

# How Much Serotonin in the CNS is Too Much?

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## 1. Introduction

Serotonin syndrome is a neurological disorder primarily associated with inappropriate uses of serotonin (5HT)-promoting drugs such as serotonin reuptake inhibitors (SRIs), monoamine oxidase inhibitors (MAOIs) or 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy) (Boyer *et al.*, 2005; Karunatilake *et al.*, 2006; Parrott, 2002; Paruchuri *et al.*, 2006). The primary cause of the syndrome is due to a global increase in brain 5HT that can potentially activate all 14 subtypes of 5HT receptors (5HTRs). However, the significance of a given subtype contributing to the syndrome is not always the same, depending on the amount of 5HT evoked by drugs, physical condition of individual patients and even surrounding environment of drug administration. As a result, signs and symptoms of the syndrome vary widely (Mills, 1995) and thus it has been a clinical challenge to get an accurate diagnosis. Regardless, it has been recognized since the early 1990's that the symptoms of the syndrome can be generally classified into 3 categories: mental state changes (mood swings), neuromuscular hyperactivity and autonomic dysfunction (Sternbach, 1991). Among these, neuromuscular and autonomic symptoms can be replicated in laboratory animals with MAOIs combined with 5HT precursors (Shioda *et al.*, 2004) or by MDMA at a high dose (Baumann *et al.*, 2008a; Spanos *et al.*, 1989). Thus, despite many difficulties in clinical research, there are some great advances in understanding serotonin syndrome thanks primarily to preclinical investigation in animals.

Increasingly, the term "serotonin toxicity" has been used interchangeably with "serotonin syndrome", particularly in MDMA-related research. This is mainly because MDMA at high doses could cause a reduction in 5HT content in the brain and possibly axonal degeneration (Bhide *et al.*, 2009; Malberg *et al.*, 1998). In fact, there is no evidence indicating that clinically relevant doses of MDMA could produce such effects or neural death although symptoms of the serotonin syndrome occur [details reviewed by (Baumann *et al.*, 2007)]. For these reasons, we avoided using the term "toxicity" in this study unless more relevant information such as levels of cell death is available in clinically relevant literature.

To address the neurological mechanisms underlying the cause of the serotonin syndrome, we will review recent research findings on how brain 5HT is dynamically altered following administration of 5HT-promoting drugs by which the syndrome would potentially be evoked. We will focus on preclinical data since most experimental studies have been carried out in rodents (rats and mice). If available, human data will also be included for comparison. Additionally, the role of 5HT receptors in the syndrome will be discussed.

## 2. Dynamic changes in 5HT concentration

Despite the fact that brain 5HT can be found in both the extracellular space and intracellular compartments, extracellular 5HT (5HT<sub>ext</sub>) is the one involved in neurotransmission in the brain. Therefore, first of all, we will review literature on 5HT<sub>ext</sub> concentration which, in normal physiological condition, likely reflects the functional activity of serotonergic neurons. While an exhaustive literature review is not possible here, representative findings will be mentioned. For comparison, data related to the level of intracellular 5HT (non-functional component) will also be included. Pharmacologically, 5HT-promoting drugs could elevate 5HT<sub>ext</sub> to a particular level resulting in improvement of behavioral response and enhancement of mood without causing mental impairment. Thus in the second part we will review literature concerning the 5HT<sub>ext</sub> level at which behavioral incompetence can be improved in response to 5HT-promoting drugs. Thirdly, we will seek evidence of the upper limit level (threshold) above which 5HT<sub>ext</sub> is too high in the brain and potentially causes serotonin syndrome.

### 2.1 Normal extracellular concentration

As a neurotransmitter, 5-hydroxytryptamine (5HT; serotonin) is synthesized mainly at serotonergic axon terminals through decarboxylation of 5-hydroxytryptophan (5HTP). Newly synthesized 5HT molecules have three possible fates as follows:

1. uptaken into vesicles by the vesicular monoamine transporter-2 (VMAT2);
2. oxidized by mitochondrial monoamine oxidase-A (MAO<sub>A</sub>) followed by aldehyde dehydrogenase into 5-hydroxyindoleacetic acid (5HIAA);
3. spontaneously released into the extracellular space through reverse transporters (Gobbi *et al.*, 1993).

