

# Neurotransmitter and Behaviour: Serotonin and Anxiety

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## 1. Introduction

There are many indications that mental disorders such as depression and anxiety disorders are directly related to mechanisms of central synaptic transmission of serotonin (5-HT).

5-HT is a peripherally and centrally occurring transmitter, which is involved in regulation of anxiety-related behaviour (Iversen 1984; Griebel 1995) and mood, but mediates also learning, appetite, food intake, sexual behaviour, sleep and influences body temperature as well as motor activity (Lucki 1998).

## 2. Central serotonergic system

In mammals 5-HT is distributed throughout the body. About 5% are located in the central nervous system (CNS). After 5-HT was found in the CNS (Twarog and Page 1953), detailed studies of the origin and projection areas of serotonergic neurons in the CNS began (Falck et al. 1962).

Only about 500 000 neurons in the CNS use 5-HT as a transmitter, but serotonergic neurons have connections to almost all structures of the brain and show a high degree of axonal branching. Dahlström and Fuxe (Dahlstrom and Fuxe 1964) demonstrated by histochemical methods that the raphe nuclei are the origin of almost all serotonergic neurons. Amin and colleagues (Amin et al. 1954) determined 5-HT levels in various brain areas and found the highest concentrations in hypothalamus, midbrain and area postrema.

5-HT receptors are distributed throughout the CNS, with different distribution patterns for the different receptor types. Most 5-HT postsynaptic receptors are located on the subsequent neurons. The release of 5-HT is regulated by presynaptic 5-HT receptors that are located either at the soma or at the nerve endings of the serotonergic neurons. Today, the "5-HT Receptor Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR) recognizes seven 5-HT receptor families with 16 receptor subtypes (Hoyer et al. 2002).

In the regulation of anxiety-related behaviour by serotonergic transmission, mainly 5-HT<sub>1A</sub> receptors, 5-HT<sub>2A/2C</sub> receptors and 5-HT<sub>3</sub> receptors are involved (Griebel 1995; Rex et al. 2007). Involvement of 5-HT<sub>1B/1D</sub> receptors in the modulation of anxiety-related behaviour is discussed.

### 3. Anxiety and anxiety disorders

Fear or anxiety has protective functions to avoid situations that cause pain, injury or even death (Vaas 2000).

In man, fear is associated with arousal, characterized by symptoms such as restlessness, tremor, less  $\alpha$ -waves and frequent  $\beta$ -waves in the electroencephalogram, tachypnea, and tachycardia, elevated systolic blood pressure, hyperemia of the skeletal muscles, decreased blood flow to the internal organs, hypermotility of the stomach, decreased salivation and mydriasis. Clinical studies however, found no uniform physiologic reaction pattern due to large individual differences (Kielholz and Battagay 1967). It was found that the decrease in the frequency of  $\alpha$ -waves in the electroencephalogram and the increase in finger tremor and respiratory rate correlated best with the perceived anxiety of the subjects.

But what about anxiety and fear in animals? If one assumes that fear is ... "an emotional reaction to the recognition or the recognition of a perceived threat, regardless of whether that risk is also a given objective" is considered (Tembrock 2000) and that animals in an aversive environment or threatening situations show similar physiological symptoms as people, it can be expected that at least highly developed animals due to physiological and ethological homologies can feel anxiety or fear (Silverman 1978).

We are aware that anxiety and fear are human emotions. However, to ease reading also in relation to animals we speak of fear, anxious and less anxious behaviour.

A distinction between pathological and "normal" anxiety is difficult. If, however, continued intense fear without real danger and threat perception occurs, or the fear response is "unreasonable" compared to the sources of threats, they get disease value.

Anxiety disorders are among the most common mental disorders. Up to 15% of all people suffer during their life from an anxiety disorder (lifetime prevalence) (Kessler et al. 2010).

Treatment of anxiety disorders and consequences of the disease cause high costs and are connected with severe social problems (Wittchen and Jacobi 2005). One in four patients with generalized anxiety disorder is not in a position to meet its daily life requirements (Becker and Hoyer 2000). The course of anxiety disorders without adequate treatment is chronic recurrent and a spontaneous remission was found in only about 14% of the patients (Wittchen 1991).

### 4. Pharmacotherapy of anxiety disorders

A rational pharmacotherapy with anxiolytics is the basis for successful treatment of anxiety disorders, often combined with psychotherapy (Bandelow et al. 2005). In general, the drug therapy lasts for at least 12 months.

In the search for effective anxiolytic agents, chlordiazepoxide was synthesized in 1957 as the first benzodiazepine by Sternberg. The benzodiazepines, such as chlordiazepoxide and diazepam, were the first primary anti-anxiety agents. Until the mid-90s benzodiazepines were the most commonly prescribed anxiolytics. Despite known sedative effects and addictive potential, they are safe drugs for the short-term treatment of anxiety (Lader 2005).

During the last decade, a fundamental change has taken place in the pharmacotherapy of anxiety disorders.

Nowadays, benzodiazepines, which are still the primary acute intervention in panic disorder or drugs of second choice or for an interim treatment in generalized anxiety disorder and social phobias, are prescribed less often (Lohse et al. 2004). At present, mainly

drugs that affect the serotonergic system, such as buspirone, SSRIs or NSMRI, are recommended as first choice (Bandelow, Zohar et al. 2005) (Table 1).

Anxiety Disorders	Recommended pharmacotherapy
Panic disorder	acute: benzodiazepines chronic: non-selective monoamine reuptake inhibitors (NSMRI), SSRI
Generalized anxiety disorder	SSRI, NSMRI, selective 5-HT-norepinephrine reuptake Inhibitors (SSNRI), buspirone in treatment failure + start: benzodiazepines or opipramol
Social phobia	SSRI, moclobemide in treatment failure + to start: benzodiazepines
Obsessive compulsive disorder	NSMRI, SSRIs, in treatment failure: combination with neuroleptics

Table 1. Summary of drugs that are recommended for the treatment of anxiety and compulsive disorders by the Drug Commission of the German Medical Association and the German Society for Psychiatry, Psychotherapy and Neurology.

In Germany, phytotherapeutics, prescribed or self-prescribed by the patients, are used widely although there is no evidence-based proof of efficacy in anxiety disorders (Kinrys et al. 2009). A special role had preparations kava-kava herbal products (*Piper methysticum*). The use of kava-kava and similar "natural remedy" in the industrialized countries increased strongly. In some clinical studies, the substance proved to be similarly effective as benzodiazepines with very few adverse drug reactions (Witte et al. 2005). In higher doses, kava-kava has also sedative and hypnotic effects.

