mannigfaltiger Art und Weise definiert. Die Beschreibungen reichen von Unvermögen, unangemessene Handlungen zu unterdrücken, über Ablenkbarkeit, Aggressivität, bis hin zu Verminderung von Exekutivfunktionen und geringe Toleranz, Verzögerungen bei dem Erhalt von Belohnung zu tolerieren. Generell werden der Impulsivität zwei wesentliche Kategorien von Verhalten zugeordnet: I) Die verminderte Fähigkeit, motorische Aktivität oder Handeln zu hemmen, auch bezeichnet als „Impulsive action". II) Die verminderte Toleranz von Verzögerungen vor dem Erhalt von Belohnung. Ein solches Verhalten reflektiert impulsives Entscheiden („kaum gedacht, schon getan") und wird als „Delay aversion" bezeichnet.

Viele präklinische, von Human-neuropsychologischen Verhaltenstests adaptierte Tiermodelle tragen bedeutsam dazu bei, die neuronalen Regionen bzw. Netzwerke sowie die neuropharmakologischen Zusammenhänge, die der Symptomatik von impulsivem Verhalten zu Grunde liegen, zu verstehen. Einer der wohl am besten charakterisierten Verhaltenstests, um „Impulsive action" bei Nagern zu messen ist die 5-choice serial reaction time task (5-CSRTT). Hingegen erfolgt die Untersuchung von „Delay aversion" in einer Apparatur (T-maze oder Skinner Box), in welcher sich die Tiere zwischen einer geringen sofortigen Belohnung und einer großen allerdings verzögerten Belohnung entscheiden müssen.

Aufgrund der Verschiedenheit impulsiver Verhaltensweisen ist es unwahrscheinlich, dass lediglich eine einzelne funktionelle Störung des Gehirns das Phänomen Impulsivität erklären kann. Vornehmlich werden Strukturen in limbisch-cortico-striatalen Netzwerken wie der orbitofrontale Cortex (OFC), der Nucleus accumbens (NAc) oder der basolaterale Nucleus der Amygdala (BLA) als wichtige Orte zu Vermittlung von impulsivem Verhalten favorisiert. Obwohl die neurobiologischen Grundlagen nicht vollständig verstanden sind, weisen viele Studien darauf hin, dass das serontonerge (5-Hydoxytryptamin; 5-HT) System im Zusammenhang mit impulsivem Verhalten eine wichtige Rolle spielt, wobei die funktionelle Wirkung von 5-HT über eine Reihe unterschiedlicher Rezeptoren und deren Subtypen (5-HT_{1-7}) vermittelt wird.

Die vorherrschende neurochemische Theorie von Impulsivität deutet darauf hin, dass eine verminderte serotonerge Funktion im Gehirn von Säugern mit einer Verstärkung impulsiven Verhaltens assoziiert ist, wobei der potenzielle Einfluss anderer Neurotransmitter wie Dopamin (DA) ebenfalls berücksichtigt werden muss. Die vermutlich bedeutsame Rolle der aufsteigenden serotonergen Nervenbahnen in der Generierung bzw. Modulation von impulsivem Verhalten und die Tatsache, dass deren Dysfunktion wahrscheinlich für pathologische Impulsivität bei einigen klinischen Gegebenheiten verantwortlich ist, führte dazu, dass Auswirkungen von Manipulationen dieser Bahnen in verschiedenen Verhaltenstest untersucht wurden. Um nun die existierenden Befunde über die Beteiligung von Transmittern sowie neuronaler Netzwerke in der Impulsivitätsforschung zu erweitern, verfolgte die vorliegende Studie zwei Ziele:

1. Inwieweit sind 5-$HT_{2A/C}$-Rezeptoren an impulsivem Verhalten, gemessen als „Impulsive action“ in der 5-CSRTT, in einem möglichen OFC-BLA-Netzwerk beteiligt?
2. Sind dieselben Rezeptor-Subtypen generell daran beteiligt, impulsives Verhalten der Kategorie „Delay aversion“, gemessen im T-maze, auszulösen?

Im ersten Teil der Studie wurden drei Gruppen männlicher Wistar Ratten in der 5-CSRTT trainiert. In diesem Verhaltenstest mussten die Tiere lernen, auf kurze Lichtreize, die in einer von fünf Aperturen nach einer Verzögerung von 5s randomisiert präsentiert wurden, korrekt zu antworten. Die Verhaltensreaktion der Ratte bestand darin, die Nase in die jeweilige Apertur zu stecken, in welcher der Lichtstimulus zuvor präsentiert worden war. Diese Reaktion wurde durch das Durchbrechen einer in der Apertur befindliche Lichtschranke detektiert. Bei einer korrekten Antwort (richtige

räumliche Zuordnung von Licht und Apertur innerhalb einer bestimmten Zeit) wurde das Tier durch den Erhalt eines Futter-Pellets belohnt. Nach Erreichen einer stabilen „Baseline“ in der 5-CSRTT (>70% korrekte und <20% ausgelassene Antworten auf Stimuluspräsentationen) wurden den Tieren Mikroinfusionskanülen bilateral in die BLA und in den OFC implantiert, durch welche separat oder auch gleichzeitig der 5-$HT_{2A/C}$-Rezeptoragonist DOI (1-2,5-Dimethoxy-4-iodophenyl-2-aminopropan) (5µg/0,3µl) und der 5-HT_{2A}-Rezeptorantagonist Ketanserin (5µg/0,3µl) lokal appliziert werden konnten. Unmittelbar nach den jeweiligen Injektionen wurde das Verhalten der Tiere in der 5-CSRTT getestet. Die Ergebnisse zeigen, dass gleichzeitige Infusionen von DOI in den OFC und in die BLA impulsives Verhalten der Tiere signifikant verstärkte, wobei gleichzeitige Infusion des Antagonisten Ketanserin in beide Strukturen keine Auswirkungen auf das Verhalten der Tiere hatte. Weder DOI noch Ketanserin allein im OFC oder in der BLA vermochten impulsives Antwortverhalten zu beeinträchtigen. Die gleichzeitige Gabe der beiden 5-HT Liganden, DOI in den OFC und Ketanserin in die BLA und vice versa hatte ebenfalls keinen Effekt auf das Verhalten der Tiere.

Diese Ergebnisse deuten darauf hin, dass sowohl der OFC als auch die BLA bei der Vermittlung von Effekten des 5-$HT_{2A/C}$-Liganden DOI bei impulsivem Verhalten von Ratten in der 5-CSRTT eine wichtige Rolle spielen. Des Weiteren bekräftigen diese Daten, dass cortico-limbische 5-$HT_{2A/C}$-Rezeptoren bedeutsamer Bestandteil eines regulatorischen Netzwerkes zwischen OFC und BLA sind, welches die Impulskontrolle bei Säugern beeinflusst.

Im zweiten Teil der Studie wurden drei Tiergruppen in einem T-maze darauf trainiert, zwischen einer geringen sofortigen Belohnung und einer großen verzögerten Belohnung zu wählen (Kriterium: >70% Wahl der großen verzögerten Belohnung). In diesem auf Verzögerung basierenden Wahl-Test wurden daraufhin Effekte der 5-HT-Rezeptor-Liganden DOI und Ketanserin auf die Verzögerungs-Kapazität der Tiere untersucht. Systemische Applikation von DOI (0,1; 0,3 und 0,5mg/kg) verminderte dosisabhängig die Fähigkeit der Tiere, die Verzögerungszeit von 10s vor Erhalt der großen Belohnung zu tolerieren. Ketanserin hingegen hatte keine Auswirkungen auf die Arm- und somit Belohnungswahl. Die gleichzeitige Gabe von Ketanserin und DOI zeigte ebenfalls keine Auswirkungen, was darauf hindeutet, dass Ketanserin die erzielten Effekte von DOI unterbindet. Diese Ergebnisse zeigen, dass impulsives Verhalten im T-maze, gemessen als

die Toleranz, Verzögerungen vor Belohnungen zu tolerieren, wahrscheinlich über 5-HT_{2A}-Rezeptoren reguliert wird.

Zusammengefasst zeigt die vorliegende Studie, dass 5-$HT_{2A/C}$-Rezeptoren entscheidend an der Vermittlung von „Impulsive action“ als auch von „Delay aversion“ beteiligt sind. Die Tatsache, dass der funktionelle Status des meso-cortico-limbischen DA-Systems teilweise über das 5-HT-System vermittelt wird, zeigt, dass auch DA eine entscheidende Rolle bei impulsivem Verhalten spielt. Hinsichtlich der Beteiligung neuronaler Hirnregionen konnte gezeigt werden, dass das 5-HT-vermittelte Netzwerk zwischen dem OFC und der BLA u.a. für die Modulation von Impulskontrolle über die Aktivierung von 5-$HT_{2A/C}$ Rezeptoren verantwortlich ist. Die vorliegende Arbeit trägt dazu bei, die komplexen neuropharmakologischen Wechselbeziehungen sowie neuronalen Korrelate die bei impulsivem Verhalten von Nagern involviert sind, besser zu verstehen und erweitert die bereits existierende Kenntnisse hinsichtlich des Forschungsgebietes Impulsivität.

2 ABSTRACT

Impulsivity or diminished inhibitory control of behavior is an essential feature of neuropsychiatric diseases, but might have evolved naturally to allow individuals to adapt successfully to uncertainty, complexity and rapidly changing environments. Thus, impulsivity at "low levels" can be considered as a feature of normal behavior or personality to our everyday life, while normal individuals can be more or less impulsive. However, high levels of impulsivity are associated with a wide range of psychiatric disorders, such as Antisocial Personality Disorder (APD), Borderline Personality Disorder (BPD) or Attention Deficit Hyperactivity Disorder (ADHD). Admittedly, studies revealed that impulsivity is not a unitary construct, but a set of diverse and complex behaviors, which consists of several distinct behavioral phenomena, dissociable in their neuroanatomical as well as neuropharmacological levels. As a result of its heterogeneous nature the behavioral expressions of impulsivity range from inability to inhibit inappropriate responses, distractibility, aggressiveness, impairment of executive functions, and inability to postpone reward. Thus, impulsivity includes two major behavioral categories: I) behavior that results from diminished ability to inhibit actions, often referred to as impulsive action. II) behavior that reflects impulsive decision-making, for example intolerance to delay of gratification also known as delay aversion.

Various preclinical animal models, adapted from human neuropsychological tasks, greatly contribute to the understanding of neural correlates and the neuropharmacological basis underlying impulsivity in rodents. One of the most ubiquitous and well-characterised tasks designed for laboratory animals to assess impulsive action is the 5-choice serial reaction time task (5-CSRTT). However, one of the first used measurements of impulsive decision making in laboratory animals is the delay-to-gratification model of impulsive choice, where rats have to choose between a small and a high rewarded arm in a T-maze, whereas the high reward can only be obtained after a delay of 10-15s.

Because of the variety of impulsive behaviors, it is unlikely that a single abnormality in brain function can explain impulsivity. In particular, structures of the limbic cortico-striatal circuits such as the orbitofrontal cortex (OFC), nucleus accumbens (NAc) or basolateral nucleus of the amygdala (BLA) have been identified as important brain loci for mediating impulsive behavior.

Even though the neurobiological basis of impulsivity is not fully understood, several studies suggest an important role of the serotonin-(5-hydoxytryptamine; 5-HT) system in impulsive behavior, where actions of 5-HT are mediated via multiple receptor subtypes, classified into seven receptor families. The predominant neurochemical theory of impulsivity indicates that reduced serotonergic function is associated with an increase in impulsive behavior seen in humans, primates and rodents, although the potential importance of other neurotransmitters, like dopamine (DA) has to be considered as well. The assumed important role of ascending serotonergic pathways in "impulse control" and the fact that dysfunction of these pathways may be responsible for pathological impulsiveness in some clinical conditions, led to several attempts to examine effects of manipulated 5-HT-function in different testing methods for impulsive behavior. To extend the existing findings about transmitters and neural substrates involved in preclinical impulsivity research, the present study investigated the following hypotheses:

1) Does activation of 5-$HT_{2A/C}$-receptors mediate impulsivity via a possible OFC-BLA-network regarding the behavioral category impulsive action, measured in the 5-CSRTT?
2) Are these receptors generally involved in causing impulsivity in delay aversion, assessed using an impulsive decision-making task in a T-maze?

In the first experiment of the study three groups of male Wistar rats were trained on the 5-CSRTT. In this task rats were required to spatially discriminate brief light stimuli, presented pseudo-randomly in one of five holes after a fixed delay. After stable baseline performance, animals were equipped with bilateral guide cannulae and received infusions of the selective 5-HT_{2A}-receptor antagonist ketanserin (5µg/0.3µl), the 5-$HT_{2A/C}$-receptor agonist DOI [(±)-1-(2.5-Dimethoxy-4-iodophenyl)-2-aminopropan hydrochlorid] (5µg/0.3µl) separate and simultaneous in the OFC and the BLA immediately prior to testing. The results show that simultaneous bilateral infusion of DOI into the OFC and BLA significantly increased impulsive responding in the 5-CSRTT. In contrast, simultaneous bilateral infusions of ketanserin into the OFC and BLA only slightly increased impulsivity, and this effect did not reach level of significance. Likewise, neither DOI nor ketanserin affected impulsive responding when administered into either the OFC or the BLA. Also, simultaneous bilateral infusion of ketanserin into the OCF and DOI into the BLA and vice versa had no effect on impulsive behavior or performance in the 5-CSRTT. The results suggest that both the OFC and the BLA are implicated in mediation

of the effects of DOI on impulsive responding seen after 5-CSRTT performance. Furthermore, these data confirm that cortico-limbic 5-$HT_{2A/C}$-receptors are a major component of a regulatory network, comprising the OFC and BLA, necessary for impulse control and response selection in mammals.

Different groups of male Wistar rats were trained in a T-maze to choose between a small immediate and a large but 10s delayed reward. In this delay-based decision-making task, effects of the 5-$HT_{2A/C}$-receptor ligands DOI and ketanserin on waiting capacity were characterized. Systemic application of DOI (0.1, 0.3 and 0.5mg/kg) impaired waiting capacity in rats in a dose-dependent manner compared to vehicle, while ketanserin had no effect. When combined with ketanserin, DOI did not impair waiting capacity. These results suggest that the inability to tolerate delays of reward in a delayed reward task performed in the T-maze is probably regulated by 5-HT_{2A}-receptors.

Taken together, the present work revealed that 5-$HT_{2A/C}$-receptors are crucially involved in mediating impulsivity in both, impulsive action and impulsive decision-making or delay aversion. However, the fact that the functional status of the mesocortico-limbic DA-system is partially mediated by the 5-HT-system suggests that DA may play a regulatory role in impulsivity as well. Regarding to the involvement of neural substrates in impulsivity, it was shown that the OFC, the BLA, and presumably the network these structures form, are inter alia responsible for mediating impulsive behavior through activation and/or inhibition of 5-$HT_{2A/C}$-receptors. Overall, this work sheds more light on the neuropharmacological interactions as well as on the neural correlates involved in impulsive behavior in rodents, and extends the existing findings on this topic of research.

3 INTRODUCTION

3.1 Impulsivity: Psychiatric aspects

Impulsivity is a component of the initiation of behavior which can be both beneficial and destructive (SWANN ET AL. 2002). We all engage in impulsive acts every once a while. Such impulsive acting ranges from blurting out critical comments without thinking to buying expensive products on the spur of a moment (CHAMBERLAIN & SAHAKIAN 2007). The ability to act on impulse may allow us to grasp a valuable opportunity, or to make a disastrous decision we then regret afterwards. Thus, impulsivity at "low levels" can be considered as a feature of normal behavior or personality of our everyday life while normal individuals can be more or less impulsive (WINSTANLEY 2007; EVENDEN 1999A). On the other hand, high levels of impulsivity are associated with a wide range of psychiatric disorders. In the Diagnostic and Statistical Manual of Mental Disorders version IV (DSM-IV) several neuropsychiatric disorders are either classified as impulse control conditions or encompass impulsive symptoms in the diagnostic criteria (LEMKE & WENDORFF 2001; CHAMBERLAIN & SAHAKIAN 2007).

The first class of psychiatric diagnoses in which impulsivity is involved are the personality disorders. The Antisocial Personality Disorder (APD) and the Borderline Personality Disorder (BPD) are characterized by impulsivity related items like failure to plan ahead, irritability and aggressiveness, consistent irresponsibility, self-damaging, affective instability, intense episodic dysphoria, frequent displays of temper, or constant anger (MOELLER ET AL. 2001; EVENDEN 1999A). A diagnosis of personality disorders will be evident in adolescence or early adulthood in most cases. The fact that personality and its traits are not fully developed in pre-adolescence children makes it difficult to apply diagnoses to young kids. Indeed, there are specific childhood disorders related to and predictive of personality disorders, like attention deficit hyperactivity disorder (ADHD) (EVENDEN 1999A). The diagnostic criteria for ADHD include items explicitly related to impulsivity, like difficulty in sustaining attention or awaiting turn, interrupting or intruding other individuals. BARKLEY (1997) suggested that impaired behavioral inhibition, which impairs neuropsychological functions mediated by the prefrontal cortex, is the fundamental problem in ADHD.

The second class of psychiatric disorders involving impulsive behavior are the impulse control disorders. These include substance related disorders (drug abuse), pyromania, kleptomania or pathological gambling, whereas impulsivity can affect the occurrence, course, and treatment of this disorders (EVENDEN 1999A). Furthermore it has been linked to increased probability of suicide and aggression (DAY ET AL. 2007). Although this apparently occurrence of impulsivity and the examples of impulsive behavior in the DSM-IV diagnostic criteria for several disorders, impulsivity is not explicitly defined. This lack of specificity concerning the role of impulsive behavior in psychiatric disorders may result partial from disagreements in the literature about defining and measuring impulsivity. Furthermore, until recently, little work has been done to clarify the role of impulsivity in psychiatric illnesses (MOELLER ET AL. 2001).

3.2 Defining impulsivity

Since impulsivity is an essential component of normal behavior, it can be part of any motivated behavior. Furthermore, it can have multiple expressions, including neurophysiology, laboratory performance, and action (EVENDEN 1999B; SWANN ET AL. 2002). In order to analyze the mechanisms underlying impulsive behavior, researchers always tried to define impulsivity in operational terms for using it as basis for empirical investigations (WINSTANLEY 2007). To measure and identify different aspects of impulsive behavior in clinical psychology, self-report questionnaires have been developed. Using the Barrat Impulsiveness Scale (FOSSATI ET AL. 2007) or “The 17” (EYSENCK 1993), researchers and psychiatrists try to quantify and qualify valuable information about impulsivity in both normal and patient populations (WINSTANLEY ET AL. 2006). One problem though, using self-report measures is that they rely on the veracity of the individual accomplishment of the questionnaire (MOELLER ET AL. 2001). Secondly, these measures are not suitable for repeated use and therefore limited in there suitability for pharmacological or physiological treatment studies. Furthermore, they have no potential for use in laboratory animals, nor allowing comparative studies of the basis biochemistry of these behaviors (SWANN ET AL. 2002; MOELLER ET AL. 2001). In an effort to overcome the disadvantages of these introspective and self-reports, laboratory measures have been

developed to study impulsive behavior of both human and animal subjects (SWANN ET AL. 2002; EVENDEN 1999C).

3.3 Behavioral approaches and measurement

As mentioned above, it has been suggested that there is not just one type of impulsive behavior. Due to the range of behaviors that the term impulsivity encompasses, EVENDEN (1999C) proposed impulsivity not as a unitary construct, but more as a set of related phenomena that may have independent underlying biological mechanisms, whereas these distinct behavioral phenomena are dissociable at neuroanatomical as well as neuropharmacological levels.

However, common aspects of impulsivity comprise decreased inhibitory control, intolerance of delay to rewards, quick decision-making due lack of consideration, as well as other deficits such as poor attentional ability and hyperactivity (DARUNA & BARNES 1993; POULOS ET AL. 1998). Breaking down impulsivity into these different elements made it possible to devise different behavioral paradigms to measure these various forms of impulsive behavior in both humans and laboratory animals (WINSTANLEY ET AL. 2004A, WINSTANLEY 2007; MOELLER ET AL. 2001). The behavioral paradigms can be broadly divided in two categories: those measuring impulsive action or motoric impulsivity, and those assessing impulsive choice or impulsive decision-making, also termed as delay aversion (SWANN ET AL. 2002; WINSTANLEY ET AL. 2006).