However, newly synthesized 5HT molecules are not randomly or equally directed into these three pathways. Their direction depends on physical states and drug action properties in medication. Normally, almost all newly synthesized 5HT molecules are uptaken into vesicles against concentration gradients. Although little is known about mammalian vesicular 5HT concentrations, it is suggested that there may be 270 mM (or  $1.6 \times 10^{23}$  molecules) in a 5HT vesicle of leech Retzius neurons (Bruns *et al.*, 1995; Bruns *et al.*, 2000). Generally in the brain, over 99% of 5HT molecules are stored in the synaptic vesicles. The amount of intracellular 5HT can be estimated using homogenized brain tissue. However, it should be kept in mind that the measures likely mirror intensity of serotonergic innervations (Schaefer *et al.*, 2008), but not functional activity of neurotransmission. Nevertheless, the range of 5HT content in the homogenized brain is 1 to 2 nmol/g (Baumann *et al.*, 2008a; Bhide *et al.*, 2009; Grahame-Smith *et al.*, 1974; Malberg *et al.*, 1998). Moreover, raphe nuclei have relatively higher contents than other regions (Adell *et al.*, 1991b). A similar range is also found in the mouse brain (Kim *et al.*, 2005; Pothakos *et al.*, 2010).

Several studies have been carried out to measure 5HT content in the post-mortem human brain (Parsons *et al.*, 1992; Seidl *et al.*, 1999). In general, 5HT is in the range of 100-400 pmol/g in homogenized tissues. Since 5HT is rapidly oxidized in the post-mortem tissues, it is likely that the level in the human brain is underestimated.

5HT<sub>ext</sub> is considered to be critical in maintaining mood and other affective functioning, although normally its quantity is estimated to be less than 0.1% of total 5HT molecules in the brain. At such an exceptionally small quantity, it is a challenge to determine its level in the human brain. Indeed, a PubMed search indicates that there are no relevant data available in clinical literature. Despite this, highly sensitive approaches have been developed during the last two decades for laboratory animals. For instance, Adell et al. measured 5HT<sub>ext</sub> in the cerebrospinal fluid using conventional microdialysis in the frontal cortex, striatum, hypothalamus, hippocampus, inferior colliculus and raphe nuclei, demonstrating that the 5HT<sub>ext</sub> concentration is in the range of 0.5-2 nM (Adell *et al.*, 1991b). Thus, it appears that 5HT<sub>ext</sub> in the rat cerebrospinal fluid is at a low nanomolar level. The same conclusion has been obtained by other studies with rats and mice using zero-net-flux microdialysis (Calcagno *et al.*, 2007; Gardier *et al.*, 2003; Mathews *et al.*, 2004; Tao *et al.*, 2000).

The amount and distribution of 5HT into the extracellular space are strongly implicated in many mental health problems. Physiologically, the concentration of 5HT<sub>ext</sub> is constantly kept at a state of equilibrium, involving balanced regulation between spontaneous release, 5HT<sub>1A</sub>R feedback inhibition and reuptake mechanisms as demonstrated in *in vitro* and *in vivo* studies (Becquet *et al.*, 1990; Blier, 2001; Sharp *et al.*, 2007; Wolf *et al.*, 1986). On the other hand, the intracellular concentration in vesicles of serotonergic terminals is at a high millimolar (mM) level, implying that intracellular 5HT can rapidly elevate 5HT<sub>ext</sub> to an extraordinary level in response to 5HT-promoting drugs. The response scale can be widely variable, ranging from a several-fold to a hundred-fold increase (Rutter *et al.*, 1995; Shioda *et al.*, 2004; Zhang *et al.*, 2009). Neuropharmacological investigations into the level of increased 5HT<sub>ext</sub> in response to 5HT-promoting drugs will be highlighted in the next section.