## 5. Neurotransmitter systems and anxiety

Since the discovery of the mode of action of benzodiazepines late 70s (Möhler and Okada 1977),  $\gamma$ -aminobutyric acid (GABA) is most frequently associated with anxiety disorders and their pharmacotherapy. Benzodiazepines augment the GABAergic inhibition via GABA-A receptors (Rudolph et al. 2001). Inverse agonists at the binding site for benzodiazepines, such as the beta-carboline-3-carboxylic acid N-methylamide, have anxiety-causing effects (Moriarty 1995).

In addition to GABA, 5-HT plays an important role in the development and the persistence of anxiety disorders (Griebel 1995). Studies show that patients with anxiety disorders may have genetic polymorphisms in the 5-HT transporter (Overview: Lesch and Gutknecht 2005) or in the 5-HT<sub>2A</sub> receptor (Golimbet et al. 2004) and the 5-HT<sub>1A</sub> receptor (Gordon and Hen 2004). In patients with panic disorder the number of 5-HT<sub>1A</sub> receptors in the limbic system was shown to be reduced (Neumeister et al. 2004). Preclinical evidence also points towards an involvement of 5-HT<sub>3</sub> receptors in the regulation of anxiety (Costall and Naylor 1992; Rex, Bert et al. 2007), but clinical efficacy is still uncertain (Adell 2010).

First indications of a link between 5-HT and anxiety-related behaviour resulted from the observation that methysergide and metergoline, later on known as 5-HT antagonists, had an anxiolytic effect in animal studies (Robichaud and Sledge 1969). The same anxiolytic effect was seen following inhibition of 5-HT synthesis by para-chlorophenylalanine in rats in the

Geller-Seifter test. This conflict-reducing effect was prevented by treatment with 5-hydroxytryptophan (5-HTP), the precursor of 5-HT (Geller and Blum 1970). Therefore, it was expected that an increased activity of the central serotonergic system would be connected with anxiety and vice versa reduced activity with declined anxiety (Iversen 1984). Several studies have shown that an increase in 5-HT concentration in the brain increased anxiety and a reduction of 5-HT level reduces anxiety.

Other neurotransmitters that affect fear-related behaviour include the neuropeptide cholecystokinin (CCK), neuropeptide S, adenosine, excitatory amino acids and angiotensin. While the fear-producing effect of cholecystokinin is firm, the role of other neurotransmitters/-modulators for the development of anxiety, however, is not sufficiently understood.

CCK, one of the best characterized neuropeptides, was, like 5-HT, discovered originally in the digestive tract and found later in the CNS (Vanderhaeghen et al. 1975). Two major receptor types were discovered: CCK2 and CCK1 receptors in the brain, whereas in the periphery almost exclusively CCK1 receptors occur (Beinfeld et al. 1981).

The CCK2 receptor is involved in the regulation of fear-related behaviours in humans and animals (Fink et al. 1998). In patients with a history of panic disorder and in healthy volunteers panic attacks could be triggered by a CCK-4 injection (De Montigny 1989; Bradwejn and Koszycki 2001).

Adenosine is also involved in the regulation of anxiety-related behaviour. High doses of the nonselective adenosine receptor antagonist caffeine induce fear in healthy people (Nehlig et al. 1992) and trigger panic attacks in patients with known anxiety disorder (Charney et al. 1985). Rats treated with caffeine, were more anxious in the elevated plus-maze test (X-maze) (Kayir and Uzbay 2005) and a free exploratory paradigm (Bert et al. 2011), while an adenosine-1 agonist had an anxiolytic effect in the X-maze (Florio et al. 1998).

For long the renin-angiotensin system has been considered as a classical endocrine system, with main effects in the peripheral blood pressure regulation, before an independent renin-angiotensin system in the CNS was demonstrated (Fischer-Ferraro et al. 1971). In the CNS, angiotensinII (ATII) is acting as a neurotransmitter involved in the regulation of anxiety-related behaviour. In animal studies intraventricular administration of ATII caused anxiogenic (Braszko et al. 2003), but also anxiolytic (Holy and Wisniewski 2001) effects in the X-maze test. Both, angiotensin1-receptor (AT1) antagonists (Srinivasan et al. 2003) and AT2-receptor antagonists (Braszko, Kulakowska et al. 2003) and reduced ATII levels by ACE inhibitors reduce the fear in rats (Srinivasan, Suresh et al. 2003).

Although clinical reports point to an anxiety-reducing effects of ACE inhibitors and AT-receptor antagonists, there are no valid data in man available (Gard 2004).

## 6. Animal models of anxiety and animal anxiety tests

Anxiety tests in the clinic have the advantage that the volunteers may self-report their experiences. To trigger anxiety, the subjects receive either fear-inducing agents (caffeine, pentylentetrazol, lactate infusions or CO<sub>2</sub> inhalation), or they undergo psychological tests in an aversive environment (Graeff et al. 2003).

If animals can experience fear (Tembrock 2000), it would be possible to observe behaviour and neurochemical changes similar to changes in humans.

Animal anxiety tests are necessary for the development and characterization of novel anxiolytics. A disadvantage of animal studies is that only indirect conclusions about the emotions involved are possible by observing the behaviour and physiological side effects.

### 6.1 Historical developments

There are many animal tests for anxiety available (File 1985; Lister 1990). These tests may be divided roughly into three groups:

1. Tests in which anxiety is induced chemically, such as the drug discrimination test (Lal and Emmett-Oglesby 1983); 2. Tests based on conditioned fear / aversion, as the conditioned-emotional-response test (Davis 1990), the Geller-Seifter test, or the bird-punished-drinking test (Geller 1960) and last but not least 3. unconditioned tests, inducing anxiety by a new aversive environment, leading to behavioural inhibition. The unconditioned tests of anxiety include the X-maze (Handley and Mithani 1984), the black and white box test (Crawley and Goodwin 1980), the modified open field test (Rex et al. 1998), the social interaction test (Cappell and Latane 1969; File and Pope 1974) and the free exploratory paradigm (Griebel et al. 1993).

In unconditioned tests a conflict situation is created for the animals and changes in the innate behaviour in this aversive situation are observed. Examples for innate behaviour of animals and inhibiting factors used are: natural exploratory behaviour and avoidance of aversive open spaces without protection, co-existing curiosity and fear reactions to the appearance of a stranger of their own species, or vocalisations during sudden isolation.

The animal anxiety tests have to be validated pharmacologically with drugs whose anxiolytic or anxiety-inducing effects are known, and biologically by the variation of test conditions and their impact on the animals' behaviour (File 1985).

Since it is assumed that the different animal anxiety tests detect various forms of anxiety, for the determination of drug effects on behaviour also various anxiety tests should be used (Hagan et al. 2000).

In humans and animals two fundamentally different forms of anxiety can be distinguished: short-term changes in the emotional state (state anxiety) and a personality trait representing enduring anxiety (trait anxiety) (Cattell and Scheier 1958). In humans state anxiety and trait anxiety are differentiated with the "State Trait Anxiety Inventory" (Spielberger 1972).