3.3.1 Impulsive action (inhibitory control)

Research fields like behavioral neuroscience and cognitive psychology describe impulse control as an active inhibitory mechanism that modulates internally and externally driven pre-potent desires for primary reinforcers such as food, sex or other highly desirable rewards. This inhibitory control mechanism may provide lower cognitive mechanisms to guide behavior, while rapid conditioned responses and reflexes are transiently suppressed (NIGG 2000; WINSTANLEY ET AL. 2006). In general impulsive action or response inhibition can be defined as the inability to withhold from making a response (WINSTANLEY ET AL.

2006; EVENDEN 1999A). The most popular clinic-based measure of sustained attention, vigilance and response inhibition in humans which has been described as a highly sensitive assessment for monitoring medication effects, is the continuous performance task (CPT) (RICCIO ET AL. 2001; SWANN ET AL. 2002). In the CPT, patients are required to respond to a specific visual stimulus (e.g. the letter "X") presented on a visual display, while this stimulus has a much lower probability to appear than other stimuli (e.g. A, C, F, M). At appearance of the letter "X", subjects have to respond by pressing a button on a computer mouse. This simple response allows to detect several measures from the subject like accuracy (correct response on the button), false alarm hit rate (number of responses when a letter other than "X" occurs), processing speed (latency to press the button when the stimulus "X" is presented) and impulsivity measures (response prior to the stimulus presentation) (RICCIO ET AL. 2001).

The preclinical analogue of the CPT designed for rats is the 5-choice serial reaction time task (5-CSRTT). This widely characterized, operant-based test was originally developed to measure visuo-spatial attention (ROBBINS 2002). In this task, animals are required to be attentive and withhold from responding while monitoring five apertures for brief light stimuli (e.g. 1s or less) presented randomly therein. The apertures in the 5-CSRTT are the equivalent to the non-target letters in the CPT in humans. Analogues of the target stimulus (the letter "X" in the CPT) are the brief light stimuli (DAY ET AL. 2007; ROBBINS 2002). Subsequent to the beginning of a trial and prior to presentation of a light stimulus, there is a five second inter-trial interval during which the animals have to withhold a response at all. Any responses made during this time are described as premature responses. They are generally regarded as a measure or index of motoric impulsivity, because low levels of premature responses require the ability to inhibit actions, whereas high levels might reflect disturbances in the inhibition of behavior. Premature responses in the 5-CSRTT are potentially analogous to "false alarm" hits done in the CPT (DAY ET AL. 2007; WINSTANLEY 2007).

Comparing these two measures, human subjects watch out for the correct letter and push a computer mouse button performing the CPT while rats, doing the 5-CSRTT, have to nose-poke into the correct aperture where the light stimulus is presented to get a food reward (DAY ET AL. 2007; ROBBINS 2002).

3.3.2 Impulsive decision-making (delay aversion)

The fact that impulsive subjects are not able to take time to carry out appropriate evaluations of incoming information in order to choose behavioral responses on a detailed analysis of a given situation (KOSKINEN ET AL. 2000; EVENDEN 1998) suggests that the inability to tolerate delays of gratification or reward may also be an important aspect of impulsive behavior (SOUBRIE 1986; EVENDEN & RYAN 1999; BIZOT ET AL. 1999).

A modified version of the self-control paradigm to measure impulsive decision-making in humans is the Two-Choice test. This test assesses the tendency to choose between a smaller more immediate reinforcer versus a larger more delayed reinforcer, while the choice of the smaller more immediate reinforcer is defined as impulsive behavior. Briefly, subjects are provided with two response options, presented as letters "A" and "B" appearing on a computer screen. Selection of a letter is done by pressing the corresponding button on the response panel. The delay to reinforcement after pressing button "A" is 5s and the reward 5 cents. The delay to reinforcement after pressing button "B" is longer, but the reward is greater than for the "A" response (15 cents after a delay of 15s). Impulsivity is measured as the number of choices made for the smaller, more immediate reinforcer (letter "A"), while letter "B" responses are operationally defined as self-control (CHEREK & LANE 1999; SWANN ET AL. 2002).

A well established paradigm for testing sensitivity to delays of reward in laboratory animals was first described by THIEBOT ET AL. (1985). In this delay-based decision-making task in a T-maze rats have to choose one arm giving immediate access to a small reward (two food pellets) or a second arm, in which rats could be detained for a certain time (e.g. 10s or more) before giving access to a large reward (ten food pellets). The ability to wait for food reward is taken as an operational measure of impulsive-like behavior (BIZOT ET AL. 2007; EVENDEN 1999B). Due to the fact that this maze procedure is very time consuming, EVENDEN & RYAN (1996) developed a discrete trial operant task, similar to the T-maze task. Therein, rats are given the choice between a small reinforcer (one food pellet) and a large reinforcer (three to five food pellets). The delay associated with the large reinforcer is increased stepwise from 0 to 60s during the session. Trained rats begin each session by choosing the lever providing the larger reinforcer, but switch the preference to the smaller one, as the delay increases. This switch from the large to the small reinforcer indicates an increase in impulsive choice. Advantage of this paradigm compared to the

maze procedure is its possibility to obtain a within-session delay-discounting curve (EVENDEN & RYAN 1996; EVENDEN 1999A).

3.4 The neuroanatomical circuitry of impulsive behavior

3.4.1 Neural substrates

There has been considerably convergence in recent years on the neuroanatomical substrates of impulsivity in clinical patient groups (DALLEY ET AL. 2008). Prefrontal cortical, striatal and limbic brain regions have been strongly implicated in different forms of impulsivity, because dysfunction or damage within this substrates and their circuitry bears patterns of impulsive behavior associated with a number of psychological disorders (ROBBINS 2000, WINSTANLEY ET AL. 2006).

Likewise, experimental preclinical models of impulsivity, e.g. lesion studies in rodents, support the idea that brain areas including cortical and limbic regions, are involved in the modulation of impulsive behavior (**Fig.1**). Beside the prefrontal cortex, one prominent subcortical output structure, which is in conjunction with the prefrontal cortex, is the nucleus accumbens (NAc) (WINSTANLEY ET AL. 2006; PATTIJ & VANDERSCHUREN 2008). This neural substrate is a key node in the limbic cortico-striatal loop, a circuit that is involved in goal-directed behavior and the evaluation of emotional stimuli and events (DALLEY ET AL. 2008; WINSTANLEY ET AL. 2006). It receives information from the hippocampal system, the amygdala and, as already mentioned, from the frontal cortex. On the other hand it projects to motor output structures like the caudate putamen and the mediodorsal thalamus. Therefore, the NAc acts as a "sensori-motor interface" through which behavior can be influenced by regions of the limbic system, such as the habenula, amygdala and hippocampus (GOTO & GRACE 2005; KOCH ET AL. 2000; WINSTANLEY ET AL. 2006). Admittedly, the precise mechanisms by which limbic structures affect impulsivity are not completely understood. Furthermore, some brain areas do dissociate between distinct forms of impulsivity (impulsive action and delay aversion), which suggests that different behavioral phenomena of impulsivity rely on separate neural pathways (PATTIJ & VANDERSCHUREN 2008).

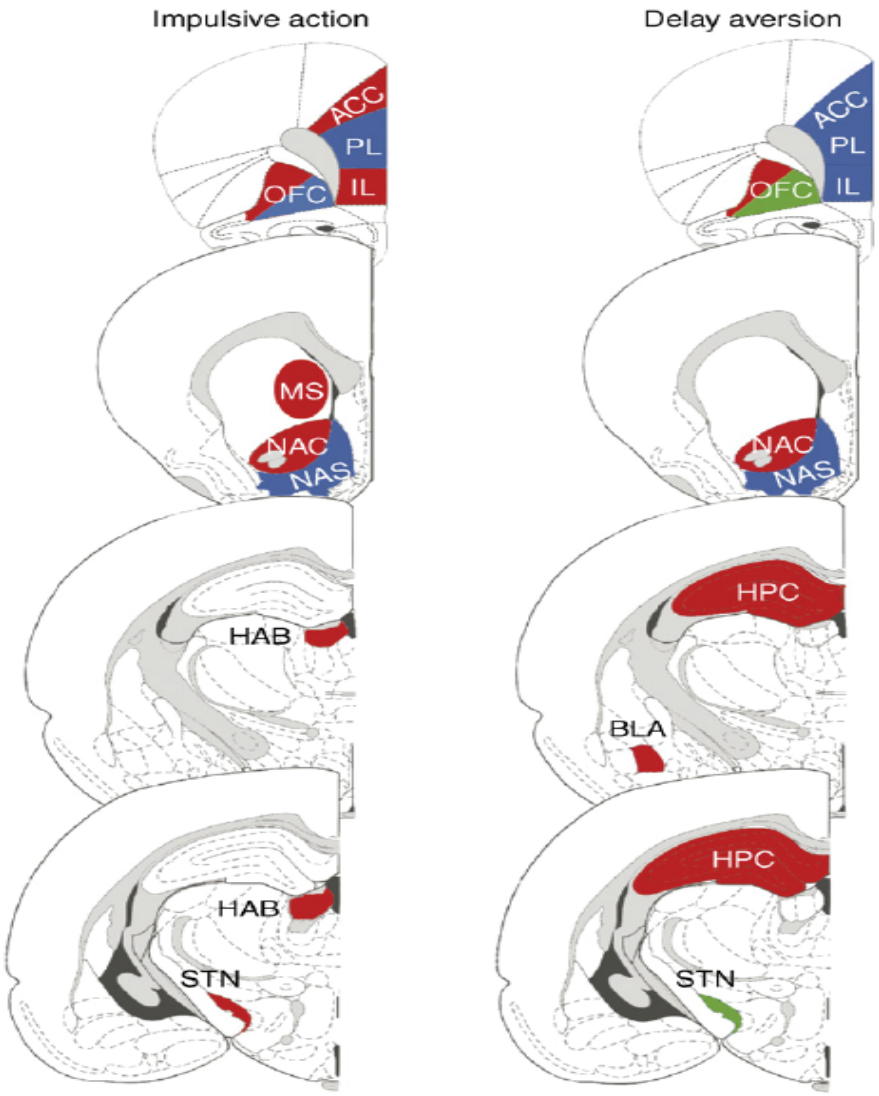

Figure 1 Schematic overview of the neuroanatomical regions in the brain involved in impulsive action (inhibitory control and delay aversion) in rats based on lesion studies. As seen in the figure, there is extensive overlap in brain regions, including cortical and limbic regions, which modulate different models of impulsivity. Some regions dissociate between models of impulsivity in terms of involvement and direction of lesion effects. For example, the infralimbic cortex seems to be primarily involved in impulsive action. Limbic regions like the basolateral amygdala or hippocampus rather modulate delay aversion. Certainly, their role in impulsive action has not been established yet. Moreover, lesions of the subthalamic nucleus for example seem to have opposing behavioral effects in different tasks measuring impulsivity, just as the orbitofrontal cortex.

red areas	=	lesions of these regions increase impulsive action or delay aversion
green areas	=	beneficial effects of lesions on impulsivity
blue areas	=	lesions of these brain regions did not affect impulsive action nor delay aversion
dark grey/black areas	=	ventricles in the brain
light grey areas	=	fibre tracts in the brain

Abbreviations: ACC - anterior cingulate cortex; BLA - basolateral amygdala; HAB - habenula; HPC - hippocampus; IL - infralimbic cortex; MS - medial striatum; NAcc - nucleus accumbens core region; NAcs - nucleus accumbens shell region; OFC - cortex; PL - prelimbic cortex; STN - subthalamic nucleus (according to PATTIJ & VANDERSCHUREN 2008).

3.4.2 The orbitofrontal cortex

The prefrontal cortex in humans and other primates consists of several functionally distinct subregions, such as the lateral prefrontal cortex (LPC), the orbitofrontal cortex (OFC), and the medial prefrontal cortex (mPFC) (BECHARA ET AL. 1999; SOTRES-BAYON 2004). The LPC, especially the dorsolateral region, is involved in working memory and executive control functions, the mPFC in extinction of fear, whereas the OFC plays a important role in reward, motivation, and emotional decision making (SOTRES-BAYON 2004).

More specifically, the OFC, comprising the ventrolateral and ventromedial frontal cortex, is integral to various corticocortical networks implicated in higer-order cognition, receiving polymodal input from sensory cortices as well as interacting with subcortical areas including the mediodorsal thalamus (ONGÜR & PRICE 2000). OFC-impairment is observable using laboratory-based tasks like the Iowa gambling task (IGT), devised by BECHARA, DAMASIO and colleagues, where patients wit OFC-lesions show poor performance. In this test subjects choose cards from four decks to accumulate points. During the course of the session they have to learn to differentiate between low-reward, low-risk card decks as well as high-reward, high-risk decks. Healthy volunteers learn to choose cards from the two decks associated with low-reward but also minor and infrequent losses. Whereas, persistent selection from the two high-rewarded, high-risk decks leads to high gains but heavy losses in long-term (BECHARA 1999; BECHARA ET AL. 2003). This pattern of risky decision-making, and its common coincidence with aberrant social behavior, is often described as impulsiveness. Additionally, it is not only observed in patients with OFC-damage, but also in pathological gamblers, substance abusers, and patients with damage to the BLA (see 1.4.3) (WINSTANLEY 2007). In terms of animal models of impulsivity, lesions of the OFC affect premature and perseverative responding in the 5-CSRTT, as well as in delayed reinforcement paradigms (CHUDASAMA ET AL. 2003; MOBINI ET AL. 2002; WINSTANLEY ET AL. 2004B), indicating a crucial involvement of the OFC in both, mediating impulsive action and impulsive decision-making (PATTIJ & VANDERSCHUREN 2008).

Amongst others, functions that require the integrity of the OFC are dependent on information provided through interconnected structures, which allow access to information regarding the context of cues and associations formed during learning processes. One strategic structure is probably the BLA. This neural substrate is important for associative

aversive learning in primates and other species (SCHOENBAUM ET AL. 1998; CARDINAL ET AL. 2003; ONGÜR & PRICE 2000).

3.4.3 The amygdaloid complex

In the early 19th century, Karl Friedrich BURDACH discovered an almond shaped mass of grey matter in the temporal pole of the human cerebral hemispheres (**Fig.2**) and called it the "*Mandelkern*" (amygdalar nucleus) (SWANSON & PETROVICH 1998).

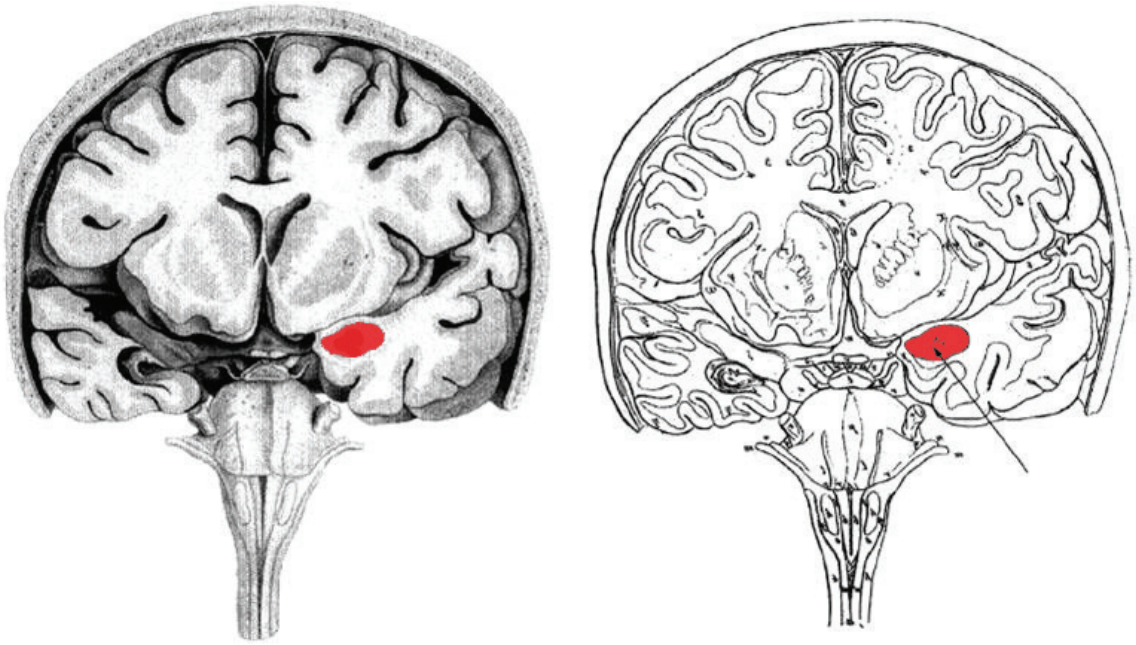

Figure 2 The first illustrations of the "Mandelkern", in the human brain. The arrow on the right hand picture points to the location of the amygdalar nucleus (red area), drawn by BURDACH. Comparison with a standard modern textbook picture (left hand) indicates that BURDACH was referring to a region now known as the basolateral complex (according to SWANSON & PETROVICH 1998).

Originally, BURDACH described a group of cells that are today known as the basolateral complex of the amygdala. Subsequently, a large number of additional structures have been identified in many species and constituted as the amygdaloid complex (SAH ET AL. 2003). In primates the amygdaloid complex can be found in the anteriomedial part of the temporal lobe, where it is located ventromedial to the striatum and anterior to the ventral portion of the hippocampal formation. The position of the complex in non primates is similar to that

in primates (see **Fig.1**), whereas in the rat and cat the temporal lobe is not as well developed (MCDONALD 1998). As already mentioned the amygdaloid complex is structurally diverse and comprises about 13 nuclei, which are further divided into subdivisions, distinguished on the basis of their cytoarchitectonics, histochemistry, and the connections they make (SAVANDER ET AL. 1997; SAH ET AL. 2003; PITKÄNEN ET AL. 2003). The nomenclature for describing the amygdaloid complex differs in the literature. Based on the nomenclature introduced by PRICE ET AL. (1987), the amygdaloid nuclei are divided into three groups: 1) the deep or basolateral group, including the lateral nucleus, the basal nucleus and the accessory basal nucleus; 2) the superficial or cortical-like group, which includes the cortical nuclei and the nucleus of the lateral olfactory tract; and 3) the centromedial group, consisting of the medial and central nuclei. Additionally, a separate set of nuclei, the intercalated cell masses and the amygdalo-hippocampal area does not belong to any of these three groups (SAH ET AL. 2003; SWANSON & PETROVICH1998).

Recent studies of forebrain circuitry in primates and rodents indicate that there are multiple, parallel cortico-striato-pallido-thalamic circuits which have discrete functions. The amygdaloid complex has extensive connections with the structures of the "limbic circuit", like the prefrontal cortex, limbic striatum, ventral pallidum and mediodorsal thalamic nucleus, which seem to be involved in behavior related to emotion, motivation and reward (MCDONALD ET AL. 1996). The general function of the amygdaloid complex is to evaluate significance of sensory stimuli and to generate appropriate emotional responses by coordinating the activity of brain areas controlling autonomic, endocrine, and somatomotoric mechanisms. This process involves associating sensory stimuli with their affective attributes (MCDONALD 1998; PITKÄNEN ET AL. 1997, 2003). Abnormal functioning or damage of the amygdaloid complex in various human diseases, such as epilepsy, Alzheimer's disease, Parkinson's disease, Urbach-Wiethe disease, schizophrenia, depression, addiction, or posttraumatic stress disorder, compromises the evaluation of social signals in the environment (PITKÄNEN ET AL. 2003). For instance, fear-motivated learning or conditioning using appetitive stimuli such as food, sex and drugs are mediated by the amygdaloid complex (MCDONALD 1998). Furthermore, beside its direct role in learning and memory, activation of the amygdaloid complex has also modulatory effects on the acquisition and consolidation of memories that evoke emotional responses (SAH ET AL. 2003).

The basolateral nuclei group of the amygdala (BLA) is one of the larger amygdaloid cell groups, which functionally interconnects with temporal lobe structures such as the hippocampus as well as cortical and striatal regions (WINSTANLEY ET AL. 2004B). It also projects to the NAc, and has extensive reciprocal connections with the OFC. Taken together, the BLA plays an important role in forming associations between affective states and environmental stimuli, such as stimulus-reward associations (CARDINAL ET AL. 2003). In animal models, rats bearing neurotoxic lesions of the BLA have difficulty in learning to avoid an aversive outcome. Furthermore, damage to this structure in both rats and monkeys is associated with deficits in the ability to adjust behavior when the value of a reinforcer is altered (SCHOENBAUM ET AL. 2003). In different work, WINSTANLEY and colleagues (2004B) showed that excitotoxic lesions of the BLA promote impulsive choice in a delayed-reward task.