## 2.2 Therapeutic elevation of 5HT<sub>ext</sub> by 5HT-promoting drugs

Many psychoactive drugs used for patients are able to elevate 5HT<sub>ext</sub> in the brain. To better elucidate the neuropharmacological range of extracellular concentrations, we focus on three categories of 5HT-promoting drugs: serotonin reuptake inhibitors (SRIs), monoamine oxidase inhibitors (MAOIs) and 5-hydroxy-L-tryptophan (5HTP). SRIs, which elevate 5HT<sub>ext</sub> by blocking 5HT from reentering synapses, have been widely prescribed for decades in the treatment for depression, anxiety and posttraumatic stress disorders. MAOIs are one of the oldest classes of antidepressants, functioning by increasing extravesicular accumulation and spontaneous release. 5HTP is the immediate precursor compound for 5HT synthesis, which is considered to be an important supplemental ingredient in promoting mood (Parker *et al.*, 2011). Altogether, 5HT<sub>ext</sub> elevation is a part of critical mechanisms in the course of medical treatment for some mental diseases although other mechanisms based on 5HT receptors and relevant intracellular signaling pathways are also involved (Sharp *et al.*, 2011). Up to date, laboratory methods for determining 5HT release in patients' brain are not available and thus how much elevation of 5HT<sub>ext</sub> is sufficient to improve mental health in humans is unknown. The relevant knowledge on the neurochemical effects of these drugs is almost exclusively obtained by animal studies.

There are several members in the SRI family, mainly (but not exhaustively) comprising selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs). For the last three decades a variety of approaches and treatment protocols have been examined on animals to investigate how

these drugs act against 5HT reuptake into serotonergic neurons in the brain. It appears that acute systemic injection could cause only a regionally selective increase in 5HT<sub>ext</sub> (Beyer *et al.*, 2008; David *et al.*, 2003; Rutter *et al.*, 1993). At clinically relevant doses, the maximum elevation evoked by SRIs is relatively low, at less than a 2-fold increase over baseline. This is because a global increase in 5HT<sub>ext</sub> by systemic injection would activate autoreceptors, namely 5HT<sub>1A</sub>Rs in the raphe that inhibit discharge-dependent release of 5HT at axon terminals, limiting further increase in 5HT<sub>ext</sub> (Hervas *et al.*, 2000; Rutter *et al.*, 1995). Related to this, 5HT<sub>1A</sub>R activation is associated with an acute anxiogenic response to SRIs, examined with laboratory animals (Birkett *et al.*, 2011; Greenwood *et al.*, 2008). Indeed, SRIs are known to have an anxiogenic effect in human patients, particularly during the early period of medical treatments (Bigos *et al.*, 2008; Browning *et al.*, 2007).

Functional activity of 5HT<sub>1A</sub>Rs can be to some extent desensitized after long-term use of SRIs (Blier, 2001; El Mansari *et al.*, 2005). As a result, 5HT<sub>ext</sub> elevation is slightly augmented, most likely to around 2-3 fold (Dawson *et al.*, 2000) but never more than 5-fold (Popa *et al.*, 2010). It has been hypothesized that the desensitized 5HT<sub>1A</sub>Rs together with increased 5HT<sub>ext</sub> but neither alone, are two required elements for antidepressant treatments (Sharp *et al.*, 2011). If the hypothesis were correct, it suggests that a less than 5-fold increase in 5HT<sub>ext</sub> in the cerebrospinal fluid is sufficient for therapeutic purposes.

In addition to 5HT<sub>1A</sub>Rs, other 5HTR subtypes could also affect the profile of SRIs, thereby affecting 5HT<sub>ext</sub> elevation (Cremers *et al.*, 2007; Jongsma *et al.*, 2005). This suggests that in the brain there exist several other feedback loops that collectively influence the effect of systemic administration of SRIs. In other words, the response to SRIs would be stronger after eliminating the feedback inhibition. Consistently, the 5HT<sub>ext</sub> response is much higher when the drug administration is locally applied in the brain (Adell *et al.*, 1991a; Hervas *et al.*, 2000; Tao *et al.*, 2000). The maximum elevation of 5HT<sub>ext</sub> by local application of SRIs can be 5-fold but less than 10-fold compared to the baseline. Although 5HT<sub>ext</sub> at this level appears to be higher than normal, there is no evidence in the literature suggesting that a 5 to 9-fold increase is associated with mental impairment such as serotonin syndrome.