To our knowledge only the free exploration test, in which home cage leaving behaviour in a real home environment is used to measure trait anxiety in rodents.

### 6.2 Recent developments and improvements

Despite the use of benzodiazepines, antidepressants and 5-HT<sub>1A</sub> agonists in the pharmacotherapy of anxiety disorders, only about 40-70% of the patients reach a symptom-free state (remission) or symptomatic improvement (response) (Kjernisted and Bleau 2004). Remission rates with SSRIs are even lower than under the conventional benzodiazepines (Pollack 2004). Therefore for the treatment of anxiety disorders, novel drugs with a rapid onset and with a constant therapeutic effect, and with fewer adverse effects are needed.

Consequently, there is still a need for improved animal tests for anxiety, which are capable to predict the anxiolytic efficacy of a drug and to mirror different facets of anxiety related disorders (Iversen 1984).

In the 80s and 90s of the last century, tests for anxiety were directed towards the discovery of drugs acting at the GABA receptors, because benzodiazepines were the gold-standard in the pharmacotherapy of anxiety disorders (Stephens and Andrews 1991). In contrast to the reliable detection of effects of GABAergic drugs on anxiety-related behaviour, it appeared that the anxiety tests available gained insufficient information regarding cholecystokinergic or serotonergic influences on anxiety-related behaviour (Griebel 1995; Rodgers and Johnson 1995). In order to assess fear-related behaviours more

comprehensively, new tests for anxiety were developed or established tests changed. Examples for this progress are the elevated zero maze test (Shepherd et al. 1994) and improvements of the open field test.

### **6.2.1 Modified open field test and its validation**

The open field has been used since about 80 years to study the locomotor and exploratory activity in laboratory rodents (Hall 1934). It could be shown that the movement pattern of animals in the brightly lit open field depends on their anxiety. The duration and frequency of the stay of animals in the central region or the amount of thigmotaxis indicate the intensity of anxiety in the animals. In assessing the behaviour of animals in the open field it has to be taken into account that a complex behaviour, which is composed of anxiety-related behaviour, neophobia, exploration, motivation, habituation and spatial learning, is analyzed.

In pre-clinical testing easy and quick tests are needed, which in the ideal case, reduce the assessment of a complex behaviour to a "yes-no decision". We modified the open field test on the basis of existing tests (Britton and Britton 1981). In our test, hungry rats were placed in a brightly lit and unfamiliar open field, with a petri dish of the usual rat chow in the middle of the open field. The hungry animals had the conflict between food supply and the fear of an unknown and aversive environment that suppresses food intake. As a parameter of an anxiety-modifying effect, we determined the percentage of rats of a group, which began to feed in the open field and could simplify the test (Rex et al. 1996; Rex, Stephens et al. 1996).

The modified open field test has been validated behaviourally and pharmacologically (Rex, Voigt et al. 1998). A shorter fasting period results in a less frequent food intake in the open field, similar to the offer of unfamiliar food.

Variations in the illumination of the test arena also changed the incidence of feeding in the open field. Increased illumination prevented the food intake entirely, while a reduction of light intensity increased the proportion of rats that fed in the aversive open field (Rex et al. 1994). These findings are consistent with other studies of rat behaviour in an open, aversive environment, showing that rats in a dimly lit open field had more social interactions than in a brightly lit open field (File and Hyde 1978; Rex et al. 2004).

We were able to show in this simple animal test not only the known anxiolytic effects of GABA agonists, such as diazepam and the  $\beta$ -carboline abecarnil (Rex, Stephens et al. 1996), but also the dose-related anxiety-reducing effects of 5-HT1A agonists, 5-HT2A antagonists and 5-HT3A antagonists. Hypnotics, antipsychotics such as haloperidol and stimulants such as amphetamine, without fear-reducing effect, did not increase the incidence of feeding in the open field (Rex, Voigt et al. 1998). To exclude false positive or false negative results locomotor activity and substance-related effects on food intake were also determined (Rex, Voigt et al. 1998).

### **6.2.2 Risk assessment in the X-maze**

Often, serotonergic drugs fail to change traditional parameters of anxiety-related behaviour (De Vry 1995; Griebel 1995). However, the risk assessment behaviour of animals treated with serotonergic drugs differed from the behaviour of the controls. Therefore, in addition to developing new anxiety tests, a more detailed analysis and precise description of the behaviour in the commonly used tests was suggested. The determination of the risk

behaviour of rats and mice in the X-maze is now common in the assessment of anxiety-related behaviour (Rodgers et al. 1997). We observed that guinea pigs after placement on the X-maze remained motionless in the centre of the test apparatus ("freezing"). The duration of freezing was shortened by anxiolytics such as diazepam, the 5-HT<sub>1A</sub> agonist 8-OH-DPAT or the CCK<sub>2</sub>-receptor antagonist L365, 260 and significantly prolonged by fear-inducing substances, such as CCK-4 (Rex et al. 1994). Similar findings were observed in rats. Rats of a more anxious strain showed a longer freezing-period compared with a less anxious strain (Rex et al. 1999).

### 6.2.3 Variation of pre-test and test procedures

The widespread use of unconditioned tests (Lister 1990) also led to varying results across different laboratories, caused by various reasons.

Variations in the construction of the experimental apparatus, for example, can affect the explorative activity of the animals. These variations include the mechanical stability of the entire experimental apparatus (Jones et al. 2002), the use of different materials of the walls of the open fields (Ohl and Keck 2003) or the use of opaque or translucent wall materials for the closed arms of X-maze (Anseloni and Brandao 1997).

Similar effects have variations in the handling and husbandry of animals before and during the test, which can alter both the spontaneous behaviour and sensitivity to anxiety-modulating drugs. Examples include: repeated "handling" (Andrews and File 1993), stress factors in the environment (also in the animal unit) (Haller and Halasz 1999), light conditions in the animal unit and in the test arena, social stress or isolation (Rodgers and Cole 1993) or test-experience of the animals (Hagan, Harper et al. 2000).

We could show that the rearing conditions in rats have an impact on the fear-related behaviour of the animals. It appears that rats that were reared either single housed or in groups, differed in anxiety-related behaviour. Animals reared in social isolation, behaved much more fearless on the X-maze (Marsden et al. 1995).

For the above reasons, a pharmacological and a behavioural validation is needed to compare own results with those in the literature. As an example for our laboratory, we established and validated the social interaction test in rats.