3.4.4 Interconnection between the OFC and the amygdala

As already mentioned, humans with OFC-damage are impaired in a number of tests of emotional reactivity to stimuli and make poor decisions as a result. Admittedly, these patients resemble amygdala-lesioned subjects in several respects. In both cases subjects are impaired in the capacity to assess and use the value of predicted outcomes to guide their actions in the Iowa gambling task (BECHARA ET AL. 1999; CARDINAL ET AL. 2003). Furthermore, the patterns of behavior these patients exhibit are often described as impulsive. Likewise, OFC and BLA lesions in rats often cause similar behavioral effects (SCHOENBAUM ET AL. 2003). Animal models of impulsive action and delay aversion show that lesion to either the OFC or the BLA affect impulsive behavior in many different ways, depending on the test procedures and tasks used (WINSTANLEY ET AL. 2004B; MOBINI ET AL. 2002; PICKENS ET AL. 2003.)

These findings in humans and animals suggest that both structures, known as important nodes in the limbic cortico-striatal loop, form a network, which is linked through many extensive and reciprocal connections (CARDINAL ET AL. 2002). This OFC-BLA-network or circuit provides the use of incentive informations to guiding behavior (SADDORIS ET AL. 2005). Admittedly, this network not only includes the BLA and the OFC, but also others regions like the NAc, where both structures are projecting to. On the other hand, BLA and OFC lesions can also have opposite effects in exactly the same paradigm,

measuring impulsive action or delay aversion, suggesting that any interaction of these structures is likely to be complex (CARDINAL ET AL. 2004). Despite a large number of studies addressing the anatomic and functional aspects of amygdaloid connectivity, modelling of OFC-amygdala-networks in normal and pathologic conditions is still in early stages. This partly arises from the lack of detailed quantitative data of amygdaloid neurons and neuronal circuits (PITKÄNEN ET AL. 2003).

3.5 The neuropharmacology of impulsive behavior

The predominant neurochemical theory of impulsivity indicates that reduced serotonin (5-hydroxytryptamine; 5-HT) function is associated with an increase in impulsive behavior in humans, primates and rodents, where the potential importance of other neurotransmitters, like dopamine (DA), norepinephrine (NE) or glutamate (GLU) has to be considered as well (SOUBRIE 1986; POULOS ET AL. 1998; TALPOS ET AL. 2006).

The assumed important role of ascending 5-HT-pathways in "impulse control" and the fact that dysfunction of these pathways may be responsible for pathological impulsiveness in a number of clinical conditions, led to several attempts to examine effects of manipulating 5-HT-function in animal models of impulsivity (SOUBRIE 1996; MOBINI ET AL. 2000). WOGAR ET AL. (1993) and MOBINI ET AL. (2000) showed that destruction of the ascending 5-HT-pathways by intra-cerebral injection of the selective neurotoxin 5.7-dihyroxytryptamine (5.7-DHT) into the raphe nuclei increased the choice of the smaller and earlier of two reinforcers in discrete-trials operant tasks. However, recent studies which use a number of different tasks, such as the 5-CSRTT, the go/no-go task or differential reinforcement of low-rate- (DRL-) schedules (WINSTANLEY ET AL. 2004C, 2006), have been in conflict with this traditional theory. The 5-$HT_{2A/C}$-receptor agonist (±)-1-(2.5-Dimethoxy-4-iodophenyl)-2-aminopropan hydrochlorid (DOI) increases premature or "impulsive" responding in the 5-CSRTT (KOSKINEN ET AL. 2000A, B) and in operant paradigms of delay of reinforcement described by EVENDEN & RYAN (1996), when administered systemically. Consistent with effects of global 5.7-DHT-lesions, premature responding also appears to be increased by the selective 5-HT_{2C}-receptor antagonist 6-chloro-5-methyl-1-[[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl]carbamyl]-indoline (SB242084) (WINSTANLEY ET AL. 2004C). Furthermore, DALLEY ET AL. (2002) showed that elevated

5-HT-levels in the frontal cortex have been associated with increased premature responding in the 5-CSRTT. On the other hand, treatment with the 5-HT_{2A}-receptor antagonist ketanserin leads to a decrease in premature responding in the 5-CSRTT (KOSKINEN ET AL. 2000A, B; PASSETTI ET AL. 2003; TALPOS ET AL. 2006) but causes no effect on impulsive responding in the operant delayed reward task, when administered systemically or locally into the medial prefrontal cortex (EVENDEN & RYAN 1996; TALPOS 2006). Similar effects have been caused by the 5-HT_{2A}-receptor antagonist (+/)2,3-dimethoxyphenyl-1-[2-4-(piperidine)-methanol] (M100907), which reduced premature responding in the 5-CSRTT as well as in the operant delayed reward task (WINSTANLEY ET AL. 2003A, 2004C; CARLI ET AL. 2006).

Taken together, these data suggest that decreased 5-HT-function enhances or reduces impulsive behavior, depending on the 5-HT-receptor subtypes and the behavioral tasks used (WINSTANLEY ET AL. 2006). Due to the fact that different tests of impulsivity probe different cognitive processes (EVENDEN & RYAN 1999), it is still unclear what generalizations towards 5-HT_2-receptor involvement can be made on the basis of measurements of impulsive behavior.

3.6 Serotonin

3.6.1 Biosynthesis and metabolism

In evolutionary terms, 5-HT is one of the oldest neurotransmitters and has been implicated in the etiology of numerous disease states such as depression, anxiety, social phobia, schizophrenia, obsessive-compulsive and panic disorders, and impulsivity (HOYER ET AL. 2002). This monoamine neurotransmitter is synthesized in 5-HT-neurons of the central nervous system (CNS), but is also present in particularly high concentrations in blood platelets and the enterochromaffin cells of the gastrointestinal mucosa (SAXENA 1995). The indole nature of 5-HT shows a high similarity to the psychedelic drug lysergic acid diethylamide (LSD), with which it interacts on smooth muscle preparations in vitro. Furthermore, 5-HT is also structurally related to other psychotropic agents (COOPER ET AL. 2003). The combination of the hydroxyl-group in position 5 of the indole nucleus and a primary amine nitrogen, serving as a proton acceptor at physiological pH, makes 5-HT a

hydrophilic substance. This attribute indicates that 5-HT has to be synthesized in the brain from another substance, named 5-hydroxytryptophan (5-HTP) which has the ability to pass the lipophilic blood-brain barrier (COOPER ET AL. 2003, SIEGEL ET AL. 2006). Thus, the amount of centrally produced 5-HT is dependent on the amount of tryptophan that is available peripherally to cross the blood-brain barrier (JONNAKUTY & GRAGNOLI 2008). Hence, the primary substrate for the synthesis of 5-HT is the naturally occurring essential amino acid L-tryptophan. Once ingested, tryptophan is converted to 5-HT via a series of chemical reactions (**Fig.3**) (BOADLE-BIBER 1993).

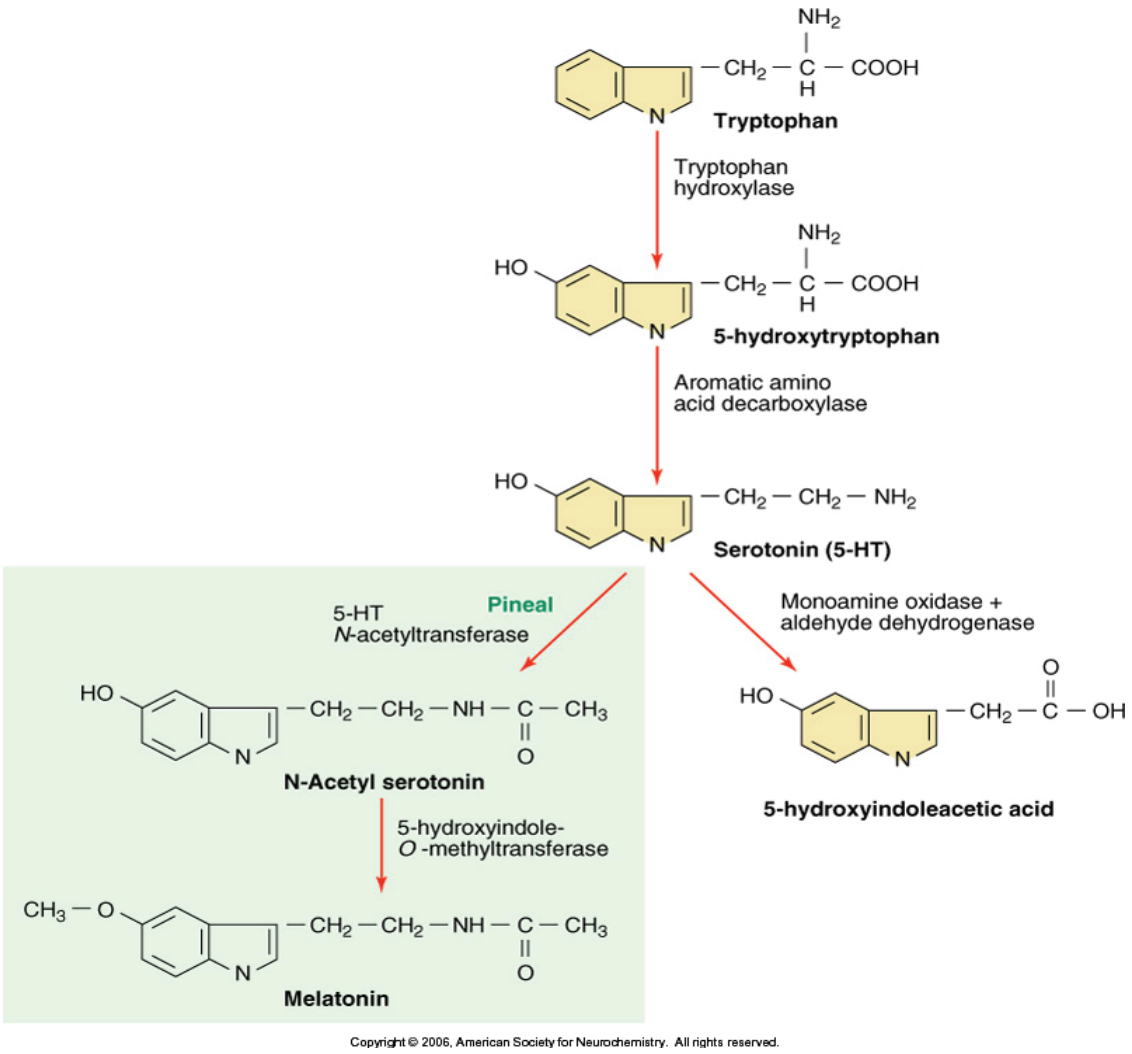

Figure 3 Synthesis and metabolic pathways of 5-HT (highlighted in yellow). In the pineal gland (highlighted in green) 5-HT is converted enzymatically into melatonin (SIEGEL ET AL. 2006).

The first step in the synthetic pathway is the hydroxylation of tryptophan to 5-HTP via the enzyme tryptophan-hydroxylase (COOPER ET AL. 2003) and constitutes the rate-limiting step in the synthesis of 5-HT (JONNAKUTY & GRAGNOLI 2008). Once synthesized from tryptophan, 5-HTP is in turn almost immediately decarboxylated to 5-HT via the enzyme L-amino-acid-decarboxylase (BOADLE-BIBER 1993). As with other transmitters, 5-HT is sequestered in storage vesicles via a vesicular-monoamine-transporter (VMAT). The fact that 5-HT requires its active transport from the cytoplasma, the VMAT uses a electrochemical gradient generated by a vesicular H+-ATPase. Therefore, a cytoplasmic amine is exchanged for a luminal proton (SIEGEL ET AL. 2006). 5-HT is held in the vesicles until it is released from 5-HT-neurons into the synaptic cleft (JONNAKUTY & GRAGNOLI 2008; BOADLE-BIBER 1993).

Action of released 5-HT in the synaptic cleft is terminated by uptake via the 5-HT-plasma-membrane-transporter (SERT, **Fig.5**), located in the membrane of 5-HT-axon-terminals (JONNAKUTY & GRAGNOLI 2008). The re-uptake is an active process that is temperature-dependent and has an absolute requirement for external Na^+ and Cl^-. More precisely, 5-HT-uptake is dependent on the maintenance of the Na^+-gradient across the plasma-membrane (SIEGEL ET AL. 2006; COOPER ET AL. 2003). Once back in the presynaptic terminal, 5-HT is restored in vesicles again via the VMAT. Cytoplasmic 5-HT is rapidly metabolized to 5-hydroxyindoleacetic acid (5-HIAA) through the actions of the enzyme monoamine-oxidase (MAO), which oxidatively deaminates the amine to 5-hydroxy-indoleacetaldehyde. This step follows the oxidization of the aldehyde to the acid by an NAD+-dependent aldehyde-dehydrogenase to form 5-hydroxyindolacetic acid (5-HIAA) (BOADLE-BIBER 1993; SIEGEL ET AL. 2006, JONNAKUTY & GRAGNOLI 2008). Understandably, this re-uptake process plays an important role in the modulation of 5-HT-neurotransmission (OLIVIER 2004).

3.6.2 Receptor classification

5-HT induces its effects through a variety of membrane-bound receptors, which are found in both the CNS and in the peripheral nervous system (PNS), as well as in a number of non neuronal tissues, such as the gut, cardiovascular system and blood (HOYER ET AL. 2002). 5-HT- receptors contain seven characteristic transmembrane regions, whereas the subtypes differ in their operational, structural and transductional properties (SAXENA 1995).

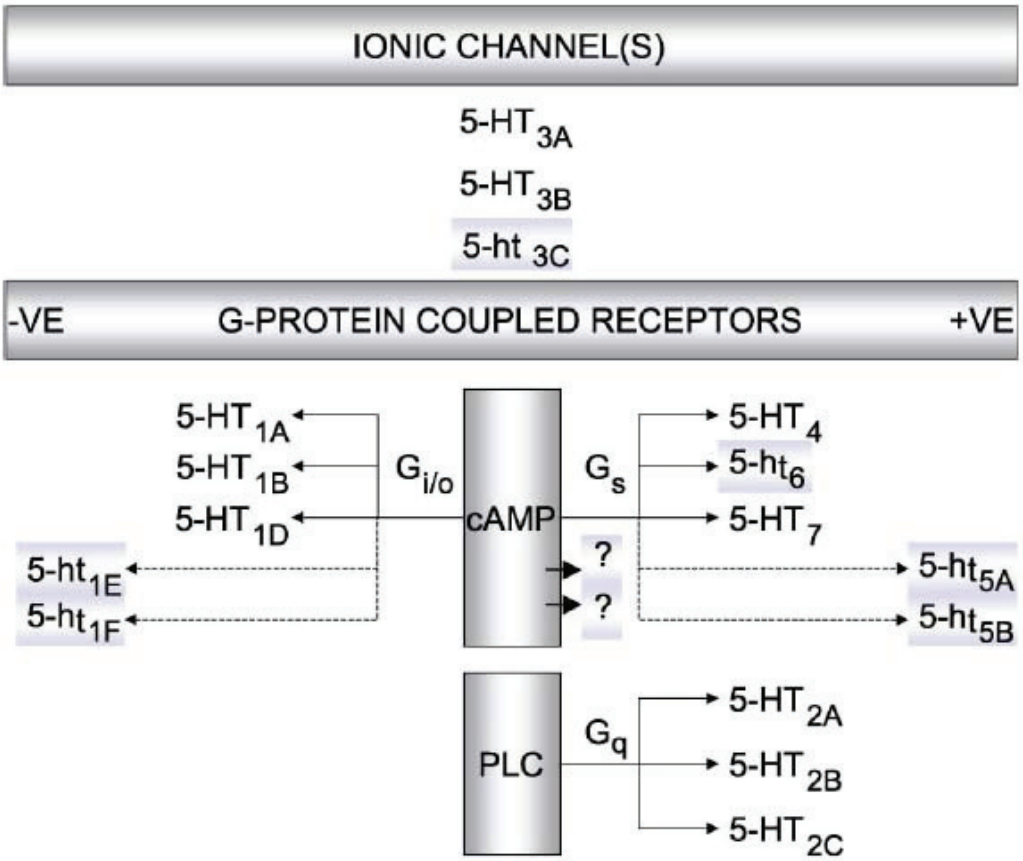

Figure 4 Graphical depiction of the current classification of 5-HT-receptors. Receptor subtypes represented by coloured boxes and lower case designate receptors that have not been demonstrated to definitively function in native systems. Abbreviations: cAMP - cyclic adenosine monophosphate; PLC - phospholipase C; -ve - negative; +ve - positive (HOYER ET AL. 2002).

With at least 14 distinct members, the 5-HT-receptors represent one of the most complex families of neurotransmitter receptors (HOYER ET AL. 2002; HANNON & HOYER 2008). For a number of years there has been no new addition to the 14 known receptors with the exception of a second ($5\text{-}HT_{3B}$) and possibly a third ($5\text{-}ht_{3C}$) subunit for the $5\text{-}HT_3$-receptor

(HOYER ET AL. 2002; HANNON & HOYER 2008; SHIH ET AL. 1995). The diverse electrophysiological actions of 5-HT-receptors in the CNS encompass two major neurotransmitter gene families, the G-protein-coupled receptors and the ligand-gated cationic channels (**Fig.4)**. The net effects of both can be categorized according to the receptor subtypes, their respective effects or mechanisms of action, and their anatomic location as described below and seen in **Fig.5** (COOPER ET AL. 2003).

The G_i/G_o-coupled 5-HT_1-receptors generally mediate inhibitory effects of 5-HT on neuronal firing through an opening of voltage-sensitive K^+-channels with no intervening second-messenger signalling (AGHAJANIAN & SANDERS-BUSH 2002). This dual coupling with both adenylate-cyclase and K^+-channels is now recognized as a hallmark of G_i/G_o-linked receptors and is likely to occur with other members of the 5-HT_1-receptor family (SANDERS-BUSH & CANTON 1995). Furthermore, direct coupling to voltage-gated Ca^{2+}-channels has been described as a third signal transduction pathway for the G_i-linked family of receptors. Activation of G_i-linked receptors leads to enhancement of K^+-channel activity and, conversely, decline of Ca^{2+}-channel activity (AGHAJANIAN & SANDERS-BUSH 2002; SANDERS-BUSH & CANTON 1995).

Excitatory effects of 5-HT are mediated by 5-HT_2-receptors and involve the closing of K^+-channels, whereas these receptors can be modulated by second-messenger systems. The membrane-bound enzyme phospholipase C (PLC) catalyzes the degradation of the inositol lipid, phosphatidylinositol-4,5-bisphosphate (PIP2), with the production of inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 then mobilizes Ca^{2+} from an intracellular storage site by interaction with specific receptors. Ca^{2+} induces multiple responses in the cell, such as activation of Ca^{2+}/calmodulin-dependent protein-kinases, enzymes which phosphorylate or dephosphorylate key protein substrates in the cell. Thus, PLC-activation induces diverse changes in the cell, leading to the regulation of many different cellular processes. On the other hand, DAG activates another kinase family known as protein-kinase C (PKC), which regulates numerous processes of cell function (AGHAJANIAN & SANDERS-BUSH 2002; COOPER ET AL. 2003; SANDERS-BUSH & CANTON 1995).

Other effects of 5-HT appear to be mediated via 5-HT_4- and 5-HT_7-receptors by a reduction in certain voltage-dependent K^+-currents mediated through the PKA phosphorylation-pathway and thus involving positive coupling of the 5-HT response to adenyly-cyclase (AC) (COOPER ET AL. 2003).

Fast excitations are mediated via 5-HT_3-receptors which differ from the other receptors of the 5-HT-family (AGHAJANIAN & SANDERS-BUSH 2002). This receptor is a member of the large family of ligand-gated cationic ion channels which do not require coupling with a second-messenger (COOPER ET AL. 2003). Instead, the receptor itself forms an ion-channel that regulates ion-flux in a G-protein-independent manner (SANDERS-BUSH & CANTON 1995).