It should be kept in mind that 5HT reuptake is one of the important pathways for 5HT metabolism. Drugs that have an effect on the reuptake would likely pose the risk of interrupting the integrity of 5HT metabolism, particularly after long-term use (Moret *et al.*, 1992; Stenfors *et al.*, 2001). It has been shown that chronic treatment with SRIs could reduce the amount of intracellular 5HT in some brain regions (Bianchi *et al.*, 2002). Whether the reduced 5HT content would ultimately affect the SRI-evoked increase in 5HT<sub>ext</sub> or whether such chronic effects on intracellular 5HT are associated with the development of treatment resistance in patients is not clear.

MAOIs are a family of drugs that inhibit the activity of the MAO-A isozyme located at the outer membrane of mitochondria in the synapses of serotonergic axon terminals, causing 5HT accumulation in the cytoplasmic compartment (Evrard *et al.*, 2002; Ferrer *et al.*, 1994). Since the capacity of the cytoplasm to retain 5HT molecules is very limited while the synaptic vesicles are full, the only place for the extravesicular 5HT is to “spillover” into the extracellular space. The mechanisms for 5HT spill are not fully elucidated, most likely through the transmitter carriers that reversely transport extravesicular 5HT into the extracellular space (Gobbi *et al.*, 1993; Silva *et al.*, 2008). Thus, administration of MAOIs

would cause an increase in  $5HT_{ext}$  proportional to that of intracellular content (Ferrer *et al.*, 1994). Normally, a maximum increase following systemic injection is about 1 to 2-fold above baseline (Ferrer *et al.*, 1994; Rollema *et al.*, 2011). The effect of MAOIs is also determined by dietary ingredients, particularly of those containing the 5HT precursor such as tryptophan and 5HTP. It deserves separate mention that one of the major side effects in the clinical use of the old generation of MAOIs is hypertensive crisis, known as “cheese reaction” due to ingestion with tyramine-rich diets. The good news is that the new generation of MAOIs has fewer side effects relevant to diet [reviewed by (Wimbiscus *et al.*, 2010)]. Clinically, while SRIs are widely used as a first-line drug to treat depression, MAOIs are often recommended for treatment-resistant depression. Similar to SRIs, the MAOI-evoked increase in  $5HT_{ext}$  is also regulated by feedback mechanisms involving  $5HT_{1A}Rs$  (Lanteri *et al.*, 2009). Hence, like SRIs, MAOIs could produce relatively higher increases after the feedback inhibition mechanism is eliminated or desensitized, showing a maximum elevation of 5-10 fold above baseline (Tao *et al.*, 1994).

5HTP is recommended as a supplemental treatment for depression and menopausal hot flush (Curcio *et al.*, 2005; Shaw *et al.*, 2002). In humans, the typical 5HTP dosage is 100-300 mg/day or 1-3 mg/kg [reviewed by (Turner *et al.*, 2006)]. In animals, 5HTP alone has no measurable effect on  $5HT_{ext}$  except at a dose of 40 mg/kg and higher (Gartside *et al.*, 1992; Perry *et al.*, 1993). This implies that newly synthesized 5HT molecules can be rapidly metabolized before being accumulated in the extravesicular compartment for spontaneous release. Specifically,  $5HT_{ext}$  was increased by 2-fold in response to 75 mg/kg 5HTP (Nakatani *et al.*, 2008) and by 4-fold in response to 100 mg/kg 5HTP (Gartside *et al.*, 1992). In summary, it appears that 5HTP alone at the dose typically used in human patients has little contribution to the level of  $5HT_{ext}$ .