### 6.2.4 Validation of the social interaction test

The social interaction test, based on the open field test, was developed by (Cappell and Latane 1969) and validated throughout (File, 1978). When two rats, unknown to each other, are placed in a test arena, there will be contacts between the two animals. The time and frequency of individual forms of active social interaction during the tests are measured. In the aversive environment of a brightly lit open field contacts between the two animals are less frequent than in a non aversive environment (File 1985). Benzodiazepines stimulate the interaction between the two rats under aversive conditions (File and Hyde 1978). The use of non-GABAergic drugs led to conflicting results (De Vry 1995; Griebel 1995).

To assess the influence of organismic test variations on the fear-related behaviour, we tested a generally more anxious rat strain (Wistar [Wist: Shoe] Dimed Schönwalde GmbH, Germany) and a less anxious rat strain (Sprague Dawley [SD: Shoe], Dimed Schönwalde GmbH, Germany). We found that the duration of individual housing before the test and the associated social deprivation, had a significant influence on the duration and frequency of

social contacts. Without a previous isolation we observed little interest in the other animal (Rex, Voigt et al. 2004).

Reduction of the aversive nature of the test arena by lower illumination or by a previous habituation to the test arena led to increased social interaction and confirmed the results of (File and Hyde 1978). Here, the change of behaviour of the more anxious Wistar rats was more pronounced. Diazepam increased social contacts only in the more anxious Wistar rats (Rex, Voigt et al. 2004). The well-known anxiogenic mCPP decreased the number and duration of mutual contacts in both rat strains (Rex, Voigt et al. 2004).

### **6.3 Impact of strain differences**

Considering the widespread use of rodents in behavioural experiments, strain differences and their impact on the findings in the assessment and comparison of results with the available literature have to be considered. Genetic differences between individual strains or substrains in rodents may produce conflicting results and lead to misinterpretation of results (Jax 2003).

It is known that the fear-related behaviour differs between various strains of rats or mice (e.g. Trullas and Skolnick 1993; Rex, Sondern et al. 1996).

During the validation of the social interaction test, we observed that anxious Wistar rats and less anxious Sprague Dawley rats differed in the severity of behavioural changes after modifications of the test (Rex, Voigt et al. 2004). It was not known to what extent these behavioural differences were genetic determined or caused by environmental conditions during breeding and animal keeping. To determine the influence of breeding, husbandry and genetic conditions on the anxiety-related behaviour, we examined the anxiety-related behaviour of inbred and outbred rats under identical and different breeding and housing conditions.

#### **6.3.1 Inbred laboratory rodents**

We compared the fear-related behaviour of inbred Fischer 344 rats supplied by two national breeders (Charles River Laboratories Inc., Germany and Harlan-Winkelmann Ltd, Germany) and from a regional vendor (Dimed Schönwalde GmbH, Germany). Because of their extensive homozygosity inbred animals should show a small variation in behaviour. In the analysis of the anxiety-related behaviour in the X-maze, black and white box and the modified open field we found small but significant differences in anxiety-related behaviour between animals from different breeders (Bert et al. 2001).

In a second set of experiments we obtained pregnant Fischer 344 rats from the three above-mentioned vendors. The F1 generations were reared in our animal house. Interestingly, the F1 generation showed despite identical housing conditions behavioural differences between the stocks and strains (Bert, Fink et al. 2001). These differences in anxiety-related behaviour seem to be primarily innate. It is possible that a long term breeding of the rats with original genetic uniformity at different places led to the formation of substrains (Jax 2003).

#### **6.3.2 Outbred laboratory rodents**

In behavioural pharmacology experiments outbred rats, like Wistar rats or Sprague Dawley rats, are used more often. Outbred rats with their greater genetic diversity are thought to reflect the genetic diversity of the human population. We observed significant differences in anxiety-related behaviour between different outbred rat strains. These behavioural



differences were detected in several unconditioned anxiety tests and are therefore not attributable to a specific test stimulus (Rex, Sondern et al. 1996). Interestingly, behavioural differences between different stocks of one strain and the behavioural differences between strains were similar (Rex, Sondern et al. 1996; Bert, Fink et al. 2001). Even when pregnant rats from different breeders were obtained and the F1 generation was raised under the same conditions, the F1 generation showed similar differences in anxiety-related behaviour. This confirms again, that behavioural differences between the rat strains can be caused primarily by genetic differences (Rex, Sondern et al. 1996; Rex, Voigt et al. 1999; Rex et al. 2007).

In a second approach, we examined the fear-related behaviour of two lines of Sprague Dawley rats with common origin. 20 years ago the Institute of Cytology and Genetics in Novosibirsk (Russia) started breeding Sprague-Dawley (SD) rats obtained from Charles River Sulzfeld, Germany. To best knowledge, there were no further deliveries from Charles River to Novosibirsk (communication with Dr. J. Geller, CEO Charles River Germany). Examination of the anxiety-related behaviour and neurochemical experiments using both stocks revealed differences in their exploratory and anxiety-related behaviour, in habituation and learning, physical development, and serotonergic neurotransmission. Therefore, rats of the same stock but obtained from different breeders should be used with caution in research involving these measures (Rex, Kolbasenko et al. 2007).

Intentionally selectively bred sublines of rat strains have been used for more than 40 years. The selective breeding includes lines of rat strains, which differ significantly in anxiety-related behaviour, such as the HAB / LAB (high anxiety-related behaviour / low anxiety-related behaviour) rats (Liebsch et al. 1998) or the Maudsley (reactive / nonreactive) rat lines (Broadhurst 1975).

Since each of the selectively bred lines start from one common rat strain, there should be only minor genetic differences between the lines, causing the change in behaviour in anxiety tests (Landgraf 2003). Genetic and pharmacological investigations of these rat lines may contribute to the elucidation of the neurobiological basis of fear and anxiety disorders.

However, it has to be taken into account, that a complex emotion like fear has a polygenic base.

## **7. Combination of anxiety tests with neurochemical analysis**

### **7.1 Brain microdialysis**

The microdialysis as an *in vivo* sampling technique allows to gain a sterile and protein-free dialysate from one or more limited regions of the brain and subsequently to analyze changes in the levels of substances in the extracellular fluid *in vivo* in anesthetized and in awake animals over time.

Introduction of brain microdialysis in awake animals represented a major advance, since substance effects in anesthetized and in awake animals may differ dramatically (Boix et al. 1992; Boix et al. 1993).

Together with the group of C.A. Marsden at the University of Nottingham, U.K. we established the method of microdialysis in an awake animal during a behavioural experiment (Rex et al. 1991; Marsden, Beckett et al. 1995). This made it possible to detect changes in the release of neurotransmitters associated to drug administration and behavioural changes. Accompanying experiments in the open field (Cadogan et al. 1994)

and on the X-maze (Rex et al. 2003) ensured that the implanted microdialysis probe with the attached tubings does not change the natural behaviour of animals.