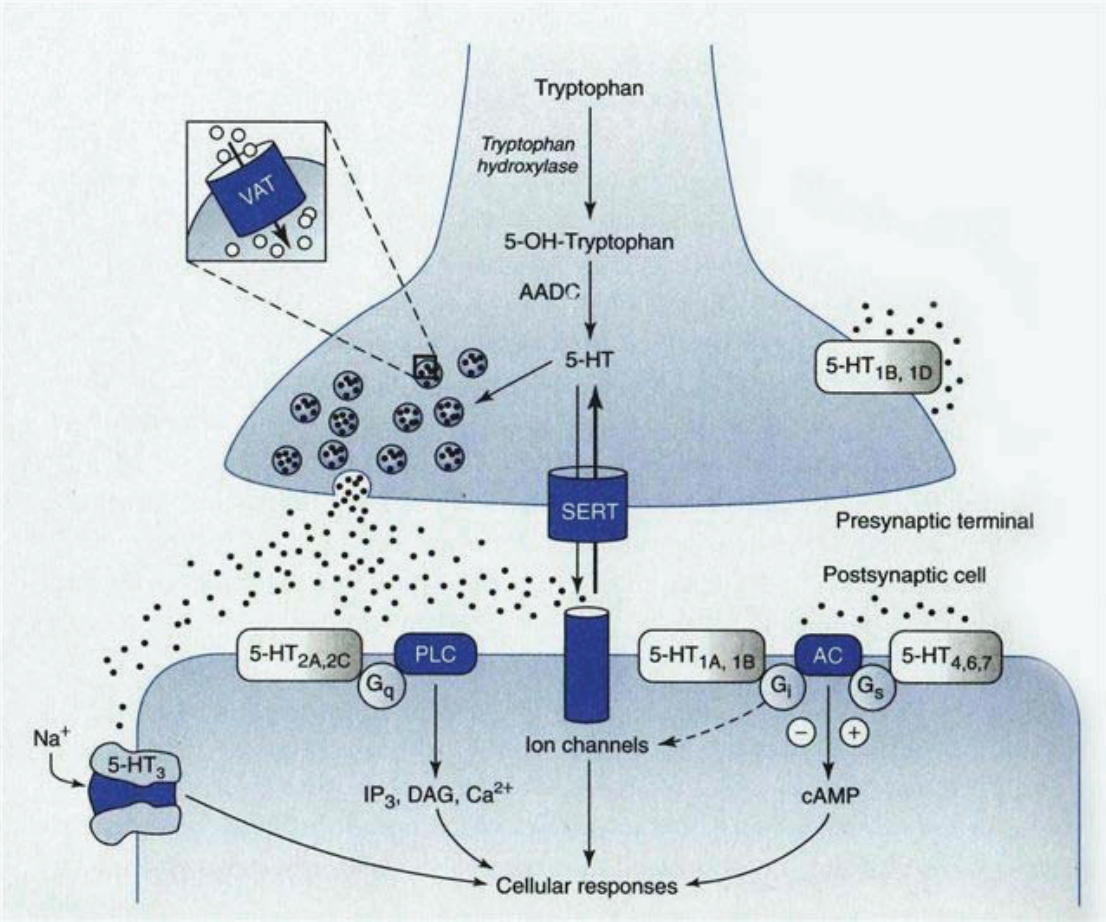

Figure 5 Schematic drawing of a 5-HT-synapse. Once released, 5-HT can interact with as many as 15 different receptors. 5-$HT_{1B/D}$-autoreceptors are located on 5-HT-presynaptic terminals, 5-HT_{1A}- autoreceptors are found on 5-HT-cell bodies and dendrites. A number of G-protein-coupled 5-HT-receptors are located postsynaptically, whereas the 5-HT_3-receptor is a ligand-gated ion channel. Abbreviations: AC - adenylyl cyclase; DAG - diacylglycerol; IP3 - inositol triphosphate; PLC - phospholipase C; SERT - plasma-membrane-serotonin-transporter; VMAT – vesicular-monoamine-transporter; AADC – amino-acid decarboxylase (COOPER ET AL. 2003).

The 5-HT_{1A}-autoreceptors which modulate impulse flow are localized presynaptically on cell bodies and dendrites (somato-dendritically) of 5-HT-neurons in the raphe nuclei and postsynaptically on many non-serotonergic neurons. Likewise, 5-HT_{1B}-(rodent) and 5-HT_{1D}-(human) receptors are localized pre- and postsynaptically, whereas they modulate stimulus-induced release of 5-HT as presynaptically located autoreceptors (COOPER ET AL. 2004, OLIVIER 2004). As indicated in **Fig.5,** every other 5-HT-receptors are presumably localized postsynaptically (ZIFA & FILLION 1992; OLIVIER 2004).

3.6.3 The serotonergic system

5-HT-containing neuronal cell bodies are mainly restricted to discrete clusters or groups of cells located along the midline of the brainstem. Their axons however, innervate nearly every area of the CNS (**Fig.6**), whereas the majority of 5-HT-somata are found in cell body groups designated as the raphe nuclei (COOPER ET AL. 2003; SIEGEL ET AL. 2006).

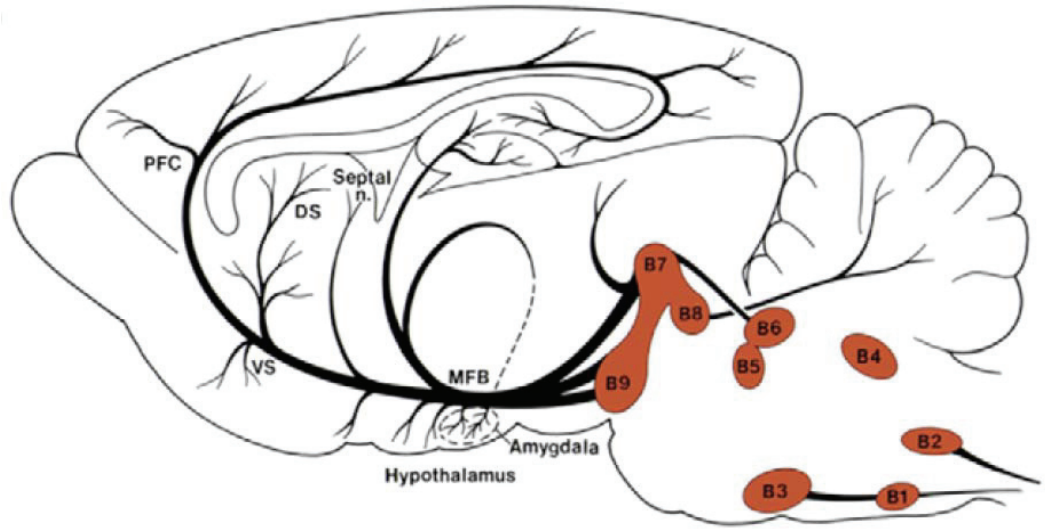

Figure 6 Neuroanatomical projections of the central 5-HT-system in rat brain (projections shown as bold branching lines). B7-B9= rostral group; B1-3= caudal group; B4-6= intermediate group; B7= dorsal raphe nucleus, B8= medial raphe nucleus. MFB, medial forebrain bundle; DS - dorsal striatum; VS - ventral striatum; PFC - prefrontal cortex; Septal n - septal nucleus (COOLS ET AL. 2008).

The description of the nine 5-HT raphe nuclei (B1-B9) was originally done by DAHLSTRÖM AND FUXE in 1964, and based on cytoarchitectural criteria such as cell body

structural characteristics and organization (COOPER ET AL. 2003). The raphe nuclei receive input from other cell body groups in the brainstem, such as the substantia nigra and ventral tegmental area (DA), superior vestibular nucleus (acetycholin; ACh), locus coeruleus (NE), nucleus prepositus hypoglossi and nucleus of the solitary tract (epinephrine; E). Other afferents include those from the hypothalamus, cortex and limbic forebrain structures such as the amygdala (SIEGEL ET AL. 2006). Beside these nine cell clusters, immunocytochemical localization of 5-HT has detected reactive cells in the area postrema, as well as in and around the interpeduncular nucleus (COOPER ET AL. 2003).

The more caudal groups (B1–3) located in lower brainstem largely project to the medulla and the spinal cord. The most rostral cell group of 5-HT-neurons in the upper midbrain (raphe dorsalis, raphe medianus, and raphe centralis superior; B7-B9) provide extensive ascending projections into the forebrain as well as into the diencephalon (COOPER ET AL. 2003; COOLS ET AL. 2008). More precisely, the B8 group (raphe medianus) appears to furnish a large component of the 5-HT-innervation of the limbic system, while B7 (dorsal raphe) projects with greater density to the neostriatum, cerebral cortices, and thalamus (COOPER ET AL. 2003). Group B9 is part of the ventrolateral tegmentum of the pons and midbrain and it forms a lateral extension of the median raphe (SIEGEL ET AL. 2006).

B4-6 are intermediate groups, which may project into both ascending and descending groups, as well as a far more extensive innervation of the cerebral cortex, which, unlike the noradrenergic cortical fibres, is quite patternless in general (COOPER ET AL. 2003).

3.6.4 Distribution of 5-HT_2-receptors

The various 5-HT-receptors are neuroanatomically localized at different sites in the CNS in rats (OLIVIER 2004), whereas in the present work only the distribution of the 5-HT_2-type will be focused on. Cellular examination of receptors in general can be achieved using techniques that deal with in situ hybridization, double-immunofluorescence staining or light microscopic autoradiographic mapping (XU & PANDEY 2000; PAZOS ET AL. 1985; BARNES & SHARP 1999).

The 5-HT_2-receptor, especially the 5-HT_{2A}-subtype is suggested to play an important role in many pathophysiological conditions, such as depression, suicide,

schizophrenia, impulsive behavior or alcoholism (XU & PANDEY 2000). Beyond this it has been implicated in the mechanism of action of hallucinogenic drugs (MORILAK ET AL. 1993). The regional localization and anatomical distribution of 5-HT_2-receptors in the brain may therefore be important to understand its functional implications in various pathophysiological conditions.

Quantitative autoradiographic mapping revealed abundance of 5-HT_2-receptors in the claustrum olfactory tubercle and layer IV of the neocortex. Likewise, this receptor subtype is present in regions of the anterior olfactory nucleus, pirifom cortex and layer I of the neocortex. Furthermore studies revealed concentrations of 5-HT_2-receptors in the caudate putamen, nucleus accumbens, layer V of neocortex, ventral dentate gyrus, the mammillary bodies, thalamus, hippocampus, brainstem, medulla, cerebellum and spinal cord (PAZOS ET AL. 1985).

In view of the present study, neurons in various structures of the cortex (frontal, insular, orbital etc.) are likewise abundant with 5-HT_2-receptors. Regarding the amygdaloid complex, 5-HT_2-receptors were found in various structures, such as the basolateral part, or the centromedial part, whereas its distribution was observed in cell bodies as well as in the fibres of neurons (XU & PANDEY 2000).

4 MATERIALS AND METHODS

4.1 Subjects

Adult male Wistar rats (250-350g), purchased from Harlan Germany (Hannover strain, Borchen, Germany), were kept in groups of five in standard Macrolon Type IV cages under controlled ambient conditions (22°C, 12h light/dark cycle, lights on at 7:00a.m.). During the light cycle a softly playing radio provided a continuous background noise and minimized disturbing effects of sudden noise in all animal facilities. The rats received free access to tap water and were maintained on their experimental body weight by controlled feeding of 12g standard laboratory rodent chow/rat/day (Nohrlin 10Z10, Nohrlin GmbH, Bad Salzuflen, Germany), where the weight of the animals was controlled individually on average. This controlled feeding schedule was continued throughout the whole testing periods, keeping the animals` body weight on approximately 85% of the free feeding weight. All behavioral testing was done during the rats light cycle between 9:00a.m. and 5:00p.m.

The experiments were performed in accordance with the NIH ethical guidelines for the care and use of laboratory animals for experiments, and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

4.2 Assessment of impulsive action

4.2.1 Apparatus

Operant testing chambers (26x26x26cm) (**Fig.7**), purchased from Campden Instruments (Campden Instruments Ltd., Loughborough, UK), were constructed of aluminium with a curved clear wall resulting in a variable depth starting at 21.5cm and reaching 25cm at its most distal point. The curved rear wall contained 9 apertures of 2.5 square cm, extended to a depth of 4cm positioned 2cm above the floor level. During the study the apertures 2, 4, 6 and 8 were closed with aluminium inserts. Within the other five apertures infra-red photocells were placed at the entrance to detect nose-poke responses. At the rear of the apertures standard light emitting diodes (LED) were located for presentation of visual

stimuli. Opposite of the curved wall, the upper half of the testing chambers was made of a hinged perspex panel, which allowed placing the animals into the chambers. Underneath this panel, a food magazine dispenser, equipped with a flap and an infrared or micro switch detector was located to detect nose entries. The magazine was equidistanced from each of the nine apertures. The testing chambers were illuminated with a house light mounted on the ceiling and housed within individual sound attenuated wooden boxes with constant ventilation and background noise provided by a small electric fan.

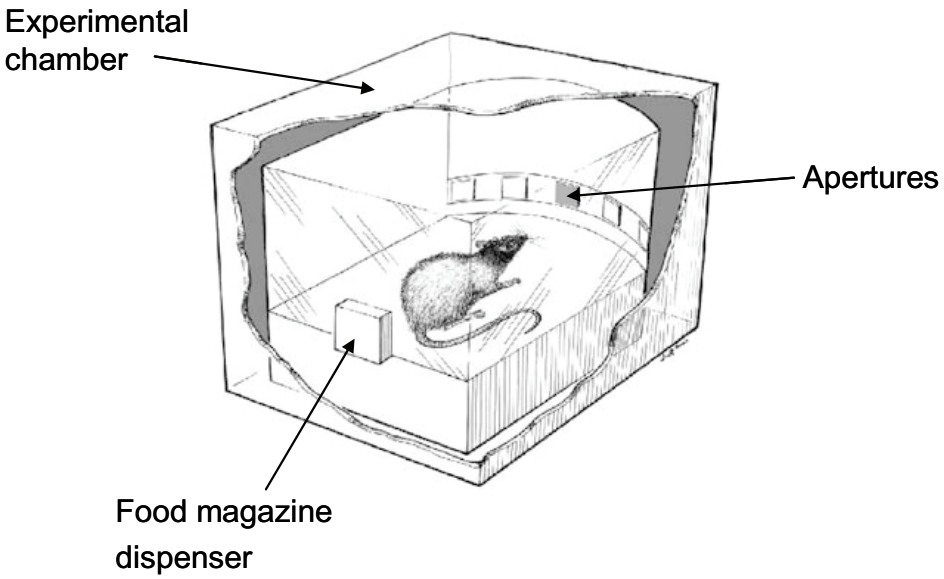

Figure 7 Schematic illustration of the operant testing chamber for the 5-CSRTT in cross section (CHUDASAMA & ROBBINS 2004).

Controlling of the chambers was provided by specific software written in Turandot (Cambridge Cognition Ltd.; Version 0.7) which was run on a personal computer with the BNC Mark 2 System (Behavioral Net Controller, Campden Instruments Ltd., Loughborough, UK).

4.2.2 General procedure

During two initial 30min habituation sessions, five to ten food pellets (Bio-Serv, UK Dustless Precision Pellets, 45mg) were placed in each open aperture and the magazine of the testing chambers to encourage rats to explore them. The training procedure of the 5-CSRTT was based on the protocol by Campden Instruments (**Fig.8**).

Each session began with the illumination of the house light and the food hopper, which signalled the beginning of the first trial. A nose poke of the animal into the food hopper initiated the first trial and triggered the random illumination of one of the five apertures after a fixed inter-trial interval.

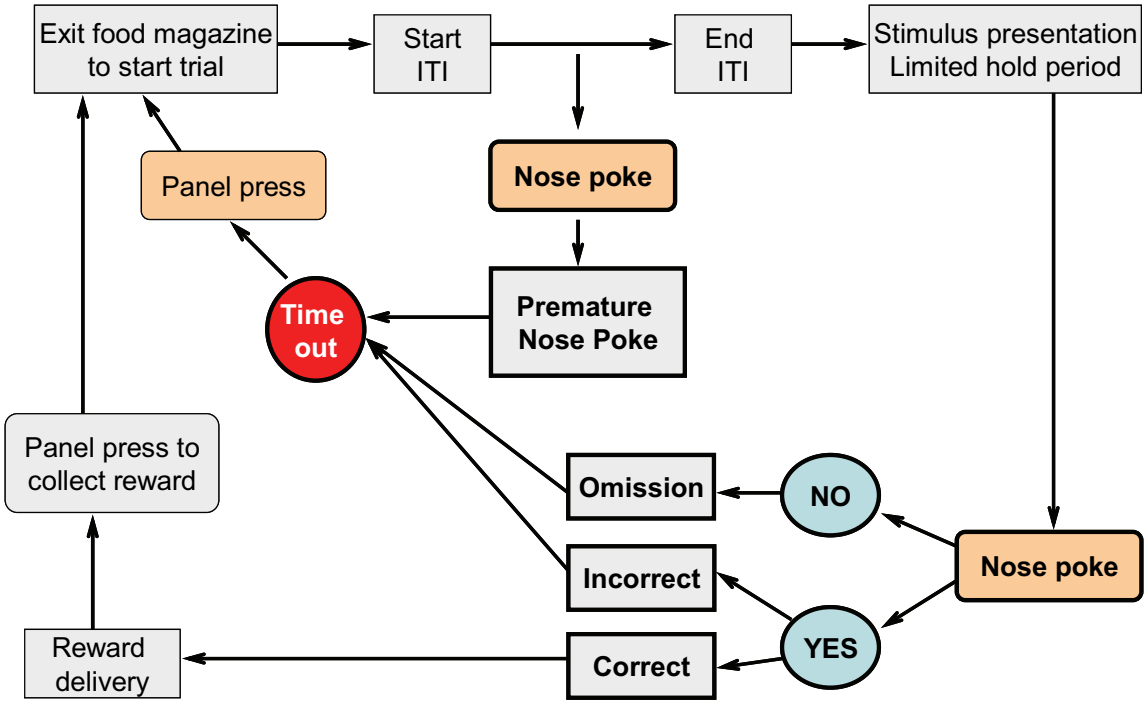

Figure 8 Schematic representation of the 5-CSRTT (according to Campden Instruments 2007).

A response into the illuminated hole during the stimulus presentation or during a 5s limited hold period afterwards led to the illumination of the magazine light and the receipt of a reward (food pellet). The collection of the food pellet, initiated the inter-trial interval before the next trial started. Response into any other not illuminated aperture (incorrect response) resulted in a 5s time out period, marked by extinction of the house light and no delivery of a reward. Failure to respond within the limited hold period (omission) likewise resulted in a time out, as did nose-pokes in any aperture made before presentation of the target stimulus (premature responses). A time out also followed after a perseverative responding, defined as additional response made in any of the five holes before the collection of the reward. Nose-pokes during the time out period reset the time out. At the end of a time out period, the light of the food hopper was turned on again, and a nose-poke response into the food hopper initiated a new trial. Additional nose-poke responses made

after the presentation of the stimulus in any aperture (perseverative responses) and additional responses made at the food magazine before or after food retrieval were recorded although not punished with time outs. Test sessions lasted over 30min or until 100 trials had been completed, which ever was shorter. Duration of the light stimulus and the limited hold period were initially set at 60s, respectively, and gradually decreased in a stepwise progression to their final length (Train8; **Tab.1**), dependent on the individual performance.

Table 1 Training stages of the 5-CSRTT (according to Campden Instruments 2007)

	Train1	**Train2**	**Train3**	**Train4**	**Train5**	**Train6**	**Train7**	**Train8**
Dauer	30 min	30 min	30 min	30 min	30 min	30 min	30 min	30 min
ITI	5 s	5 s	5 s	5 s	5 s	5 s	5 s	5 s
TO	5 s	5 s	5 s	5 s	5 s	5 s	5 s	5 s
SD	60 s	30 s	20 s	10 s	5 s	2.5 s	1.5 s	1 s
LH	60 s	30 s	20 s	10 s	5 s	5 s	5 s	5 s

Stimuli were presented randomly with a maximum of two consecutive presentations in the same aperture. Response into the food hopper during a time out was not recorded.

Rats underwent training sessions once a day until they reached criterion performance (more than 70% accuracy and less than 20% omissions) and a stable baseline (less then 10% variation in accuracy and omissions over six consecutive daily sessions). The following behavioral measures were recorded to assess task performance:

- ***% Accuracy*** (correct responses/total correct and incorrect responses)
- ***% Omission*** (omissions/total number of correct, incorrect, and omitted responses)
- ***% Premature responses*** (premature responses/total number of correct, incorrect, and omitted responses)
- ***Perseverative responses*** (additional response(s) made in the apertures after the initial correct response; perseverative responses into the food hopper were defined as the number of additional responses into the food hopper after trial initiation
- ***Latency to make a correct response***
- ***Latency to collect the reward***
- ***Total number of trials***

4.2.3 Surgery

Rats were anesthetized with chloral hydrate (Sigma, Deisenhofen, Germany, 360mg/kg i.p., followed by an additional injection of 120mg/kg after about 1h if necessary) and secured in a stereotaxic frame (Kopf Instruments, Tujunga, California, USA) with the incisor bar set at -3.3mm relative to the interaural line to assure a flat skull position. Therefore, the cross points of the rats skull markers, *lambda* and *bregma*, also bench marks according to PAXINOS AND WATSON`S "Stereotaxic Brain Atlas", became inplane (**Fig.9**).