### 2.3 Dangerous elevations of $5HT_{ext}$ : How much $5HT_{ext}$ in the CNS is too much?

There is no doubt that  $5HT_{ext}$  is tightly regulated as a part of a homeostasis, most likely remaining at a low nanomolar concentration for maintaining normal brain function. Hypothetically,  $5HT_{ext}$  in patients with major depression is too low to stimulate 5HTRs for an affective process (such as mood). 5HT-promoting drugs could lift mood by correcting  $5HT_{ext}$  level. The amount of 5HT elevation is still at a small scale when drugs are used alone (monopharmacy).

However,  $5HT_{ext}$  could be overcorrected, particularly with medication switching and polypharmacy. Despite decades of clinical research, no laboratory test is available to monitor brain  $5HT_{ext}$  during a drug regimen in humans. It has been suggested that the change in brain  $5HT_{ext}$  is in parallel with changes in peripheral 5HT or 5HIAA measured in blood and urine (Alvarez *et al.*, 1999; Bianchi *et al.*, 2002; Celada *et al.*, 1993). Metabolically, peripheral 5HT is however independent of 5HT in the CNS; how changes in peripheral 5HT in response to drug treatment can infer changes in the brain is not yet established.

Although drug treatments would improve the mental well-being scale in terms of psychological evaluations, patients are at risk of having “excessive”  $5HT_{ext}$  in the brain. Thus, it is important to determine a safe range of  $5HT_{ext}$  concentrations for improvement in mood without causing excessive  $5HT_{ext}$ -induced side effects. It has been suggested by *in vivo* microdialysis studies that 5HT-promoting drugs usually produce a nanomolar (nM) level of

5HT<sub>ext</sub> in the cerebrospinal fluid. However, *in vitro* studies demonstrate that a micromolar ( $\mu\text{M}$ ) concentration is required to have postsynaptic effects on 5HT<sub>R</sub>-containing neurons (Cornelisse *et al.*, 2007; Marinelli *et al.*, 2004). This discrepancy in 5HT<sub>ext</sub> levels possibly represents the difference in microenvironments between the cerebrospinal fluid and synapses in the extracellular space. Conceivably, most 5HT molecules released from synapses are rapidly taken back by transporters while a small portion is diffused away from the synapses. If this is the case, synaptic 5HT concentration must be much higher than that in the cerebrospinal fluid measured by microdialysis. In addition, 5HT<sub>ext</sub> released from synapses is not evenly distributed *in vivo* across postsynaptic cell membranes. The amount of distribution depends on the distance from the release site. The concept of concentration differences in microenvironments is also supported by other studies. For instance, utilizing fast-scan cyclic voltammetry at a time resolution of 100 msec, it has been observed that 5HT<sub>ext</sub> at the extrasynaptic site was rapidly ( $\sim 5$  s) increased from nM to  $\mu\text{M}$  in response to electrical stimulation (Bunin *et al.*, 1998). In contrast, comparable stimulations could only induce a 1-2 fold increase in 5HT<sub>ext</sub> in the cerebrospinal fluid determined by microdialysis at intervals of 20 min (McQuade *et al.*, 1997). This suggests that the 5HT<sub>ext</sub> concentration in the extrasynapse compartment is much higher than in the cerebrospinal fluid, supporting the view of microenvironmental variation. Note that such high concentration of 5HT<sub>ext</sub> at synapses and/or extrasynaptic sites may not cause a neural disorder because: 1) the time duration of the effect is usually only a few milliseconds; 2) numbers and types of synaptic neurons involved in the action are highly localized; 3) the neurotransmitter can be rapidly removed by 5HT reuptake transporters. However, it could be problematic if there are prolonged and widespread effects on many 5HT<sub>R</sub>-containing neurons.