The microdialysis has been used in animal anxiety tests such as the X-maze (Rex, Marsden et al. 1991; Rex et al. 1993; Voigt et al. 1999), social interaction test (Cadogan, Kendall et al. 1994), and the Vogel-test (Matsuo et al. 1996). For our studies, the microdialysis probes were implanted into brain regions involved in the regulation of anxiety-related behaviour, such as the prefrontal cortex or the hippocampus (Rex et al. 2008).

## 7.2 Brain serotonin concentrations

Although the relationship between anxiety and the central serotonergic transmission system is not simple, it can be assumed that changes in the activity of the serotonergic transmission system may lead to a change in the anxiety-related behaviour as well as in brain 5-HT concentration. Therefore, we investigated the relationship between anxiety-related behaviour and 5-HT concentrations in brain regions, which are involved in the regulation of anxiety-related behaviour, such as the prefrontal cortex, the ventral hippocampus and the raphe nuclei. Both, intracellular stored 5-HT and the released, extracellular located, 5-HT are measured. In general, after release into the synaptic cleft 5-HT is transported back into the presynaptic terminal by the 5-HT transporter and metabolized mainly to 5-hydroxyindole acetic acid (5-HIAA), which also can be determined. Changes in the ratio of 5-HT and 5-HIAA in the tissue indicate functional changes in central serotonergic transmission system.

## 8. Anxiety and serotonin

### 8.1 Traditional concept of anxiety and serotonin

25 years ago, the role of the central serotonergic transmission system in the regulation of anxiety-related behaviour could be summarized as follows: An increased availability of 5-HT or stimulation of postsynaptic 5-HT receptors is associated with anxiety. A reduced availability or release of 5-HT or blockade of postsynaptic 5-HT receptors triggers an anxiolysis (Iversen 1984). Earlier studies in panic disorder patients with elevated 5-HT plasma levels supported the 5-HT hypothesis of anxiety disorders (Giannini et al. 1983).

Contrary to this paradigm, in animals a local application of 5-HT into the dorsal raphe nucleus had anxiety-reducing effects. In own studies, destruction of serotonergic neurons in the raphe nuclei by the neurotoxin 5,7-DHT reduced 5-HT tissue levels without changing the anxiety-related behaviour (Rex, Thomas et al. 2003).

These results indicate that the relationship between anxiety-related behaviour and central serotonergic transmission system is not as clear as originally described by Iversen (Iversen 1984).

### 8.2 Strain differences in anxiety and central serotonin

If there is a connection between anxiety-related behaviour and the 5-HT concentration in the CNS, it should be possible that rat strains that differ in their anxiety-related behaviour also differ in the 5-HT concentration in the brain, too. In rats of different strains (Rex, Sondern et al. 1996), the 5-HT levels in brain tissue were determined. It could be shown that rats with a more anxious behaviour had increased 5-HT levels in projection areas of the central serotonergic transmission system (Bert, Fink et al. 2001; Rex, Voigt et al. 1999; Rex, Voigt et al. 2004).

### 8.3 Anxiogenic drugs and serotonin release

#### 8.3.1 SSRIs

The 5-HT-releasing drug fenfluramine increases 5-HT concentration in the rat cortex substantially (Thomas et al. 2000) and leads to a more anxious behaviour on the X-maze (File and Guardiola-Lemaitre 1988).

The acute administration of NSMRIs or SSRIs, which increase 5-HT levels in the synaptic cleft leads to a more fearful behaviour in rats on the X-maze (Bagdy et al. 2001). This fear-enhancing effect of SSRIs is also observed in humans (Stahl 1998). It is particularly pronounced at the beginning of therapy (Gunnell et al. 2005) and generally it declines within two to three weeks.

#### 8.3.2 CCK-receptor agonists

As described, CCK2-receptor ligands are also involved in the regulation of anxiety-related behaviour (Crawley and Corwin 1994; Fink, Rex et al. 1998). We have shown that the CCK2-receptor agonist CCK-4 induced in rats and guinea pigs a clear anxious behaviour on the X-maze, in the modified open field, in the black and white box and ultrasonic vocalisation test (Rex, Barth et al. 1994; Fink, Rex et al. 1998). Since both, CCK and 5-HT, affect the anxiety-related behaviour, an interaction between the two neurotransmitter systems was suggested. In the modified open field test we could show that the 5-HT<sub>1A</sub> agonist 8-OH-DPAT reduced as an anxiolytic and consequently as a "functional antagonist" dose-dependently the anxiogenic effect of CCK-4 (Rex et al. 1996).

During exposure to the X-maze, CCK-4 increased the release of 5-HT up to threefold (but not in home cage), whereas administration of the CCK1-receptor agonist A71738 and the non-selective CCK1/2-receptor agonist CCK-8s, did not affect the fear-related behaviour nor did change the 5-HT release compared to control animals (Rex and Fink 1998). The anxiogenic effect of CCK-4 in tests for anxiety could be antagonized by the selective CCK2-receptor antagonist L365,260 (Rex et al. 1993; Rex and Fink 1998). We could show an anxiolytic-like effect of L365,260 that abolished not only the effects of CCK2-receptor agonists, but had even own anxiety-reducing effects in animal anxiety tests (Hughes et al. 1990; Fink, Rex et al. 1998).

Our results can be summarized as follows: CCK-4 affected the function of central serotonergic transmission system only slightly or not at rest, but stimulated during acute fear the release of 5-HT in the cortex and hippocampus.

### 8.4 Arousal and serotonin release

There is general agreement that aversion, based on animal models of anxiety in animals leads to activation in the limbic system and cortex (arousal reaction). So far, it is uncertain whether the increased release of 5-HT during the test for anxiety is caused by anxiety or by the arousal reaction.

Besides the results of various *in vivo* microdialysis experiments that confirm our hypothesis of an involvement of central serotonergic system in anxiety-related behaviour (Wright et al. 1992; Rex, Marsden et al. 1993; Matsuo, Kataoka et al. 1996; Rex, Voigt et al. 1999; Rex, Voigt et al. 2004), other studies suggested a link between stress-related hyperactivity of rats and the increased release of 5-HT in projection areas (Linthorst et al. 2002).

We could show that the mild stressor "white noise" (Buller et al. 2003), did not increase hippocampal 5-HT release in anxious rats, although the rats were highly active during

exposure to white noise (Rex et al. 2005). Comparing our results with other findings in which increased 5-HT release was found during a forced stay in an inescapable stressful situation, like the forced swim test, we think that these situations most likely cause fear in this animals. This is supported by the results of Linthorst and colleagues who interpret an extremely increased 5-HT release in some animals as an anxiety / panic-stimulated release (Linthorst, Penalva et al. 2002).