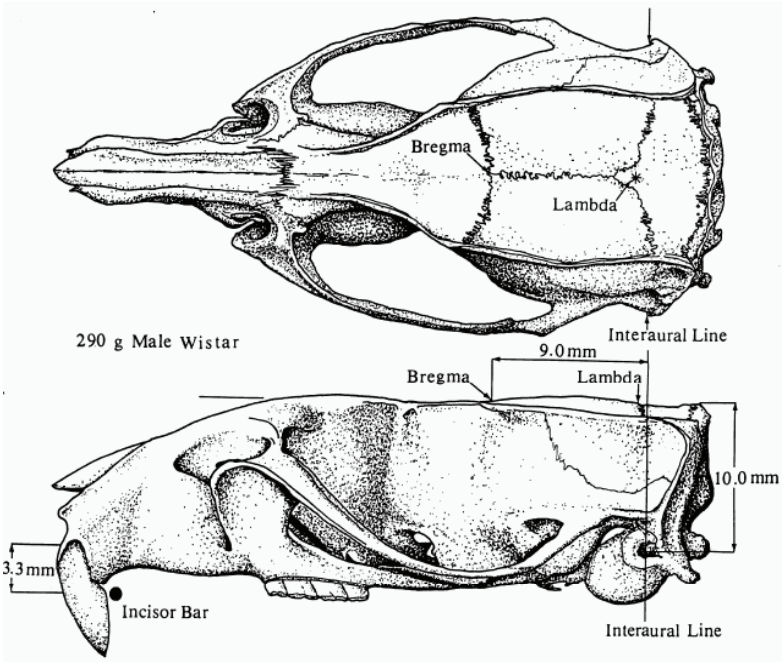

Figure 9 Dorsal and lateral views of the skull of a 290g Wistar rat. Positions of bregma, lambda, the plane of the interaural line as well as the distance between the incisor bar and the horizontal plane ("flat skull position") are shown (PAXINOS & WATSON 1998).

After rostrocaudal opening of the scalp, the periost of the skull-cap was removed with a raspatory. The bare skull-cap was cleaned with hydrogen peroxide (10% H_2O_2 Sigma-Aldrich, Germany), which also led to hemostasis of minor bleedings. According to PAXINOS & WATSON (1998), the coordinates for implanting guide cannulae were determined on the basis of lambda and bregma. The skull was trepanated with a dental drill (KaVo Typ 4912, Elektrotechnisches Werk GmbH, Leukirch, Germany) at these coordinates. Bilaterally, 23gauge stainless steal guide cannulae (Braun, Melsungen, Germany) were implanted aiming 2mm above the intended injection site at regions of the

OFC and BLA. The coordinates used for the final injection sites were as follows (from bregma): OFC= anteroposterior +3.2mm, lateral ±2.6 mm and dorsoventral -5mm; BLA= anteroposterior +2.8mm, lateral ±4.7mm and dorsoventral -8.2mm (PAXINOS & WATSON 1998). To secure all mounted material, anchor screws were fixed to the skull, and the guide cannulae were embedded in dental acrylic (Paladur, Heraeus Kulzer, Weinheim, Germany), which was applied to the exposed skull surface. Finally, the scalp was sewed and the guide cannulae were closed by removable obturator plugs of the same length before and between the experiments. After surgery animals were housed individually for two days with free access to food and tap water. After five days of recovery rats were retrained on the 5-CSRTT until they re-reached stable baseline performance over six consecutive daily sessions before starting with the microinjections.

4.2.4 Microinfusion procedure

Before starting the procedure, rats were gently restrained whilst the obturator plugs were removed. Custom made injectors (external diameter 0.4mm), connected with microliter syringes (Scientific Glass Engineering (SGE) GmbH, Darmstadt, Germany) via 50cm flexible FEP (fluorethylene-propylene) tubes (external diameter 0.65mm), were inserted into the four guide cannulae and left in place for 60s before starting the infusion (**Fig.10**).

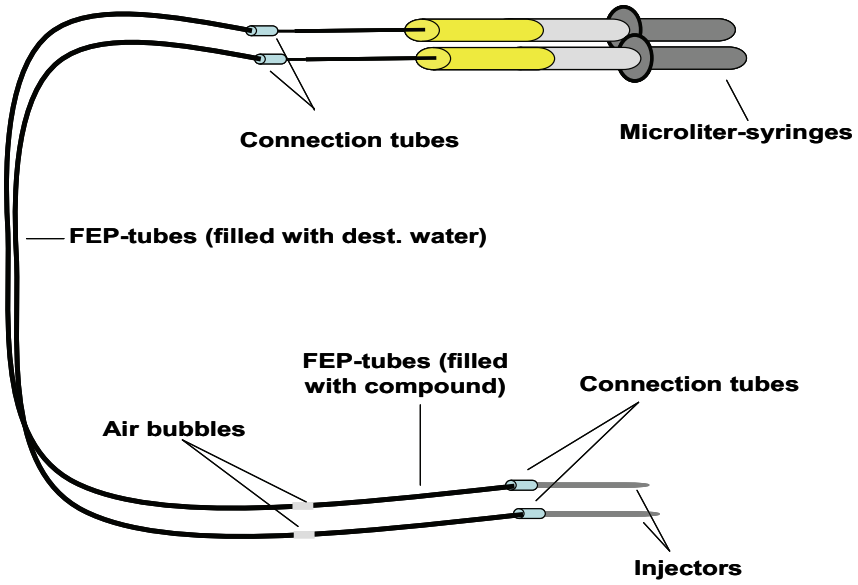

Figure 10 Schematic illustration of the 2x-syringe-system for intracranial microinfusion. All infusions were given in the behavioral testing room and were carried out on unanaesthetized, unrestrained rats.

Simultaneous bilateral infusion of 0.3µl compound solution into the OFC and BLA followed, while 0.1µl compound was injected over a period of 30s. The injectors were left in place for a further minute to allow the solution to diffuse in the local vicinity of the injector tip. Afterwards, the injectors were removed carefully and the obturator plugs replaced. The rats were directly placed into the test chambers and the 5-CSRTT started immediately. Parenchymal infusion of the solutions was controlled via movement of assigned bubbles within the injection tubes.

4.2.5 Experimental design

The parameters of the task during drug testing were identical to those used during training (stimulus duration= 1s, ITI= 5s, limited hold= 5s). Due to the fact that rats should obtain a maximum of four infusions into the same brain area in order to minimize effects of mechanical tissue damage, we run three experiments where animals were divided into different treatment groups (**Tab.2**).

Table 2 Combination of drug administration divided into three different treatment groups.

Group 1 (n= 13)

OFC	BLA
Vehicle	DOI
DOI	Vehicle
DOI	DOI
Vehicle	Vehicle

Group 2 (n= 7)

OFC	BLA
Vehicle	Ketanserin
Ketanserin	Vehicle
Ketanserin	Ketanserin
Vehicle	Vehicle

Group 3 (n= 12)

OFC	BLA
DOI	Ketanserin
Ketanserin	DOI
Vehicle	Vehicle

Microinfusions into the OFC and BLA were done simultaneously within each group (BLA= basolateral nucleus of the amygdala; OFC= orbitofrontal cortex).

The bilateral infusions of the compounds into the OFC and the BLA were carried out simultaneously via two 2x-syringe-systems (see **Fig.10**). These syringe-systems provided the accomplishment of four infusions at the same time. Depending on the particular treatment group, rats received combined bilateral infusions of the compounds DOI and ketanserin or saline according to a latin-square design. The microinfusion procedure started after the animals showed a stable baseline performance over five consecutive test sessions (accuracy >70%, omissions <20%) and continued every other day, whilst the days between drug testing were used for normal training to re-establish the animals' baseline performance. Admittedly, baseline performance was not affected by the microinfusion procedure at any time.

4.2.6 Histology

Upon completion of the behavioral experiments, subjects were anaesthetized with a lethal dose of chloral hydrate (720mg/kg i.p.) and perfused transcardially with 0.01M phosphate buffered saline (PBS) followed by 4% paraformaldehyde (Formaldehyde (37%) in 0,2M phosphate buffer, pH 7,4; 4°C; Serva Electrophoresis GmbH, Heidelberg, Germany). For sufficient perfusion pressure the two perfusion bottles (PBS and paraformaldehyde) were placed 100-120cm above the overdosed animal, which was secured in a supine position on a paraffin wax block. An incision was made right below the thorax approximately 8cm along the sternum. The exposed end of the sternum was grasped with a hemostat, and the diaphragm was cut laterally on both sides. Additional cranial cutting across the ribs and parallel to the lung allowed access to the thorax. Adjacent, a catheter needle which was connected to the perfusion bottles via two perfusion tubes was inserted directly into the left ventricle of the rats` heart and clamped in place. At last the perfusion tube leading to the PBS bottle was opened, and the right cardiac auricle was punctured, allowing the escape of return circulation. After washing out the blood of the systemic circulation, perfusion was switched from PBS to paraformaldehyde to assure fixation of the tissue.

The perfused brains were removed from the skull and immersed in 30% sucrose in 0.1M phosphate buffer (pH 7.4) for 24h minimum. Coronal sections of 50µm in range of the OFC and BLA were cut on a freezing microtome (Jung CM 3000, Leica Instruments GmbH, Nussloch, Germany) at a temperature of -20°C, and kept in 50mM PB (pH=7.6). Sections were put in a gelatine-chromalaun solution and transferred on object slides. For

rehydration, sections were placed into a descending sequence of alcohol (96%, 80%, 70%, 50% ethanol and aqua dest. 3min each), Nissl-stained with Thionin (Sigma-Aldrich, Steinheim, Germany) for 70-90s, dehydrated in an ascending sequence of alcohol (50%, 70%, 80%, 96% ethanol), and fixed in terpineole and Rot-Histoli (Carl Roth GmbH & CO., Karlsruhe, Germany). Finally sections were covered with Entellan (Merck, Darmstadt, Germany) and cover slips. All cannulae locations were histological verified using a light microscop (Axioskop 2 mot., Carl Zeiss, Göttingen, Germany) and injection sites were mapped onto schematic standardized coronal sections of a rat brain stereotaxic atlas (PAXINOS &WATSON 1998).

4.3 Assessment of impulsive decision-making (delay aversion)

4.3.1 Apparatus

Experiments were performed in a T-maze that was build of grey plastic (**Fig.11**). It consisted of a starting runway (60cm lenght), a choice area and two goal arms (58cm length), where at the end of each arm a metal food well was inserted into the floor (goal area).

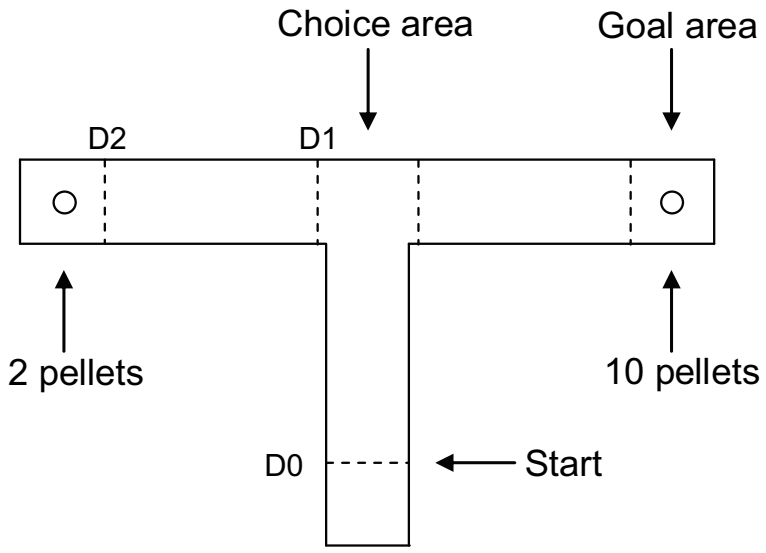

Figure 11 Schematic representation of the delay-based decision-making apparatus (T-maze). Guillotine doors are marked as D0, D1 and D2.

Width of all arms was 15cm, height 30cm. The maze was elevated 80cm above the floor. At the entry of the starting runway (D0), at the choice area (D1) and at each end of the arms in front of the goal areas (D2), removable, intransparent guillotine doors were installed which allowed the closing of each area. One of the goal areas (left or right, depending of the rats`choice) was always provided with a large reward (ten food pellets), whereas the other one was rewarded with a small reward (two food pellets). The food pellets were placed in the food wells before each trial was initiated.

4.3.2 General procedure

The experimental procedure was similar to the ones used in previous studies which revealed effects of different drugs in the delay-based decision-making task (THIÉBOT ET AL. 1985; BIZOT ET AL. 1988, 1999, 2007). During two 5min habituation sessions, five to ten food pellets were placed in the food wells of the T-maze to encourage the animals to explore the apparatus.

Pre-training In the pre-training sessions one arm of the maze (left or right, depending of the rats`choice) was always provided with a large reward. After closing doors in front of the goal areas (D2), the rat was placed in the starting runway. Immediately after passing the choice area and entering one of the two arms, a door was closed behind the rat (D1). Simultaneously, the door at the goal area was opened. As soon as the rat entered the goal area, it was closed again. After all pellets were consumed, the rat was replaced in its home cage for an intertrial interval (ITI) of 1-2min. Animals underwent pre-training sessions of seven trials (one session per day) until they selected the arm provided with the large reward in more than 70% of the trials (5 of 7).

Training Like in the pre-training, animals had to perform seven-trial training sessions (one session per day), where a delay was introduced before accessing the large reward. After closing doors in front of the goal areas (D2), the rat was placed in the starting runway. Immediately after entering on of the two arms, a door was closed behind the animal and the choice area (D1). By choosing the arm provided with the small reward the door at the goal area was opened immediately. In contrast, the door at the goal area leading to the large reward was only opened after a delay of 10s. When animals reached the baseline criterion, choosing the large reward in more than 70% of the trials, the test sessions began. Since the task was performed as a forced choice paradigm, animals

received a reward after performance of any trial (large or small, depending on the animals' choice). Consequently, there were no missed trials during the entire study.

4.3.3 Experimental design

Experiment 1 After showing a stable baseline performance over five consecutive test sessions, rats were tested over six serial daily sessions of seven trials each with three different doses of DOI and ketanserin (0.1, 0.3 and 0.5mg/kg subcutaneously administered according to a latin-square design) in the delay-based decision-making task. As indicated in **Fig.12** Drug testing continued every other day, whilst the days between drug testing were used for normal training sessions to re-establish the animals' baseline performance (more than 70% choice of the large, delayed reward).

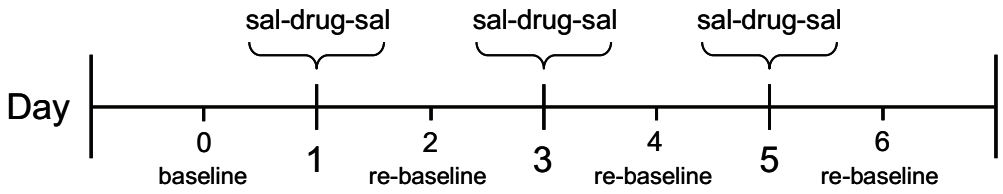

Figure 12 Experimental procedure of drug testing illustrated in a time-table. Drug testing began after stable baseline performance. On drug testing days animals were tested six times (two control-pre sessions, two drug sessions and two control-post sessions). The intervening days were used to re-establish the baseline performance (more than 70% choice of the large, delayed reward).

Drug testing started with DOI and continued with ketanserin after a wash-out-period of at least one week had elapsed. To prevent possible contamination of following drug sessions, animals were required to perform pre- and post-drug sessions with saline before getting tested with different drug doses on the other days. 30min before starting the first two sessions, animals received injections of saline (control-pre sessions). Likewise, injections of DOI and ketanserin were given 30min before the following two sessions (drug sessions). Finally saline injections were administered 30min before the last two sessions (control-post sessions). Taken together, animals received a total of six injections on each drug testing

day, i.e. one injection before each test session. Thus, animals were required to perform six test sessions on each of the three drug testing days.

Experiment 2 A second group of rats (n=6) was subjected to the task in the absence of any delay of reward under control-pre, drug (DOI, ketanserin: 0.1, 0.3, 0.5mg/kg) and control-post conditions. Drug testing started after achievement of a stable baseline performance over five consecutive daily sessions (more than 70% choice of the large reward). Like in *experiment 1* drug testing started with DOI, whereas a wash-out-period of at least one week elapsed until the testing with ketanserin was initiated.

Experiment 3 To test whether ketanserin blocks the effects of DOI, another group of rats (n=8) was subjected to the task after combined injections of DOI and ketanserin under conditions as described in *experiment 1*. In this test ketanserin was injected 2min prior to DOI (KOSKINEN ET AL. 2000B). The administration of the three different doses of DOI and ketanserin (0.1, 0.3 and 0.5mg/kg for both compounds) was performed according to a latin-square design. In this experiment, animals received a total of 12 injections on each drug testing day, two injections before each test session (two saline injections or DOI and ketanserin injection). Thus, animals were required to perform six test sessions on each of the three drug testing days.

In all experiments animals received the injections (saline, DOI, ketanserin, DOI and ketanserin) immediately after completing the last trial of the respective session. Thus, the time interval between two testing sessions was always 30min.

4.4 Drugs

Ketanserin tartrate and DOI hydrochloride were purchased from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany) (**Fig.13**).

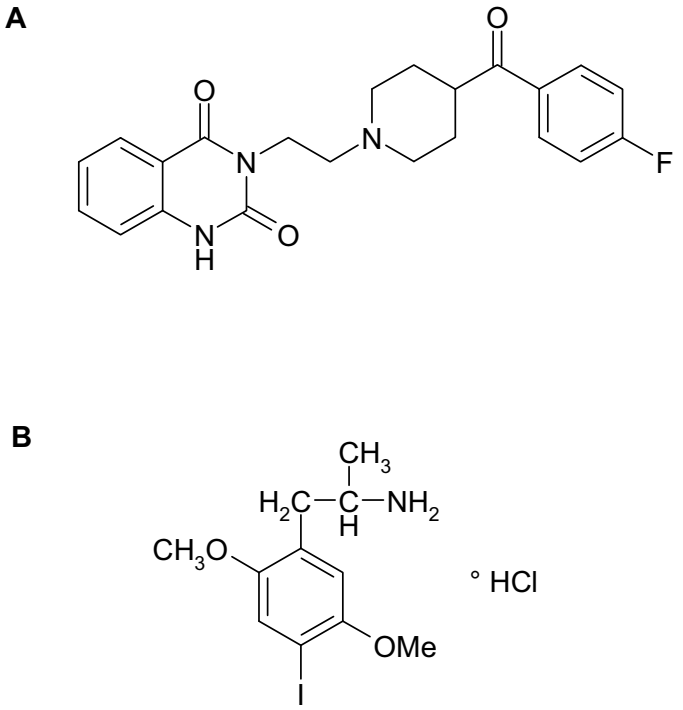

Figure 13 Chemical structures of the 5-HT-receptor ligands ketanserin (A) and DOI (B).

The selective $5HT_{2A/C}$-receptor agonist DOI and ketanserin, a selective $5HT_{2A}$-receptor antagonist, were dissolved in 0.9% saline. All drug doses are expressed in terms of the free base. Both solutions were made freshly on each treatment day. The form of application was dependent of the task used. Drugs were administered subcutaneously (s.c.) in a volume of 1ml/kg bodyweight at doses of 0.1, 0.3 and 0.5mg/kg in the delayed reward task, and intracranial bilaterally at a dose of 5µg/0.3µl in the 5-CSRTT. The 5-HT-receptor antagonist ketanserin was used in the present study because it is approximately 1000-fold more selective for 5-HT_{2A}- receptors than for 5-HT_1-receptors. Furthermore, it is 100-fold more selective for 5-HT_{2A}-receptors than 5-HT_{2C}-receptors. To a minor extent ketanserin also binds to H_1-histamine- and α_1-adrenergic-receptors (LEYSEN ET AL. 1985; HOYER ET AL. 1994).

4.5 Statistical analysis

All statistical analyses were conducted using SigmaStat (Sigma Aldrich, Statistical Software, Version 2.0) for Windows. According to the experiments conducted with the 5-CSRTT following measures were investigated: the percentage of correct responses made (number of correct responses/total correct and incorrect responses); percentage of responses omitted (number of omissions/total number of correct, incorrect, and omitted responses); percentage of premature responses (number of premature responses/total number of correct, incorrect, and omitted responses), latency to make a correct response, latency to collect reward, perseverative responses and the total number of trials completed . Within each injection group data of the mentioned measures were analyzed using repeated-measures one-way analysis of variance (ANOVA), followed by post hoc Tukey`s *t*-tests for pairwise comparisons. Level of significance was set at $p<0.05$.