Except for MDMA, few 5HT-promoting drugs acting alone could elevate 5HT to high levels for a long period. This may occur mainly when two or more 5HT metabolic pathways are simultaneously disrupted during medication switching or polypharmacy, resulting in “excessive” 5HT<sub>ext</sub> that exerts an adverse effect on mental health. For instance, Shioda *et al.* investigated the interaction between MAOIs and SSRIs for understanding changes in 5HT<sub>ext</sub> relevant to serotonin syndrome (Shioda *et al.*, 2004). In their investigation, male Wistar rats received co-injection of tranylcypromine (3.5 mg/kg), a nonselective MAOI, and fluoxetine (10 mg/kg), an SSRI. Hypothalamic 5HT<sub>ext</sub> was determined by microdialysis while serotonin syndrome was estimated by measuring body-core temperature and neuromuscular activity. The environmental temperature was set at  $23 \pm 1$  °C. Under this condition, drug interaction caused an increase in 5HT<sub>ext</sub> by 40-fold above the pre-drug level, lasting at least 6 hours. There were obvious signs of serotonin syndrome including hyperthermia, head shakes and tremor, suggesting that 5HT<sub>ext</sub> at this level causes both physical and behavioral problems. Since each drug alone could only evoke a 2-3 fold increase, it appears that their combined effect is not simply additive, but synergistic. Other neurotransmissions, for instance, dopaminergic and glutamatergic systems that usually are not affected by single drug treatments are also elevated. This suggests that neural circuits consisting of several neuronal systems are involved in the syndrome, which is beyond the scope of this analysis and will not be discussed further here.

It is critical to determine the threshold level of 5HT<sub>ext</sub> responsible for evoking serotonin syndrome. Baumann *et al.* examined the harmful potential of MDMA by scoring behavior signs of serotonin syndrome (e.g., flat-body posture and forepaw treading) in correlation with elevated 5HT<sub>ext</sub> in the frontal cortex and nucleus accumbens of male Sprague-Dawley

rats (Baumann *et al.*, 2008a; Baumann *et al.*, 2008b). In their behavioral and neurochemical studies, animals received a first injection of MDMA at the dose of 1 mg/kg followed by a second injection of 3 mg/kg 60 min later. The ambient temperature in which animals were examined was  $22 \pm 2$  °C. As a result,  $5HT_{ext}$  was increased by 5- to 8-fold in response to 1 mg/kg and 18- to 33-fold following 3 mg/kg. While MDMA at 1 mg/kg had no effect on animal behavior, 3 mg/kg of MDMA were able to induce symptoms of the serotonin syndrome, suggesting that the  $5HT_{ext}$  threshold to evoke the serotonin syndrome may be in a range between 9 to 18-fold.

Compared to other 5HT-promoting drugs, a single injection of MDMA at clinically relevant doses has a transient effect on 5HT, lasting only 15-30 min in terms of peak effect. To obtain a significant response, many investigators employ multiple injections to mimic the binge use of MDMA in humans. Theoretically, multiple injections could produce several 5HT peak responses which would complicate the elucidation of causal relationships between 5HT and behavioral effects. To simplify the analysis, 5HT precursors (e.g., 5HTP) in combination with MAOI (e.g., clorgyline) are more applicable for elucidating the 5HT threshold. Many studies have demonstrated that a single injection of 5HTP combined with clorgyline was sufficient to produce a dose-dependent increase in  $5HT_{ext}$  in the brain, causing serotonin syndrome (Ma *et al.*, 2008; Nisijima *et al.*, 2004; Nisijima *et al.*, 2000; Nisijima *et al.*, 2001; Shioda *et al.*, 2004). To obtain the threshold level of  $5HT_{ext}$ , we designed a 5HTP dosing regimen in clorgyline-pretreated rats. Male Sprague-Dawley rats were examined under controlled ambient temperature ( $22 \pm 1$  °C) and humidity (70%) in test chambers (Ma *et al.*, 2008; Zhang *et al.*, 2009). Animals received 2 mg/kg clorgyline, 2 hours before injection of 5HTP at the dose range of 1 to 25 mg/kg.  $5HT_{ext}$  was determined in the frontal cortex and hypothalamus while behavioral responses were recorded with a 4-level scale by scoring the severity of tremor, head shakes, forepaw treading, hindlimb abduction, myotonia and Straub tail.