It was also assumed by others that the increased release of 5-HT during the stay on the X-maze (Rex, Marsden et al. 1993), in the aversive open field (Cadogan, Kendall et al. 1994) or during the forced swim test (Linthorst, Penalva et al. 2002) is caused not by fear but just by exposure to a new unfamiliar environment per se. To check whether a new, but not aversive environment also causes an increased release of 5-HT, we performed microdialysis studies with anxious rats in a non-aversive version of the X-maze. By closing the open arms the remaining arms represented with their high walls rather a protective environment for the animals. The initial stay of the animals in the modified X-maze did not increase 5-HT release, although the animals actively explored the new environment (Rex, Voigt et al. 2005).

Another argument for an anxiety-related increased 5-HT release in hippocampus and cortex emerged from studies in which behavioural strain differences have been analyzed. In several microdialysis studies, we could measure an increased release of 5-HT during the stay on the X-maze only in anxious rat strains. In rats with a non-anxious behaviour, the release of 5-HT in the X-maze test under the same conditions was not increased as much (Rex, Voigt et al. 1999; Rex, Voigt et al. 2004).

Together, our results suggest that acute fear increases the extracellular 5-HT concentration in the serotonergic projection areas and there is a connection between the amount of released 5-HT and the anxiety of the animals.

## 9. Anxiolysis and serotonin

### 9.1 Role of 5-HT<sub>1A</sub>, 5-HT<sub>1B/1D</sub> receptors

The release of 5-HT is regulated by presynaptic somatodendritic 5-HT<sub>1A</sub> receptors and presynaptic 5-HT<sub>1B/1D</sub> receptors at the nerve endings of serotonergic neurons (De Vry 1995).

The functional significance of postsynaptic 5-HT<sub>1A</sub> receptors and 5-HT<sub>1B/1D</sub> receptors is still not completely clarified (Göthert and Schlicker 1997). Differentiation of effects mediated by pre- or postsynaptic receptors with the existing substances is difficult. While a stimulation of presynaptic 5-HT<sub>1A</sub> receptors and 5-HT<sub>1D</sub> receptors by agonists reduces 5-HT release in the projection areas (Rex, Fink et al. 1993; De Vry 1995; Rex et al. 1997) antagonists at the 5-HT<sub>1A</sub> and 5-HT<sub>1D</sub> receptors do not always induce an increase of 5-HT release (Roberts et al. 1999), as 5-HT is released tonically only to a small extent.

Also own microdialysis studies showed that blockade of autoreceptors without previous stimulation of 5-HT release had no measurable effect. If guinea pigs stayed in the familiar cage, neither the selective 5-HT<sub>1A</sub> antagonist WAY 100635 nor the 5-HT<sub>1B/1D</sub>-Antagonist GR 127 935, changed release of 5-HT in the ventral hippocampus and the prefrontal cortex (Rex et al. 1996; Rex, Voigt et al. 2008).

Whereas in the resting state the tonic 5-HT release and therefore the effects of antagonists on 5-HT<sub>1A</sub> and 5-HT<sub>1B/D</sub> receptors are small, in an aversive environment, such as the X-maze

the release of 5-HT was stimulated. Treatment with the 5-HT<sub>1B/1D</sub>-antagonist GR 127935 led to a quicker but short-lasting increase in extracellular 5-HT concentration in the cortex, compared to treatment with saline (Rex, Fink et al. 1996).

In other studies in which a drug-induced increased extracellular 5-HT concentration was examined, the regulatory function of 5-HT<sub>1A</sub> and 5-HT<sub>1D</sub> receptors could be shown more clearly. If pre-treatment with a SSRI increased the 5-HT concentration in the synaptic cleft, both, 5-HT<sub>1A</sub> antagonists (Hughes et al. 2005), as well 5-HT<sub>1B/1D</sub>-antagonists had a potentiating effect on 5-HT release in the CNS. These results suggest that control of the firing rate of serotonergic neurons and thus of transmitter release by presynaptic 5-HT<sub>1A</sub>- and 5-HT<sub>1B/1D</sub>-receptors is pronounced when 5-HT release is stimulated.

5-HT<sub>1A</sub> agonists such as buspirone and tandospirone are used in the treatment of anxiety disorders. Their anxiety-reducing effect is explained by stimulation of presynaptic 5-HT<sub>1A</sub> receptors and the subsequent reduction in 5-HT release (Stahl 1998).

However, the role of postsynaptic 5-HT<sub>1A</sub> receptors for an anxiolytic effect is still not clear. So we used a neurobiochemical approach to try to discriminate the function of presynaptic and postsynaptic 5-HT<sub>1A</sub> receptors in vivo.

### **9.2 5-HT<sub>1A</sub> receptors and brain metabolic activity**

It is known that the energy metabolism in the CNS is linked closely to the activity of the cells. Determination of the redox status of cells allows conclusions about the functional state of these cells (Ames 2000). Complementary to established methods, the laser-induced fluorescence spectroscopy offers the possibility of "on line" - measurements of the metabolic state of intact tissues.

After verification that in vivo laser-induced fluorescence spectroscopy detects changes in mitochondrial/metabolic activity in the CNS, we determined the mitochondrial activity in the ventral hippocampus after administration of the 5-HT<sub>1A</sub> agonist 8-OH-DPAT (Rex and Fink 2006).

Systemic administration of 8-OH-DPAT induced dose-dependent changes in mitochondrial activity of hippocampal neurons. In the highest dose 8-OH-DPAT significantly increased the NADH fluorescence, while the middle dose had no effect on NADH fluorescence and the lowest dose resulted in a slight but not significant increase in NADH fluorescence.

We interpret the results as follows: The highest dose of 8-OH-DPAT stimulates postsynaptic 5-HT<sub>1A</sub> receptors in the ventral hippocampus and thus inhibiting the activity of the following neurons, leading to an increase in NADH fluorescence. Our conclusions are sustained by electrophysiological studies, in which higher doses of 8-OH-DPAT lowered activity in the hippocampus and cortex (Tada et al. 1999).

Since 8-OH-DPAT has in behavioural tests an anxiety-reducing effect in doses that affect the postsynaptic 5-HT<sub>1A</sub> receptors it cannot be excluded that the postsynaptic 5-HT<sub>1A</sub> receptors play a role in the regulation of anxiety-related behaviour.

## **10. Anxiolytic drugs and serotonin**

It was shown that aversive stimuli and substances with an anxiety-enhancing effect increase the 5-HT release in some serotonergic projection areas, such as the ventral hippocampus and the prefrontal cortex. Therefore, we investigated whether anxiolytics of different drug classes inhibit 5-HT release in general.