Likewise, dose-dependent effects of DOI and ketanserin on arm-choice in the delayed reward task were statistically evaluated within each treatment group using analyses of variance (ANOVAs). Comparisons were done between the mean total number of choices of the large-but-10s-delayed reward during the two control-pre sessions, the two drug sessions and the two control-post sessions within each compound treatment. In case of $p<0.05$, post-hoc a Tukey`s *t*-test for pairwise comparisons followed.

5 RESULTS

5.1 Effects of intra-OFC, intra-BLA infusions of DOI in the 5-CSRTT

Fig.14 shows the localization of injector tips in the OFC and in the BLA upon completion of the experiments of *group 1*. If the cannulae position regarding the OFC and BLA was incorrect the respective animals were excluded from the further evaluation. Rats were also taken out of the evaluation if the histological examination revealed local physical tissue damage beyond or around the injector tract. Usually, repeated injections, according to the experimental design did not cause extensive damage and only some animals showed signs of infection (reactive microglia, extensive tissue loss). Consequently, these subjects were not included in the results. Therefore, all valid injector tips used for evaluation were located in regions of the OFC and BLA.

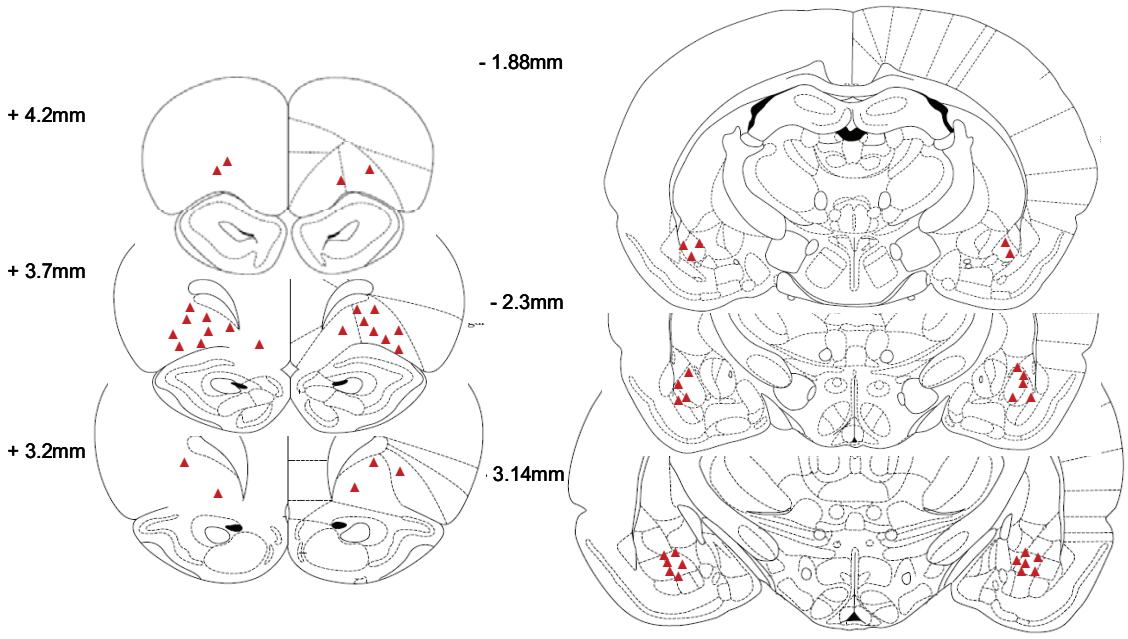

Figure 14 Location of injector tips in the OFC (left hand series) and BLA (right hand series) depicted on schematic drawings according to the atlas of PAXINOS & WATSON (1998) in distance to bregma as indicated by numbers. Injector tips are depicted as red triangles (n=13).

To analyze impulsive behavior in rats, effects of bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist DOI into the OCF and BLA were assessed using the 5-CSRTT task. **Fig.15** shows the percentage scores of accuracy, premature responses and omitted trials. Microinjection of DOI into either the OCF or the BLA, simultaneously with bilateral injection of vehicle into the respective opposite structure did not affect the performance in the 5-CSRTT. However, ANOVA revealed a significant difference in premature responding after simultaneous bilateral infusion of DOI into the OFC and BLA [$F_{(3,36)}$=3.095; P=0.039]. Post hoc Tukey´s *t*-test revealed that DOI significantly increased premature responding compared to vehicle injection. Accuracy and omissions were not affected in this case of injection combination [% accuracy: $F_{(3,36)}$=0.381; P=0.767/ % omissions: $F_{(3,36)}$=0.521; P=0.670] When administered into the OFC, DOI slightly increased premature responding as seen in **Fig.15**. However, this effect did not reach the level of significance.

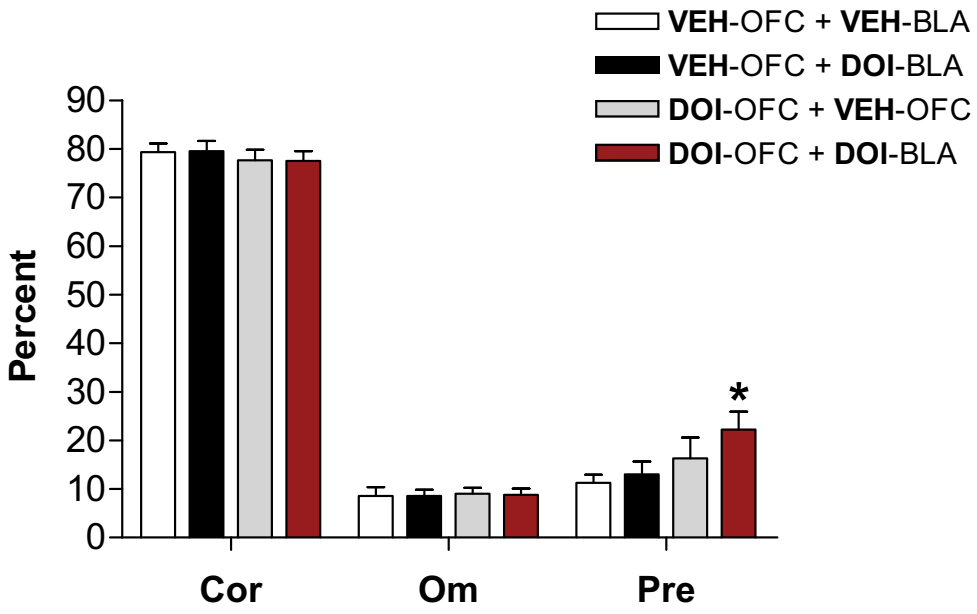

Figure 15 Effect of local bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist DOI (5 µg/0.3 µl) into the OFC and the BLA on impulsive behavior in rats. Bars show percentage scores of accuracy (Cor), omissions (Om), and premature responses (Pre) during performance of the 5-CSRTT task. Data are means ±S.E.M.. Significant differences between vehicle and DOI are indicated as asterisks (n=13; post-hoc Tukey´s *t*-test, $p<0.05$).

Tab.3 indicates that the additionally recorded measures, i.e. the latency to make a correct response, the latency to collect the reward, perseverative responses and the number of trials completed were also not affected in this case of combined drug treatment.

Table 3 Effects of local bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist DOI (5 µg/0.3 µl) into the OFC and the BLA on additional measures in the 5-CSRTT in rats.

Drugs	Perseverative responses	Correct latency	Panel latency	Trials completed
VEH+VEH	1.692 ± 0.382	0.887 ± 0.038	1.228 ± 0.089	100 ± 0
VEH + DOI	1.538 ± 0.606	0.855 ± 0.027	1,234 ± 0.076	100 ± 0
DOI+VEH	2.462 ± 0.666	0.875 ± 0.038	1.249 ± 0.078	96.692 ± 2.257
DOI+DOI	2.462 ± 0.573	0.876 ± 0.026	1.231 ± 0.09	95.692 ± 2.688
ANOVA;F[3,36]	1.474	0.430	0.148	2.035
P		>0.1		

Each first mentioned compound in the table description was injected into the OFC, each second mentioned into the BLA; VEH= vehicle. Data are means ±S.E.M. (n=13).

5.2 Effects of intra-OFC, intra-BLA infusions of ketanserin in the 5-CSRTT

Fig.16 shows the localization of injector tips in the OFC and in the BLA upon completion of the experiments of *group 2*. Animals were taken out from the further evaluation if the cannulae position regarding the OFC and BLA was incorrect or if the histological examination revealed local physical tissue damage beyond or around the injector tract. Repeated injections according to the experimental design did not cause extensive damage. All injector tips used for evaluation were located in regions of the OFC and BLA. Contrary to the other two testing groups, the number of animals in this group was diminished. This fact is attributed to the not happened re-establishment of the baseline performance (>70% correct, <20% omissions) between the drug testing days, which was set as criterion for further drug-testing. Five of twelve animals showed this phenomenon and had to be removed from the study.

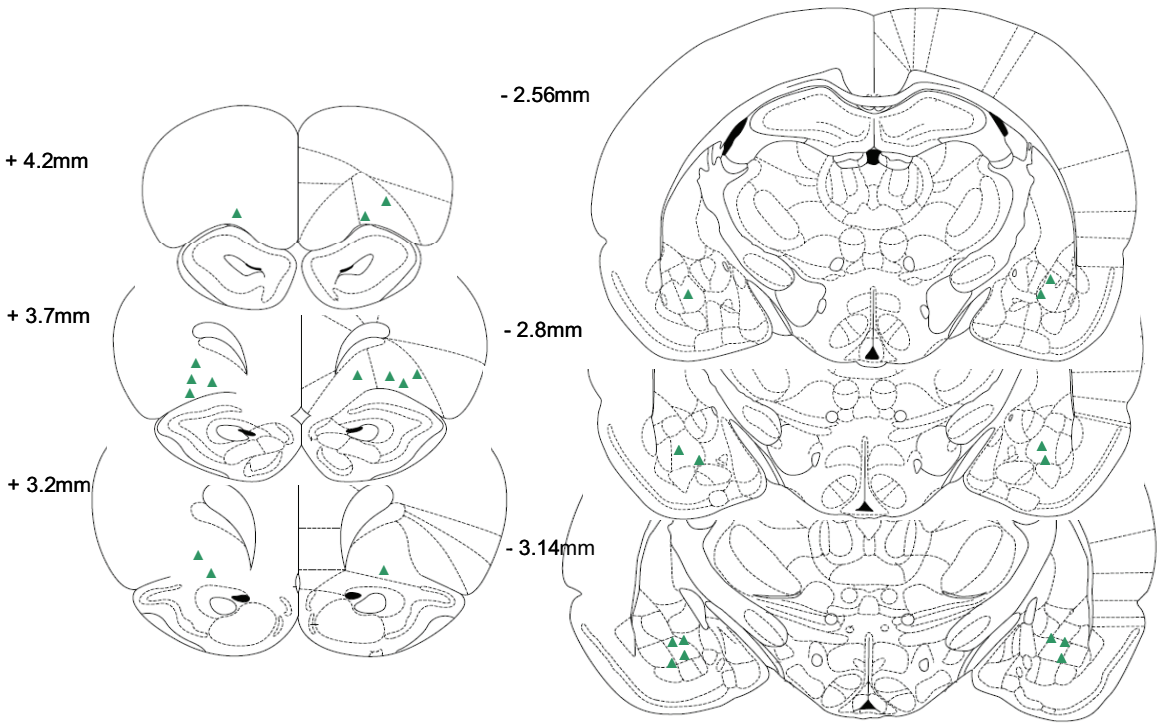

Figure 16 Location of injector tips in the OFC (left hand series) and BLA (right hand series) depicted on schematic drawings according to the atlas of PAXINOS & WATSON (1998) in distance to bregma as indicated by numbers. Injector tips are depicted as green triangles (n=7).

In *group 2* effects of bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist ketanserin into the OCF and BLA on impulse control were investigated. **Fig.17** shows the percentage scores of accuracy, premature responses and omitted trials. Bilateral microinjection of ketanserin into either the OCF or the BLA together with injection of vehicle into the respective opposite structure did not affect the performance of the 5-CSRTT. Compared to vehicle, simultaneous infusion of ketanserin into the OCF and into the BLA slightly decreased accuracy but also marginally increased premature responding as seen in the figure. However, ANOVA revealed that non of the effects reached the level of significance [% accuracy: $F_{(3,18)}$=2.411; P=0.1, % premature responses: $F_{(3,18)}$=0.469; P=0.707]. Omissions were also not affected in this case of injection combination [$F_{(3,18)}$=0.691; P=0.569].

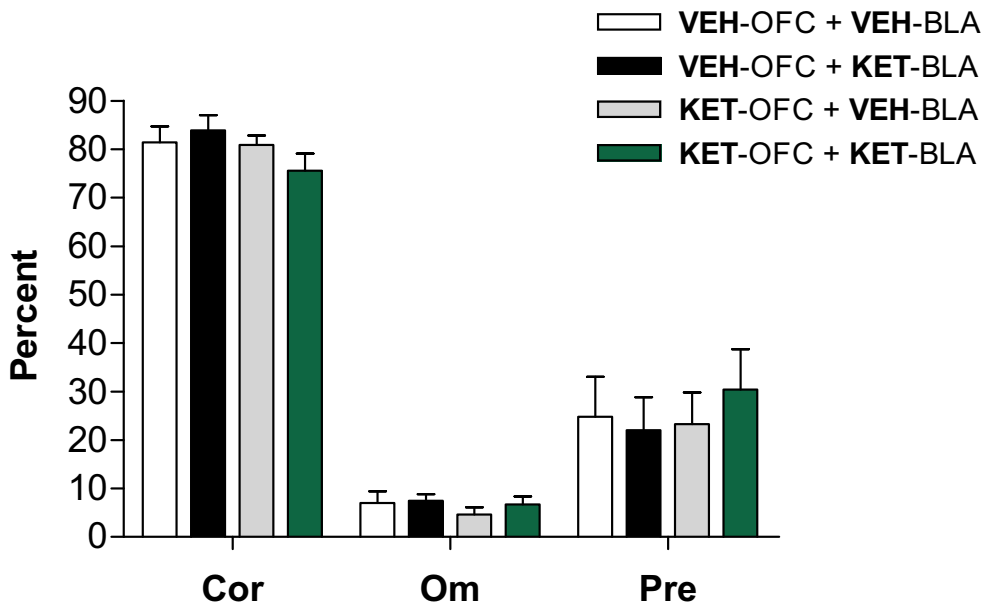

Figure 17 Effects of local bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist ketanserin (5µg/0.3µl) into the OFC and the BLA on impulsive behavior in rats. Bars show percentage scores of accuracy (Cor), omissions (Om), and premature responses (Pre) during performance of the 5-CSRTT. VEH= vehicle; KET= ketanserin. Data are means ±S.E.M. (n=7).

Tab.4 indicates that the additionally recorded measures i.e. the latency to make a correct response, the latency to collect the reward, perseverative responses and the number of trials completed were not affected in this case of combined drug treatment.

Table 4 Effects of local bilateral infusion of the 5-$HT_{2A/C}$-receptor antagonist ketanserin (KET; 5µg/0.3µl) into the OFC and the BLA on additional measures in the 5-CSRTT in rats.

Drugs	Perseverative responses	Correct latency	Panel latency	Trials completed
VEH+VEH	3.286 ± 1.658	0.844 ± 0.058	1.217 ± 0.057	98.857 ± 1.143
VEH + KET	3.429 ± 1.043	0.875 ± 0.026	1,227 ± 0.057	98.571 ± 1.020
KET+VEH	3.857 ± 2.123	0.852 ± 0.031	1.177 ± 0.045	99.857 ± 0.143
KET+KET	1.143 ± 0.404	0.88 ± 0.031	1.254 ± 0.065	96.286 ± 3.714
ANOVA;F[3.18] **P**	1.396	0.232 >0.1	1.396	0.664

Each first mentioned compound in the table description was injected into the OFC, each second mentioned in the BLA. VEH = vehicle; KET = ketanserin. Data are means ±S.E.M. (n=7).

5.3 Effects of intra-OFC, intra-BLA infusions of ketanserin and DOI in the 5-CSRTT

Fig.18 shows the localization of injector tips in the OFC and in the BLA upon completion of the experiments of *group 3*. Similar to *group 1* and *2,* animals were excluded from further evaluation if the cannulae position was incorrect or if histological examination revealed local physical tissue damage beyond or around the injector tract. The majority of the injector tips were confined to the intended regions of interest the OFC and the BLA. Usually, repeated injections according to the experimental design did not cause extensive tissue damage and only some animals showed signs of infection (reactive microglia, extensive tissue loss). Consequently, these subjects were not included in the results. Therefore, all valid injector tips used for evaluation were located in regions of the OFC and BLA.

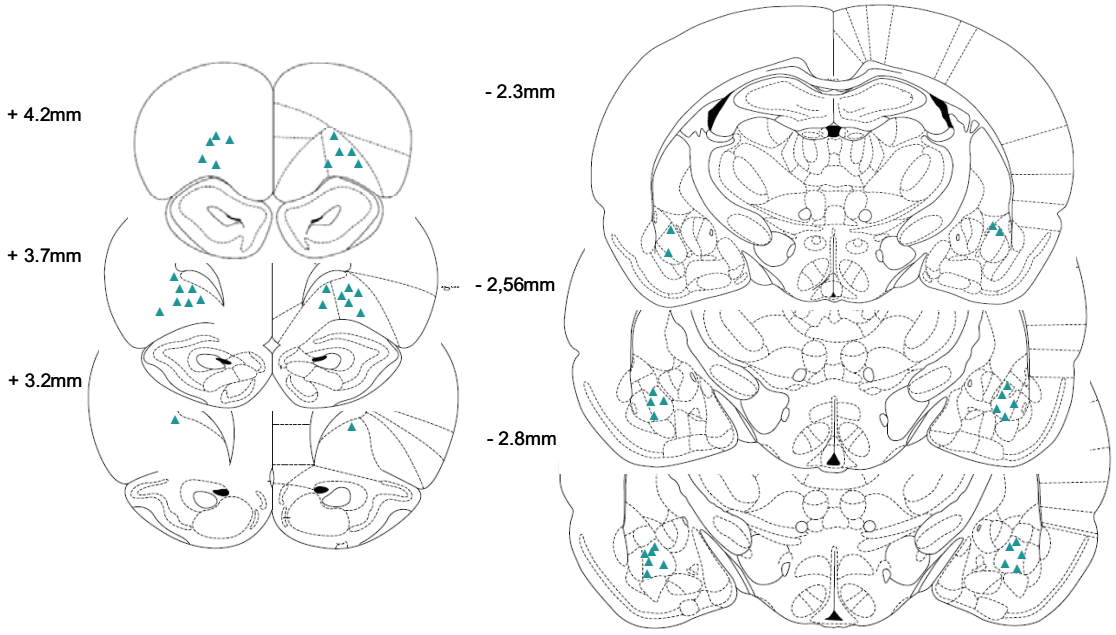

Figure 18 Location of injector tips in the OFC (left hand series) and BLA (right hand series) depicted on schematic drawings according to the atlas of PAXINOS & WATSON (1998) in distance to bregma as indicated by numbers. Injector tips are depicted as blue triangles (n=12).

This third part of the investigation on impulsive action analyzed possible effects of simultaneous bilateral infusion of ketanserin into the OCF and DOI into the BLA and vice

versa, while assessing performance in the 5-CSRTT. **Fig.19** shows the percentage scores of accuracy, omissioned trials and premature responses. Compared to vehicle, the drug administration of simultaneous bilateral infusion of ketanserin into the OCF and infusion of DOI into the BLA slightly decreased premature responding. Likewise, this drug combination infused vice versa only showed a modest decrease in premature responding. Admittedly, neither one the effects reached the level of significance [ANOVA; $F_{(2,22)}$=1.552; P=0.234]. Just as in *group 1* accuracy and omissions were not affected in this case of injection combination [% accuracy: $F_{(2,24)}$=0.0904; P=0.914, % omissions: $F_{(2,24)}$=0.259; P=0.774].