### 10.1 Different classes of anxiolytic drugs and serotonin release

Benzodiazepines and 5-HT<sub>1A</sub> agonists are therapeutically used anxiolytics. CCK2 antagonists were effective in various animal anxiety models, such as the X-maze (Rex, Barth et al. 1994), the modified open field (Rex, Voigt et al. 1998), social interaction and the elevated zero maze (Revel et al. 1998). Therefore, we investigated the effects of diazepam (Rex, Marsden et al. 1993; Rex et al. 1993a), 8-OH-DPAT (Rex et al. 1993) and the CCK2 antagonist L 365.260 (Rex, Fink et al. 1994) on 5-HT release in the prefrontal cortex using microdialysis during the X-maze test.

As shown before, exposure to the X-maze resulted always in a marked increased 5-HT release (Rex, Marsden et al. 1991; Marsden, Beckett et al. 1995). Pretreatment with diazepam or 8-OH-DPAT or L 365.260 reduced this aversion induced increase in 5-HT release in the prefrontal cortex and caused a less anxious behaviour of the animals on the X-maze.

Under non-aversive conditions, like before and after exposure to the X-maze, diazepam, 8-OH-DPAT and L 365.260 decreased basal 5-HT release in the prefrontal cortex.

Both the reduction in the aversion induced 5-HT release and the anxiolytic effects of diazepam, 8-OH-DPAT and L 365.260 were antagonized by pretreatment with the benzodiazepine antagonist flumazenil, the non-selective 5-HT<sub>1</sub> antagonist methiothepine and the selective CCK2-receptor agonist CCK-4, respectively (Rex, Fink et al. 1993; Rex, Marsden et al. 1993; Rex, Marsden et al. 1993).

Our results indicate a receptor mediation of the observed effects and they are consistent with the hypothesis that an acute stay in an aversive environment is associated with increased 5-HT release and an anxiolytic effect with a decreased release of 5-HT. The results are supported by *in vitro* studies showing that both, diazepam and the 5-HT<sub>1A</sub> agonist buspirone as well as the CCK2 antagonist GV 150 013, decreased the electrically stimulated release of 5-HT from cortical slices significantly (Siniscalchi et al. 2001).

### 10.2 Benzodiazepines and serotonin concentrations

Already in the 70s it has been shown that benzodiazepines reduce 5-HT synthesis (Dominic et al. 1975) and the 5-HT turnover (Wise et al. 1972).

After systemic administration chlordiazepoxide inhibited the firing rate of serotonergic neurons in the dorsal raphe nucleus (Trulson et al. 1982) and after injection into the dorsal raphe chlordiazepoxide reduced anxious behaviour in the conditioned-emotional-response test (Thiebot et al. 1980).

The dorsal and median raphe nuclei have a high density of GABA receptors. Microinjections of GABA in the dorsal raphe nucleus and in serotonergic projection areas, such as the amygdala and the hippocampus, reduced the firing rate of serotonergic neurons. This reduced firing rate was reduced even further by administration of benzodiazepines (Gallager 1978). On the other hand, the anxiety-reducing effect of benzodiazepines in a conflict test was abolished by the intraventricular administration of 5-HT (Wise, Berger et al. 1972).

We have seen "antiserotonergic" effects of diazepam on both the tissue concentration as well as the 5-HT release (Rex, Marsden et al. 1993; Bert, Fink et al. 2001). In the rat brain diazepam reduced tissue concentrations of 5-HT in serotonergic projection areas, such as cortex and hippocampus which confirmed the results of Pei and colleagues (Pei et al. 1989). Interestingly, diazepam had a greater effect in animals with high tissue concentrations of 5-HT, while the effect was smaller in animals with low 5-HT levels.

In a Wistar rat strain (HsdCpb: WU, Harlan, Germany), which showed a non-anxious behaviour in different anxiety tests, diazepam reduced the already low 5-HT levels in hippocampus and cortex only marginally and did not change the anxiety-related behaviour. In these non-anxious rats exposure to the X-maze did not increase 5-HT release in the hippocampus (Rex, Voigt et al. 1999).

In the more anxious Wistar rats (Han: Wist (SYN WI), Institute for Risk Assessment, Germany) and Fischer rats (F344/NHsd, Harlan, Germany) with higher 5-HT concentrations in the tissue and aversion-induced release of 5-HT on the X-maze (Rex, Voigt et al. 1999), diazepam induced an anxiolytic-like behaviour on the X-maze and reduced the concentration of 5-HT in the hippocampus and frontal cortex significantly (Bert, Fink et al. 2001).

Since similar doses of diazepam in rat strains with different 5-HT concentrations in the brain have different effects on anxiety-related behaviour (Bert, Fink et al. 2001), it can be assumed that the effect of benzodiazepines depends of the activity of the serotonergic neurotransmission system and the GABAergic system interacts with the central serotonergic transmission system.

To further test the hypothesis of a close link between anxiolytic effects and a decreased release of 5-HT, we examined an anxiolytic effective herbal product.

### 10.3 Herbal anxiolytic kava-kava

The herbal product kava-kava had been widely used as an anxiolytic. Although kava pyrones have been identified as the pharmacologically active ingredients of kava-kava (Singh and Blumenthal 1997), the mechanism of action has not been clarified (Smith et al. 2001). The kava-kava pyrones bind probably not to the GABA-A receptors (Garrett et al. 2003). We examined the effects of kava-kava in the X-maze and the social interaction test. In both tests a standardized kava-kava preparation caused dose-dependent anxiety-reducing effect in rats, similar to the effect of diazepam (Rex et al. 2002). Our results were later confirmed in mice (Garrett, Basmadjian et al. 2003).

In a second experiment we determined the concentration of 5-HT in a tissue sample (punch), containing the dorsal and the medial raphe nucleus and in tissue samples of the ventral hippocampus and the prefrontal cortex. Kava-kava led to a reduction in 5-HT concentration in the cortex and hippocampus.

Since the tissue concentration is not related strongly to the release of 5-HT, we investigated the release of 5-HT in the ventral hippocampus following administration of kava-kava. Using *in vivo* microdialysis we showed for the first time that kava-kava, like 8-OH-DPAT and diazepam, decreased the 5-HT release in the projection areas.

## 11. Long term reduction of serotonin and anxiolysis

Under the premise that a correlation between the amount of tissue concentration of 5-HT and the expression of anxiety-related behaviour exists, it could be assumed that a reduction of 5-HT concentration in the CNS would be associated with anxiolysis. The 5-HT concentration in the brain can be reduced by destruction of serotonergic neurons for a long time.

Systemic para-chloroamphetamine reduced the 5-HT content in the CNS of rats and these animals showed an anxiolytic-like behaviour in the Geller-Seifter test (Geller and Blum 1970).

Intracerebral administration of the neurotoxin 5.7-DHT, causing destruction of serotonergic neurons, reduced the 5-HT content in the CNS and induced anxiolytic-like effects in conditioned anxiety tests (Iversen 1984; Soderpalm and Engel 1992; Thiebot, Jobert et al. 1980). These studies confirmed seemingly a simple relation between 5-HT and anxiety.