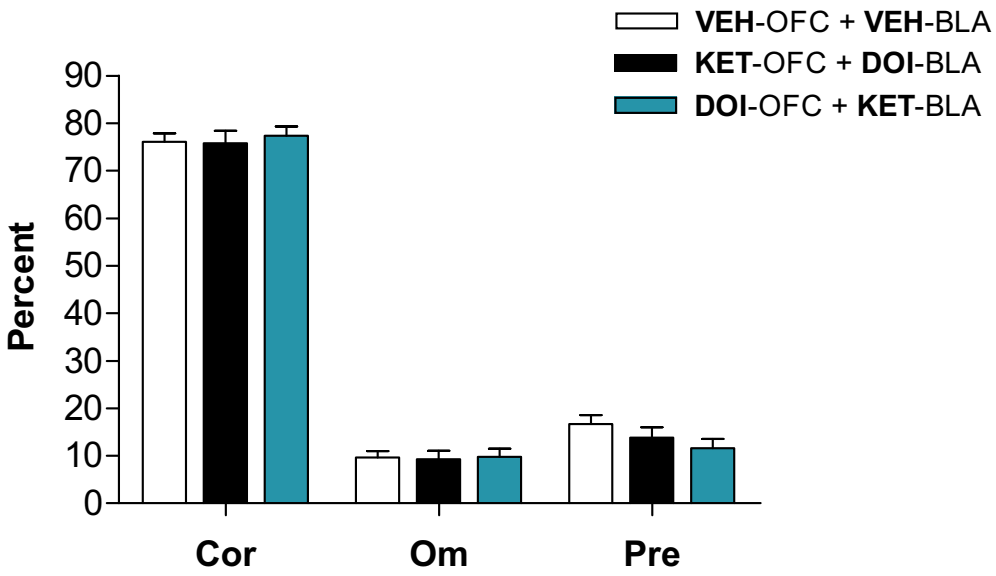

Figure 19 Effects of local bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist DOI (5µg/0.3µl) into the OFC and ketanserin (5µg/0.3µl) into the BLA and vice versa on impulse control in rats. Bars show percentage scores of accuracy (Cor), omissions (Om), and premature response (Pre) during the 5-CSRTT. VEH= vehicle; KET= ketanserin. Data are means ±S.E.M. (n=12).

Furthermore, **Tab.5** indicates that the additionally recorded measures such as the latency to make a correct response, the latency to collect the reward, perseverative responses and the number of trials completed were also not affected in this case of combined drug injection.

Table 5 Effects of local bilateral infusion of the 5-$HT_{2A/C}$-receptor agonist DOI (5µg/0.3µl) into the OFC and ketanserin (5µg/0.3µl) into the BLA and vice versa on impulse control in rats.

Drugs	Perseverative responses	Correct latency	Panel latency	Trials completed
VEH+VEH	4.667 ± 2.186	0.914 ± 0.039	1.349 ± 0.071	97.083 ± 2.227
KET + DOI	4 ± 1.966	0.88 ± 0.031	1,357 ± 0.079	99.417 ± 0.583
DOI + KET	4.083 ± 1.535	0.907 ± 0.0416	1.249 ± 0.078	98.750 ± 1.25
ANOVA;F[2,22]	0.303	0.895	0.33	0.654
P		>0.1		

Each first mentioned compound in the table description was injected into the OFC, each second mentioned in the BLA. VEH= vehicle; KET= ketanserin. Data are means ±S.E.M (n=12).

5.4 Effects of DOI in the delay-based decision-making task

Treatment with DOI decreased the number of choices of the large delayed reward in a dose dependent manner (**Fig.20**). The percentage choice of the large delayed reward was significantly different between pre-control sessions, drug sessions and post-control sessions in the DOI treatment group at doses of 0.1mg/kg [$F_{(2,22)}$=5.044; P=0.016], 0.3mg/kg [$F_{(2,22)}$=20.967; P<0.001], and 0.5mg/kg [$F_{(2,22)}$=38.209; P<0.001].

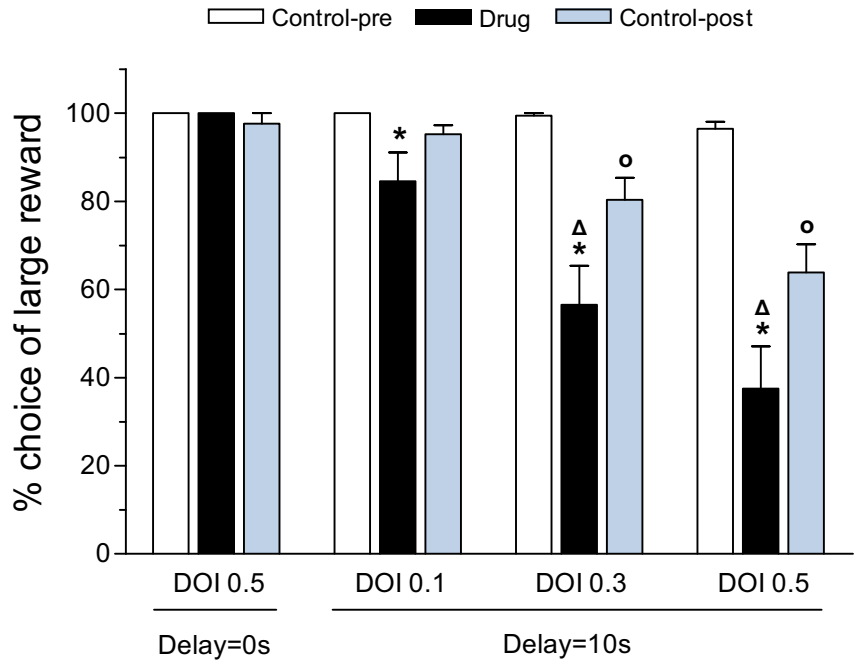

Figure 20 Effects of DOI (mg/kg) on the choice of the large reward in rats. *Left-hand series* of histograms: large reward without delay (n=6); *three right-hand series* of histograms: large reward delayed by 10s (n=12). Bars show the percentage choice of the large reward during control-pre sessions, drug sessions after DOI injection and control-post sessions. Data are means ±S.E.M.. Significant differences between drug sessions and control-pre sessions are indicated as asterisks, differences between drug sessions and control-post sessions as triangles, differences between control-pre and control-post sessions as circles (post hoc Tukey`s *t*-test $p<0.05$).

Post hoc Tukey`s *t*-test revealed that DOI decreased the number of choice of the large delayed reward compared to pre-control sessions (vehicle) at doses of 0.1mg/kg (p=0.014), 0.3mg/kg ($p<0.001$) and 0.5mg/kg ($p<0.001$). Likewise, DOI decreased the percentage choice of the large delayed reward compared to post-control sessions (vehicle) at doses of 0.3mg/kg (Tukey`s *t*-test: $p$=0.005) and 0.5mg/kg (Tukey`s *t*-test: p=0.002). Furthermore,

the percentage choice of the large delayed reward was significantly reduced in post-control sessions compared to pre-control sessions at doses of 0.3mg/kg (p=0.023) and 0.5mg/kg DOI (p<0.001), indicating a dose related carry-over effect of DOI. In some animals treated with 0.5mg/kg DOI, a distinct reduction of motor activity was observed but not quantified.

5.5 Effects of ketanserin in the delay-based decision-making task

The baseline performance before ketanserin treatment was marginally decreased compared to the baseline performance before DOI treatment.

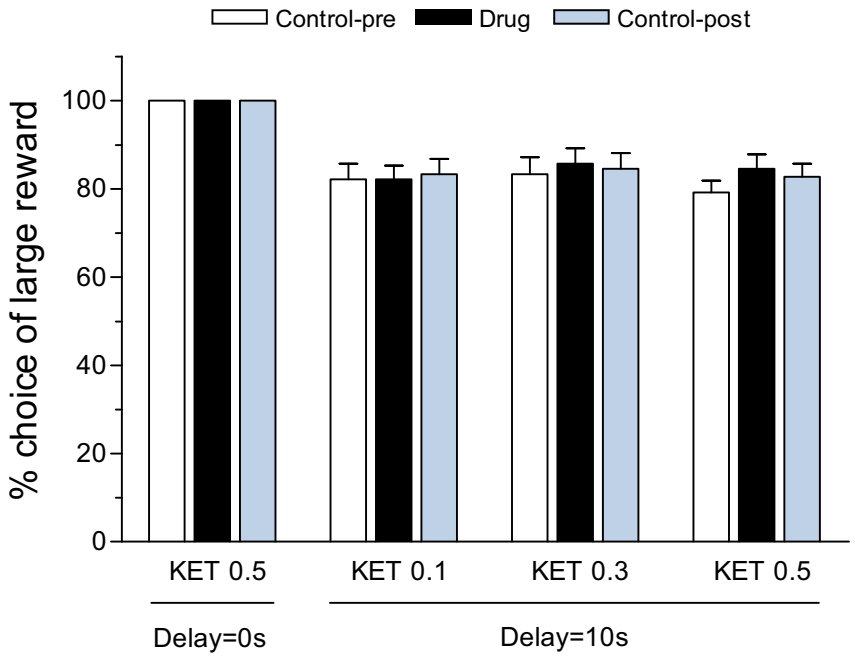

Figure 21 Effects of ketanserin (KET, mg/kg) on the choice of the large reward in rats. *Left-hand series* of histograms: large reward without delay (n=6); *three right-hand series* of histograms: large reward delayed by 10s (n=12). Bars show the percentage choice of the large reward during control-pre sessions, drug sessions after ketanserin injection and control-post sessions. Data are means ±S.E.M..

However, since the baseline criterion of more than 70% choice of the large delayed reward was still accomplished, the conditions for testing were not affected. As indicated in **Fig.21**, ketanserin had no significant effect on the percentage choice of the large delayed reward in pre-control sessions, drug sessions and post-control sessions at doses of 0.1mg/kg

[$F_{(2,22)}$=0.478; P=0.626], 0.3mg/kg [$F_{(2,22)}$=2.200; P=0.135], and 0.5mg/kg [$F_{(2,22)}$=2.783; P=0.084]. In the absence of a delay neither DOI nor ketanserin (each drug 0.1, 0.3, 0.5 mg/kg) had an effect on the choice of the large reward (only data for the highest dose 0.5 mg/kg are shown in **Figs.20** and **21**).

5.6 Effects of DOI vs. ketanserin in the delay-based decision-making task

As indicated in **Fig.22**, the effect of DOI described in *experiment 1* was antagonized by ketanserin at doses of 0.1mg/kg [$F_{(2,22)}$=0.478; P=0.626], 0.3mg/kg [$F_{(2,22)}$=2.200; P=0.135], and 0.5mg/kg [$F_{(2,22)}$=2.783; P=0.084]. The selective 5-HT_{2A}-receptor antagonist ketanserin was used for the combination study with DOI because it had no effect on its own (see *experiment 1*).

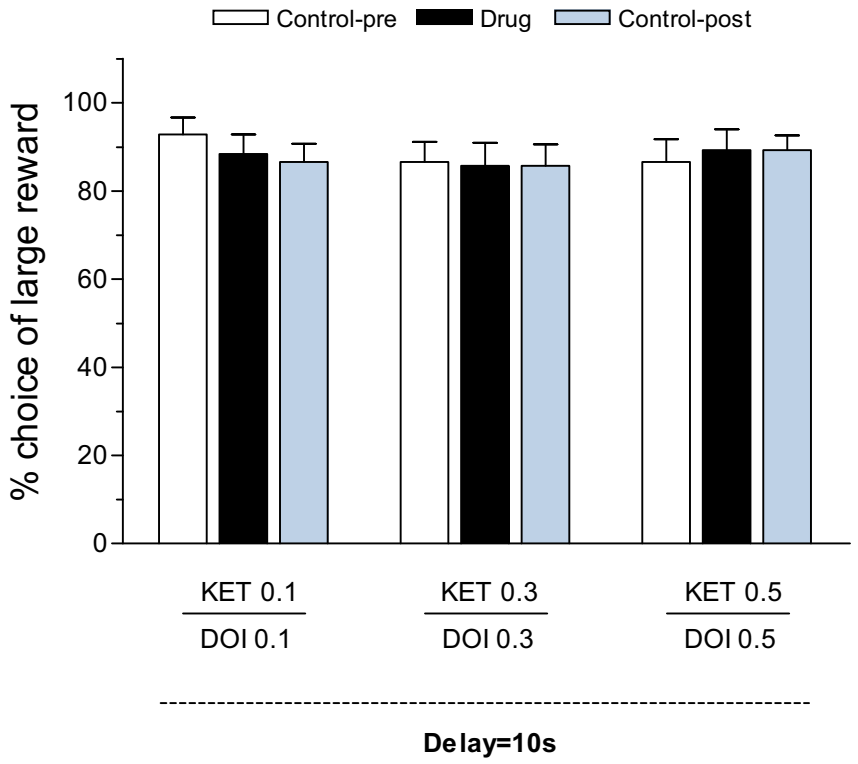

Figure 22 Effects of combined administration of ketanserin (KET, mg/kg) and DOI (mg/kg) on the choice of the large reward in rats (n=8). Bars show the percentage choice of the large reward (delayed by 10s) during control-pre sessions, drug sessions after combined ketanserin-DOI injection and control-post sessions. Data are means ±S.E.M..

6 DISCUSSION

In preclinical research into the neurobiology of neuropsychiatric disorders, animal models provide the possibility to investigate the neural basis of behavioral malfunctions. Admittedly, it is neither possibly to display specifically human aspects, such as personality or language, nor the disease in its entirety. However, substantial aspects of neuropsychiatric disorders are indeed feasible in valid animal models, whereas the validity is exemplified by three distinct criteria: (1) *face validity* postulates the comparability of behavior between human and animal, (2) *predict validity* shows that clinical effective compounds are also effective in the respective animal model, (3) *construct validity* indicates that neural constructs, which may be responsible for pathological states, can be copied in animal models via similar incidents such as neurotoxin-induced brain lesions (KOCH 2006).

Beside the achieved data, the present work introduced and established two new behavioral paradigms in the laboratory. With the 5-CSRTT and the delay-based decision-making task in a T-maze, two methods are available to asses both, impulsive action and delay aversion in rodents. Because of their almost identical adoption from originally human neuropsychological tasks these animal models show a high level of validity. The following discussion will demonstrate that the 5-CSRTT and the delay-based decision-making task in a T-maze provide excellent possibilities to examine neural substrates as well as neuropharmacological interactions of impulsive behavior.

PHILLIP SOUBRIÉ suggested that the 5-HT-system plays a key role in the regulation of behavioral inhibition and impulsivity (SOUBRIÉ 1986). Consistent with previous data, the present study supports this hypothesis of the involvement of the 5-HT-system in impulsive-related behavior in rats (BIZOT ET AL. 1988; THIÉBOT ET AL. 1985). High levels of impulsive behavior are associated with a number of psychiatric disorders, whereas the definition of impulsivity is controversial in so far as there is not just one type of impulsive behavior. JOHN EVENDEN proposed that impulsivity is not a unitary construct, but a set of diverse and complex behaviors, such as distractibility, aggressiveness, inability to plan, inability to inhibit inappropriate responses, and impaired executive functions (EVENDEN 1999A,C).

A considerable amount of data suggests that decreased 5-HT-function can enhance or reduce impulsive behavior, depending on the 5-HT-receptor subtypes and the behavioral tasks used (WINSTANLEY ET AL. 2006). However, due to the fact that different tests of impulsivity probe different cognitive processes (EVENDEN AND RYAN 1999) it is still unclear what generalizations towards 5-HT_2-receptor involvement can be made on the basis of measurements of impulsive behavior.

Summarized, the present work revealed that 5-$HT_{2A/C}$-receptors are crucially involved in mediating impulsivity in both, impulsive action and impulsive decision-making or delay aversion. However, the fact that the functional status of the mesocortico-limbic DA-system is partially mediated by the 5-HT-system suggests that DA may play a regulatory role in impulsivity as well. Regarding to the involvement of neural substrates in impulsivity, it was shown that the OFC, the BLA, and presumably the network these structures form are inter alia responsible for mediating impulsive behavior through activation and/or inhibition of 5-$HT_{2A/C}$-receptors. Overall, the present work reveals new aspects on the neuropharmacological interactions as well as on the neural correlates involved in different tasks assessing impulsive behavior in rodents.

6.1 Impulsive action

In the first part of the study, using local intra-cerebral microinjections, we were able to show that a network comprising the OFC and BLA is important for mediating DOI-induced impulsivity in the 5-CSRTT. Therefore, our data confirm that cortico-limbic 5-$HT_{2A/C}$-receptors are a major component of a regulatory network, comprising the OFC and BLA, necessary for impulse control and response selection in mammals.

The 5-CSRTT was initially developed to investigate effects of various systemic drug administration as well as the effects of specific neurochemical lesions on diverse aspects of attention that are relevant for neurological disorders such as ADHD or schizophrenia (CARLI 1983; ROBBINS 2002). In addition to attention, the 5-CSRTT has recently been used to assess trait impulsivity, whereas this measure has been validated in part by its capacity to predict susceptibility to cocaine reinforcement and by its sensitivity to drugs used in human ADHD-treatment (DALLEY ET AL. 2008; NAVARRA ET AL. 2008). The rat version of the task requires animals to discriminate brief light flashes presented

pseudorandomly in one of five holes and to make a nose-poke response in the correct spatial location in order to receive a reward. Rats have to monitor the horizontal array of apertures and to withhold from responding until the onset of the stimulus (ROBBINS 2002; BARI ET AL. 2008).

Taken together, the 5-CSRTT allows the discrimination between drug effects on attentional processes from those for food motivation and behavioral activation through the recording of many dependent variables, such as accuracy of discrimination, latency to collect reward, latency to respond correct, and the overall tendency to respond (HARRISON ET AL. 1997). The main strengths of the 5-CSRTT are a high level of *construct* and *face validity*, a severe control over behavioral contingencies, accurate and automatized data acquisition and its proven reliability, due to its widespread use in many laboratories (BARI ET AL. 2008). In general, the accuracy of stimulus discrimination in the 5-CSRTT provides an index of attentional capacity, consistent of sustained attention (vigilance), divided spatial attention, and selective attention.

In the present study three groups of male Wistar rats were trained on the 5-CSRTT. Over all three groups tested in different drug administration designs with DOI and ketanserin, good attentional scores were obtained throughout. Normally, the indication for a good attentional performance of the animals is reflected by a high number of correct target detections, few omissions and a relatively fast latency to respond. As indicated in **Figs.15, 17** and **19** all animals showed a high number of correct responses (>75%) and a relatively low number of omitted responses (<10%). Contrary, a low response accuracy (chance performance=20%) would have suggested inattention that may indeed be influenced by other sensory, motor or motivational processes (CHUDASAMA & ROBBINS 2004).

The response times calculated in this task are valuable indices of the speed of information processing, readiness, decision-making and general motivation of the rats. Response times assessed in the present study, such as the latency to collect the reward and the latency to respond correctly were similar in their low values after drug treatment in all three groups and its diverse drug combinations (**Tab.3-5**). High reward collection latency indicates a decrement in the motivation for food, where the response latency reflects the animals' motivation for the task in general (ROBBINS 2002). Thus, as seen in the results, the motivational state of animals was not affected by the compound injections.

Additional nose-poke responses in any of the five apertures or in the food magazine following a correct response were recorded as perseverative responses. An increased in perseverative responding reflects the dysfunction of another inhibitory mechanism engaged by the 5-CSRTT, often interpreted as a form of compulsive behavior (CHUDASAMA & ROBBINS 2004). In the present study none of the combined drug administrations of DOI and ketanserin into the OFC and the BLA had a significant effect on perserverative responding. Thus, compulsive-like behavior in the 5-CSRTT does not seem to be mediated via 5-$HT_{2A/C}$-receptors within the OFC-BLA-network.

Prior to the stimulus presentation, the rat is required to withhold from making inappropriate premature nose-poke responses into the five apertures. An increase in premature responding is particularly interesting. It occurs while the animal is anticipating the occurrence of the visual target, presumably when the ability to control pre-potent responding is impaired. Thus, this measure reflects the rats' inability to wait and provides a valuable index of impulsivity, the tendency to act without foresight, which may be a product of impaired response inhibitory control (BIZOT & THIEBOT 1996; CHUDASAMA & ROBBINS 2004; ROBBINS 2002). The present data show that simultaneous DOI-induced activation of 5-$HT_{2A/C}$-receptors into the OFC and the BLA led to a significant increase in premature responding of rats in the 5-CSRTT compared to vehicle, indicating greater impulsivity.