However, studies in which unconditioned tests were used and/or individual nuclei in the raphe region were lesioned, showed contradictory results. While 5.7-DHT reduces anxiety after intraventricular application (Griebel 1995), administration of the neurotoxin into either the median or the dorsal raphe nucleus had no effect on behaviour in several anxiety tests (Griebel 1995, Thomas et al 2000). Only in the social interaction test (File et al. 1979) a lesion of the dorsal raphe nucleus caused a less anxious behaviour of the animals.

In our studies, neither the lesion of the median raphe nucleus (Thomas, Fink et al. 2000) nor of the dorsal raphe nucleus (Rex, Thomas et al. 2003) alone changed the fear-related behaviour of the rats. The 5-HT levels in the projection areas, such as cortex and hippocampus were decreased significantly (Thomas, Fink et al. 2000; Rex, Thomas et al. 2003) and comparable to the effects of 5.7-DHT lesion on 5-HT concentrations in the CNS described in the literature (Tabatabaie and Dryhurst 1998).

For the first time not only the 5-HT levels in the tissue, but also the release of 5-HT in lesioned and non-lesioned rats in the familiar cage and during exposure to the X-maze were determined. Rats in which either the dorsal or the median raphe nucleus was lesioned by 5.7-DHT, differed in the tissue concentrations of 5-HT, but not in anxiety-related behaviour and in the aversion induced 5-HT release, compared to untreated control animals (Rex, Thomas et al. 2003). A reason, that the lesion of only one of the two major raphe nuclei did not relate to the aversion induced 5-HT release and the behaviour of animals, could lie in the overlapping innervations' of the hippocampus and the cortex by the dorsal raphe nucleus and the median raphe nucleus. The lesion of one of the raphe nuclei can be compensated by the innervations' of the other raphe nuclei, at least in part.

A lesion of almost all serotonergic neurons by intraventricular administration of 5.7-DHT (Griebel 1995) or simultaneous lesions of the dorsal and of the median raphe nuclei (Thomas, Fink et al. 2000), however, interrupt all serotonergic projections to the hippocampus. The 5-HT concentrations were reduced much more and there was no increased release of 5-HT observed during the exposure to the X-maze and the rats behaved "anxiety-free" on the X-maze. The destruction of serotonergic neurons in the raphe nuclei prevents storage of 5-HT in the nerve endings in the projection areas. Consequently, 5-HT is not released due to aversion and thus no anxious behaviour was induced.

The findings presented so far support the hypothesis that there is a relationship between decreased release of 5-HT and less anxious behaviour of animals.

We could also show that reduced 5-HT release does not necessarily change the anxiety-related behaviour. The non-selective 5-HT<sub>1</sub> agonist 5-carboxamidotryptamine (5-CT) reduced the basal release of 5-HT and prevented the increased 5-HT release on the X-maze, similar to the 5-HT<sub>1A</sub> agonist 8-OH-DPAT. Nevertheless, we observed no change in anxiety-related behaviour compared to control animals after treatment with 5-CT (Rex, Fink et al. 1996).

At the present it can be generalized that anxiolytics reduce the release of 5-HT in the brain. However, a reduced concentration of 5-HT and reduced 5-HT release under resting conditions does not automatically lead to an anxiety-free behaviour of animals.



## 12. Conclusions

Similar to humans, anxiety-related behaviour in animals appears to be influenced by genetic factors and environmental conditions. Changes in housing and breeding conditions and/or variations in experimental conditions and the experimental procedure may change the behaviour of the animals profoundly. Strain differences have a strong influence on the anxiety-related behaviour in the animals. Additionally, separate maintaining and breeding of rodents over several generations may lead to the development of sublines with different anxiety-related behaviour.

Further developments of animal anxiety tests, the knowledge of their limits and the evaluation of additional ethological behavioural parameters can be used to detect changes in anxiety-related behaviour more accurately.

A correlation between the level of 5-HT concentration in the CNS and the anxiety of rat strains, but not general activation, could be proved experimentally. Anxious rats have higher tissue levels of 5-HT in projection areas of neurons originating in the median and the dorsal raphe nuclei as rat strains with "fearless" behaviour. Anxiolytics decrease extracellular 5-HT levels in the projection areas of the serotonergic neurons in the CNS, especially in more anxious rat strains.

Studies using in vivo microdialysis while performing tests for anxiety in awake and freely moving animals with permanently reduced 5-HT concentrations in specific brain regions showed that not only the absolute level of 5-HT concentration in the CNS, but also the amount of 5-HT released during an aversive situation, can be related to the behaviour of the animals.

Anxiolytics of different drug classes reduce the serotonin release during an aversive situation, whereas anxiety-causing substances increase the serotonin release under the same conditions significantly. However, a reduced release of serotonin does not always lead to fearless behaviour in rodents.

The central serotonergic transmission is also influenced by other neurotransmission systems, e. g. GABA and CCK systems..

In summary our results demonstrate the major role of the serotonergic neurotransmission in the regulation of anxiety-related behaviour. Studies on the role of the serotonergic system under aversive and non-aversive conditions may lead to a better understanding of the mechanisms involved in the development of anxiety disorders and the possible development of novel therapeutic approaches in the treatment of anxiety disorders, too.

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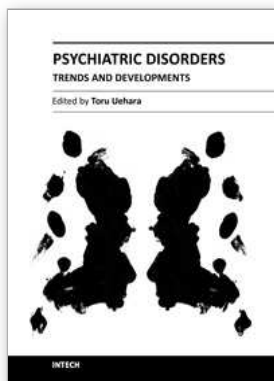
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## Psychiatric Disorders - Trends and Developments

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Due to their prevalence, pervasiveness and burden inflicted on men and women of today, psychiatric disorders are considered as one of the most important, severe and painful illnesses. This impairment of cognitive, emotional, or behavioural functioning is in some cases tragic. Aside from knowing the physical organic factors, such as infections, endocrinal illnesses or head injuries, the aetiology of psychiatric disorders has remained a mystery. However, recent advances in psychiatry and neuroscience have been successful in discovering subsequent pathophysiology and reaching associated bio-psycho-social factors. This book consists of recent trends and developments in psychiatry from all over the world, presented in the form of multifarious and comprehensive articles. The first two sections of the book are reserved for articles on schizophrenia and depression, two major illnesses present in this field. The third section of the book is reserved for addiction psychiatry, related not only to socio-cultural but also biological alterations. The last section of the book, titled Biological Neuropsychiatry, consists of three topics - updated molecular biology, fundamental neuroscience and clinical neuropsychiatric conditions. Doubtlessly, this book will be fruitful for future developments and collaboration in world psychiatry.

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