Global forebrain 5-HT-depletion induced by intra-ventricular administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) consistently increased premature responding in the 5-CSRTT without affecting attention (HARRISON ET AL. 1997; WINSTANLEY ET AL. 2003B). This increase is similar to that seen after 5-HT_{2C}-receptor blockade with the antagonist SB242084 in rats. Thus, the loss of 5-HT-signaling via the 5-HT_{2C}-receptor may contribute to the effects induced by 5-HT-depletion (HIGGINS ET AL. 2003; FLETCHER 2007). Furthermore, previous data revealed that activation of 5-HT_2-receptors with the 5-$HT_{2A/C}$-receptor agonist DOI can enhance (KOSKINEN ET AL. 2000A, B; FLETCHER ET AL. 2007), while blockade with 5-HT_{2A}-receptor antagonists, such as ketanserin (FLETCHER ET AL. 2007; PASSETTI ET AL. 2003) and M100907 (WINSTANLEY ET AL. 2003A) can reduce premature responding in the 5-CSRTT without affecting accuracy when drugs were given systemically. The data of the present study concur with these reports on the effects of drugs when administered systemically. Consistently, we could first of all attest that the expression of impulsive behavior in terms of premature

responding in the 5-CSRTT is likely governed by the balance of activity at multiple 5-HT-receptors, 5-HT_{2A}-receptors in particular, rather than by absolute levels of the neurotransmitter 5-HT. Additionally, the present data provide important supplemental information on the localization of the receptors involved.

Recent studies tried to discover possible neural sites mediating impulsivity and established amongst others that lesions of the OCF and the BLA affect premature and perseverative responding in the 5-CSRTT (CHUDASAMA ET AL. 2003; MOBINI ET AL. 2002; WINSTANLEY ET AL. 2004B; **Fig.1**). Admittedly, the precise circumstances of the neurochemical processes which may underlie these behavioral changes are not fully understood. Previous reports show that directly administered DOI into the anterior cingulate cortex had only minor effects on the performance of rats in the 5-CSRTT (KOSKINEN ET AL. 2000A). Likewise, intra-NAc infusion of DOI failed to alter premature responding (KOSKINEN & SIRVIO 2001). These loci alone seem therefore not to be the primary site for DOI to induce premature responding.

To mention antagonistic investigations, intra-PFC-infusions of selective 5-HT_{2A}-(M100907) or 5-HT_{2C}-(SB242084) receptor ligands did not reveal any major effects, whereas local blockade of selective 5-HT_{2A}- and 5-HT_{2C}-receptors with these compounds in the NAc induces a decrease and increase in premature responding, suggesting opposing effects of the two receptor subtypes on the neurochemical processes that underlie impulsive behavior (ROBINSON ET AL. 2008). Until present, it is not known definitively which brain regions mediate the effects of 5-$HT_{2A/C}$-receptors in impulsivity, but earlier studies suggest that multiple regions are involved (TALPOS ET AL. 2006).

With regard to possible brain regions that may be involved in mediating DOI-induced impulsivity in the 5-CSRTT, the present work favours the OFC, the BLA or rather the network these structures presumably form as important neural sites. We could show that DOI-induced activation of 5-$HT_{2A/C}$-receptors neither in the OFC nor in the BLA alone affects impulsive responding or accuracy under baseline task conditions. Likewise, blockade of selective 5-HT_{2A}-receptors in the OFC and the BLA after ketanserin infusion has no effect on the task performance, indicating that none of these receptors in the OFC and in the BLA play a critical role in performing the 5-CSRTT.

In contrast, simultaneous DOI-induced activation of these receptors in both, the OFC and the BLA, lead to an increase in impulsive responding. Therefore, we assume the existence of a prominent interaction between the OFC and the BLA, which seems to be

involved in mediating DOI-induced impulsive behavior in the 5-CSRTT. The theory of this interaction is supported by recent fMRI data in humans, which revealed a role for 5-HT in modulating connectivity between the PFC and the amygdala in subjects carrying the "short" allele of the 5-HT-transporter gene (NEW ET AL. 2007).

The fact that only simultaneous activation of 5-$HT_{2A/C}$-receptors in both structures increased impulsive responding in rats furthermore suggests, that the OFC-amygdala-network does not work in opposite towards the action of 5-HT, but more in a synergistic manner. On the other hand, simultaneous infusions of the selective 5-HT_{2A}-receptor antagonist ketanserin into the OFC and BLA only tended to increase premature responding, which suggests that blockade of this receptor within these regions may only play a minor role in mediating 5-CSRTT performance under baseline task conditions. This may also be the reason, why neither infusion of DOI into the OFC and ketanserin into the BLA and vice versa show effects on premature responding or on the task performance per se (**Fig.19, Tab.5**).

There is considerable evidence implicating DA-mechanisms in inhibitory response control (ROBBINS 2002; WINSTANLEY ET AL. 2006; DALLEY ET AL. 2008). OFC and BLA are on the one hand known as important nodes in the limbic cortico-striatal loop on the other hand they form a network (CARDINAL ET AL. 2002) which provides the use of incentive information to guide behavior (SADDORIS ET AL. 2005). This network certainly includes others regions like the NAc, where both structures are projecting to (CARDINAL ET AL. 2002). Neurochemical and behavioral studies indicate that 5-HT_2-receptor subtypes differentially modulate DA as well as NE function within the NAc, since selective 5-HT_{2A}-(M100907) and 5-HT_{2C}-(SB242084) receptor antagonists showed differential effects on NAc-DA following in vivo microdialysis experiments (DI MATTEO ET AL. 2001; PORRAS ET AL. 2002; DE DEURWAERDÈRE ET AL. 2004). Furthermore, M100907 does attenuate an amphetamine or DOI-induced DA-release (ROBINSON ET AL. 2008). These studies show that in contrast to 5-HT_{2A}-receptors, 5-HT_{2C}-receptors exert a tonic inhibitory control on basal DA-neuron activity in the brain (PORRAS ET AL. 2002).

If the NAc per se or accumbal DA release are possibly involved in the impairment of inhibitory control seen after DOI-induced 5-$HT2_{A/C}$-receptor activation in the present study, remains unclear. However, it is evident that the present findings rely on 5-$HT_{2A/C}$-receptor activation within the 5-HT-modulated network between OFC and BLA.

In conclusion, the present data demonstrate that impulse control of rats measured in the 5-CSRTT can be impaired by extensive co-activation of 5-$HT_{2A/C}$-receptors in the OFC-amygdala-network. However neither the OFC nor the BLA alone seem to be primary sites for this action, since local infusion of DOI into these structures separately did not affect premature responding. The fact that only simultaneous activation of 5-$HT_{2A/C}$-receptors in both structures increased premature (impulsive) responding in rats suggests that the OFC-amygdala-network does work in a synergistic manner towards the action of 5-HT. Moreover, cortico-limbic 5-$HT_{2A/C}$-receptors have been confirmed as a major component of a regulatory network, comprising the OFC and BLA, necessary for impulse control and response selection in mammals.

6.2 Impulsive decision-making

Consistent with previous data, the results of the second part of the study support the hypothesis of the involvement of the 5-HT-system in rats' ability to tolerate a delayed reward, which has been proposed to index impulsive-related behavior (BIZOT ET AL. 1988; THIEBOT ET AL. 1985). We could show that DOI-induced activation of 5-$HT_{2A/C}$-receptors impairs the performance in the delay-based decision-making task in a T-maze, obvious in rats` shifting to the small immediate rewarded arm, indicating impulsive choice. Secondly, we showed that the selective 5-HT_{2A}-antagonist ketanserin counteracts a DOI-induced impairment of this task performance and therefore prevents impulsive behavior. In the maze procedure used in this work, rats were confronted with two alternative choices, an immediately available small reward vs. a large but delayed reward. Previous work clearly established that drugs like antidepressants enhance waiting capacity in rats under 25s delay conditions in this task (BIZOT ET AL. 1988; POULOS 1998), whereas benzodiazepines reduced rats` ability to wait when subjected to a 15s delay condition (THIÉBOT ET AL. 1985; BIZOT ET AL. 1999). Consequently, under long delay conditions this procedure only allows detecting drug-induced improvement of waiting capacity, but not reduction of tolerance to delay of reward. At conditions with shorter delays (15s or less), as used in the present study, rats usually choose the large but delayed reward on 65-70% of the trials. Thus, conditions with short delays provide a suitable measurement for drug-induced impairment

of waiting capacity or better impulsive behavior (THIÉBOT ET AL. 1985, 1999, 2007; POULOS 1998).

The performance of the delay-based decision-making task in a T-maze is probably governed by a number of factors such as hunger, visuo-spatial cues, reduced food intake, or learning and memory. Certainly, in the present study a DOI-induced shift to the small, immediate reward was observed at doses that did not affect the rats' choice when no delay was introduced in the task. Therefore, the factors hunger or reduced food-intake were irrelevant in this task performance. On the other hand, the phenethylamine DOI is a hallucinogen, which shares many properties with the psychostimulant lysergic acid diethylamide (LSD) (COOPER ET AL. 2003). This can lead to the assumption, that the shifting to the small immediate reward may be attributed to DOI-induced effects on perception of visuo-spatial cues, or learning and memory. However, this explanation is unlikely, since DOI did not affect the choice of the large reinforcer, without a delay (**Fig.20**).

However, animal studies indicate that an increase in impulsivity during performance of the described T-maze procedure can be elicited by decreased 5-HT-function (BIZOT & THIÉBOT 1996). Moreover, elevated 5-HT-activity induced by selective 5-HT-reuptake inhibitors such as clomipramine and citalopram (BIZOT ET AL. 1988), or by the 5-HT-releaser dexfenfluramine, decreases impulsivity in this delay of reward paradigm (THIÉBOT ET AL. 1985; POULOS ET AL. 1998). Therefore, the beneficial effects of drugs that increase 5-HT-neurotransmission in certain diseases such as bulimia nervosa or OCD in humans may be caused by improving impulse control in these patients (BIZOT & THIÉBOT 1996).

Interestingly, the findings in the present study suggest the opposite, since the 5-$HT_{2A/C}$-receptor agonist DOI increased impulsive behavior while the 5-HT_{2A}-receptor antagonist ketanserin did not affect it at all (**Fig.21**). How can these findings be reconciled with the notion that 5-HT normally reduces impulsivity? To investigate the role of 5-HT in impulsive behavior most of the studies deal with lesions of particular brain structures, such as the dorsal raphe nuclei or the substantia nigra. The resulting global 5-HT-depletion then arises from the destruction of 5-HT-projections of these structures to multiple forebrain areas (BIZOT ET AL. 1999). In contrast, EVENDEN & RYAN (1999) revealed an increase in impulsivity in an operant delay aversion paradigm after systemic treatment with the 5-$HT_{2A/C}$-receptor agonist DOI. Likewise, KOSKINEN ET AL. (2000A, B) observed a DOI-

induced enhancement of impulsive behavior but in terms of premature responding in the 5-CSRTT (impulsive action), whereas this effect was mediated through activation of 5-HT_{2A}-receptors rather than 5-HT_{2C}-receptors. These findings show that impulsive behavior is not just mediated by absolute levels of 5-HT but by multiple different 5-HT-receptors. In the above mentioned studies DOI affected impulsive behavior just over a narrow dose range of 0.05 to 0.02mg/kg, while in the present study it caused clear-cut effects in delay aversion over a broader dose-range (0.1, 0.3, 0.5mg/kg).

Notably, HIGGINS ET AL. (2003) could show that an increase in rats impulsive behavior in the 5-CSRTT was also seen after treatment with the 5-HT_{2C}-receptor antagonist SB242084. In contrast, blockade with 5-HT_{2A}-receptor antagonists like ketanserin (FLETCHER ET AL. 2007; PASSETTI ET AL. 2003) and M100907 decreased impulsive behavior in the 5-CSRTT (WINSTANLEY ET AL. 2003A). Based on such findings for the 5-CSRTT, it was assumed that a similar decrease in impulsive responding might be seen in the delayed reward task. But, this kind of effect was not observed in our study. Instead, ketanserin did not affect impulsive responding in the delayed reward task, a result similar to the findings of an investigation by TALPOS ET AL. (2006). Likewise, combined injection of DOI and ketanserin did not affect the choice of the large delayed reward and therefore impulsive behavior of rats. Apparently, this pre-treatment with the antagonist inhibited a possible DOI-induced increase in delay aversion in this task. Results of a combined study, which involve DOI and ketanserin, have only been evaluated in tasks assessing impulsive action in the 5-CSRTT (KOSKINEN ET AL. 2000A, B) but not yet in a delay aversion task.

Based on the agonist and antagonist used in the present study, it can be assumed that that DOI-induced impulsivity in the delayed reward task in a T-maze is probably mediated through activation of 5-HT_{2A}-receptors rather than 5-HT_{2C}-receptors. For sure 5-HT_{2C}-receptors are involved in mediating impulsivity in delay aversion tasks as well, but not in the context of DOI-induced impulsivity (TALPOS ET AL. 2006). Taken together, these findings of agonist-/antagonist-studies underline, that impulsive behavior is not just mediated by absolute levels of 5-HT but more by multiple 5-HT-receptors. Furthermore, it suggests that distinct tasks probably measure different aspects of impulsivity, which are mediated by the diverse neural substrates.

Indeed, $5HT_{2A}$- and 5-HT_{2C}-receptors appear to be promising targets for therapeutic drug action in disorders of impulsive behavior (CARLI ET AL. 2006; HIGGINS ET AL. 2003;

KOSKINEN ET AL. 2000A, B; PASSETTI ET AL. 2003; WINSTANLEY ET AL. 2003A; FLETCHER ET AL. 2007). Interestingly, there is direct support for the notion that 5-HT/DA-interactions may contribute to the expression of certain impulsive behaviors (WINSTANLEY ET AL. 2003B), since results of in vivo microdialysis obtained an amphetamine-induced increase of 5-HT as well as DA and NE (KUCZENSKI & SEGAL 1989; WINSTANLEY ET AL. 2006). The hypothesis of 5-HT/DA-interactions arised on one hand from striking similarities between 5-HT- and DA-compounds and, more precisely, their beneficial therapeutic implication in the treatment of neuropsychiatric disorders, such as ADHD or depression. Secondly, effects of stimulant administration and lesion-induced brain 5-HT-depletion, as well as the synergism of these treatments in some cases, suggests that the behavioral effects of this 5-HT-depletion may be due to the removal of an inhibitory influence on DA-neurotransmission (HARRISON ET AL. 1997).

In different animal models acute administration of a moderate dose of the indirect DA-agonist methamphetamine (1.0mg/kg) decreased impulsive behavior, whereas chronic administration of larger doses such as 4.0mg/kg increased it (RICHARDS ET AL. 1999). The acute effects of this psychostimulant in rats are therefore consistent with the decrease in impulsive behavior seen after amphetamine- or methylphenidate-treatment in ADHD children. The increase in impulsivity observed after chronic methamphetamine administration is concordant with behavioral effects, such as higher impulsivity, observed in stimulant abusers (WADE ET AL. 2000). VAN GAALEN ET AL. (2006B) showed that the acute beneficial effect of amphetamine on delay aversion could be blocked by the DA-D_2-receptor antagonist eticlopride. On the other hand, data revealed that DA-re-uptake inhibitors, like methylphenidate (BIZOT ET AL. 2007) or GBR12909 (VAN GAALEN ET AL. 2006A) reduced impulsive behavior in the delayed reward task, similar to the action of the 5-HT_{2C}-receptor antagonist SER-082 (TALPOS ET AL. 2006). Moreover, the 5-HT/DA-interaction hypothesis is supported by the role of many atypical antipsychotic drugs with antagonistic effects on 5-HT-receptors, such as clozapine, which appear to increase DA-levels, whereas this action is not only exerted via DA-receptors, but also via 5-HT-receptors. (CHUDASAMA & ROBBINS 2004).

It has been shown that 5-HT_{2A}- and 5-HT_{2C}-receptors exert opposite effects on DA-release of mesocortico-limbic and nigro-striatal DA-neurons, where they may control it by acting at different levels of DA-neuron regulatory mechanisms (ESPOSITO 2006). In particular, studies indicate a selective constitutive involvement of 5-HT_{2C}-receptors in

inhibitory influence of 5-HT on the activity of mesocortico-limbic and nigro-striatal DA pathways (DE DEURWAERDÈRE ET AL. 2004; ESPOSITO 2006). It can therefore be assumed that the decrease in impulsive decision-making seen after 5-HT_{2C}-receptor blockade (TALPOS ET AL. 2006) may be attributed to an increase in DA-release. The fact that the selective 5-$HT_{2A/C}$-agonist DOI induced impulsive behavior, which was shown to be counteracted by the selective 5-HT_{2A}-antagonist ketanserin, makes it unlikely that this effect was elicited via 5-HT_{2C}-receptors and therefore bears on DA-function. Furthermore, the DOI-induced enhancement in impulsivity observed in this work seems to be mediated through an over activation of 5-HT_{2A}-receptors. However, systemic drug treatment studies aiming a particular transmitter system, must never disregard other transmitter systems distal of the manipulated receptors.

Yet, another study identified, that the metabotropic glutamate-receptor (mGluR2) interacts with the 5-HT_{2A}-receptor through specific transmembrane helix domains and forms functional complexes in the brain cortex. When targeted by hallucinogenic drugs such as LSD and psilocybin, this 5-HT_{2A}-mGluR2-complex triggers unique cellular responses. A possible involvement should therefore be considered in further investigations, characterizing the action of serotonergic compounds in neuropsychiatric disorders (GONZÁLEZ-MAESO ET AL. 2008).

According to the assessment of tolerance to delay of reward, several already mentioned studies, which use an operant procedure for measuring impulsive choice between immediate and delayed rewards, examined effects of the 5-HT_2-receptor ligands DOI (EVENDEN & RYAN 1999) and ketanserin (TALPOS ET AL. 2006). The advantage of an operant procedure is that multiple delays can be used, whereas the present T-maze method provides only a single delay. A possible disadvantage of the T-maze procedure, which did not occur in the present study, though, is that the drug-testing has to be carried out in a particular time window during the training. Otherwise the rats may no longer show a response to the treatment (EVENDEN & RYAN 1996).

However, the present investigation shows that there are marginal differences between the results of the T-maze versus the operant procedure, after injection of 5-HT_2-receptor ligands. EVENDEN & RYAN (1999) revealed that systemic administration of DOI in an operant procedure of the delayed reward task had no effect on tolerance to delay of reward at a dose of 0.3mg/kg compared to vehicle. Only a dose of 1mg/kg DOI increased switching to the small, immediate reward. **Fig.20** indicates that DOI led to a switching to

the small reward already at a dose of 0.1mg/kg in the present study. Moreover, in the investigations of EVENDEN & RYAN (1999) and TALPOS ET AL. (2006) even vehicle treatment increased the switching to the small reward at a delay of 10s, which was not observed in the present work.

EVENDEN (1999A, C) proposed that different tests of impulsivity may assess diverse cognitive processes. The present results together with recent studies (e.g. FLETCHER ET AL. 2007) indicate that the expression of different types of impulsive behavior, such as premature responding in the 5-CSRTT or delay aversion in delayed reward tasks are regulated by 5-HT_2-receptors. Furthermore, our findings indicate that the ability of compounds that modulate 5-HT-function to interfere with waiting capacities seems to be dependend on the task used (maze versus operant procedure) and on the receptor subtype which is involved.

DOI-induced impulsive behavior in the delayed reward task is probably regulated by 5-HT_{2A}-receptors, although the literature favours the 5-HT_{2C}-subtype for mediating impulsivity in delay aversion in general (e.g. TALPOS ET AL. 2006). However, it still remains unclear what generalizations concerning the receptor subtypes can be made according to impulsivity. Since effects of 5-HT_{2C}-antagonists in impulsive choice tasks revealed an important role for DA as well, the involvement of other neurotransmitters and their systems has to be considered as well.

7 CONCLUSION

Taken together, the present work revealed that 5-$HT_{2A/C}$-receptors are crucially involved in mediating impulsivity in both, impulsive action and impulsive decision making or delay aversion. However, the fact that the functional status of the mesocortico-limbic DA-system is partially mediated by the 5-HT-system suggests that DA may play a regulatory role in impulsivity as well. This hypothesis only applies in case of the systemic drug administration part of the study. If DA is possibly involved in the impairment of inhibitory control seen after DOI-induced 5-$HT2_{A/C}$-receptor activation in the local drug infusion part, is unlikely. Regarding to the involvement of neural substrates underlying impulsivity, it could be shown that the OFC, the BLA, and presumably the network these structures form, are inter alia responsible for mediating impulsive behavior through activation and possibly inhibition of 5-$HT_{2A/C}$-receptors.

Anyway, the present results indicate a crucial role of 5-$HT_{2A/C}$-receptors in psychiatric disorders where co-morbid impulsivity is often present, including ADHD, OCD, schizophrenia, and substance abuse.

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