Specifically,  $5HT_{ext}$  was potentially elevated to as high as 100-fold above baseline following a single injection of 5HTP into clorgyline-pretreated rats, consistent with the results of previous studies (Shioda *et al.*, 2004). In fact, this level of  $5HT_{ext}$  is already well over the threshold for inducing a serotonin syndrome. Our data showed that a 55-fold increase in  $5HT_{ext}$  caused the severe syndrome manifested by high hyperthermia and all other typical signs described in animals (Jacobs *et al.*, 1975) or severe symptoms resembling those described in humans (Mills, 1995). An open question is whether such a high  $5HT_{ext}$  level is really evoked in the human brain despite drug interactions.

Our studies further demonstrated that a 10-fold increase in  $5HT_{ext}$  was sufficient to cause a mild syndrome, showing hypothermia, head shakes and myoclonus but no signs indicative of advanced or severe syndrome (e.g., tremor, forepaw treading or hindlimb abduction) (Ma *et al.*, 2008; Zhang *et al.*, 2009). This suggests that the brain is most likely intolerant of the double digit increase in  $5HT_{ext}$  in terms of fold-change in the cerebrospinal fluid. There was no syndrome-related behavioral changes in response to a 5- to 9-fold increase although head shakes were still apparent, supporting the conclusion that a single digit increase is highly tolerable. The head shaking behavior disappeared when the increased  $5HT_{ext}$  decreased to 5-fold and less, consistent with suggestion that a less than 5-fold increase in  $5HT_{ext}$  in the brain is essentially safe. Clearly, more investigation into this important area of research is needed. It is crucial to relate changes in behavior to changes in  $5HT_{ext}$  level, particularly after long-term interactions between 5HT-promoting drugs.

### 3. Involvement of 5HT receptors

There are at least 14 subtypes of 5HT receptors (5HTRs) in the brain (Green, 2006). One may wonder what the role is an individual subtype in the development of the serotonin syndrome. It should be kept in mind that 5HT is the natural agonist for these subtypes in the brain; and involvement of individual subtypes depends on not only the availability of the agonist but also the binding affinity to the agonist. It has been recognized for many years that the strength of 5HT affinity can be widely different. For instance, a  $\mu\text{M}$  concentration may be required for binding 50% of low affinity 5HT<sub>2A</sub>Rs whereas a nM concentration for high affinity 5HT<sub>1A</sub>Rs (Delpiaz *et al.*, 1995; Peroutka *et al.*, 1981; Peroutka *et al.*, 1983). Thus, the role of each subtype in the syndrome is complicated, and depends on multiple factors.

For better comparison between subtypes,  $pK_d$  or  $pK_i$  values are commonly used to indicate the strength or the selectivity of ligands to a given subtype. It is worthy pointing out that their values are not always reliable or consistent between tests due to variables in experimental conditions. Despite this, they provide trends for estimating affinity between receptor and ligand. It is well known that affinity values are strongly associated with functional activity of receptors in the brain (Clemett *et al.*, 1999; Knight *et al.*, 2004; Rossi *et al.*, 2008). Although few studies are available to completely map the affinity between 5HT and its receptor subtypes under the same condition, a valuable reference is found from the database provided by the International Union of Basic and Clinical Pharmacology or IUPHAR (Table 1). Based on their database, the 14 subtypes can be ranked in the order of affinity strength from high (sub-nM; or  $10^{-9}$  M) to low activity ( $\mu\text{M}$  or  $10^{-6}$  M). Although so many subtypes are available in the brain, it appears that they are not functioning simultaneously. Their neurological function is selectively elicited by 5HT in a concentration-dependent manner. For instance, 5HT at the concentration of approximately  $10^{-9}$  M could competitively displace 50% of competitors at the 5HT<sub>1A</sub>R site, implying that a sub-nM concentration is able to activate 5HT<sub>1A</sub>Rs. In support of this, as demonstrated *in vitro* in dorsal raphe slices, the inward current indicative of 5HT<sub>1A</sub>R activity can be elicited by 5HT at concentration less than 1 nM with the EC<sub>50</sub> at 30 nM (Penington *et al.*, 1993), closely in line with results obtained by radioligand binding assays. By contrast, other subtypes such as 5HT<sub>2A</sub>Rs are unlikely to be affected by 5HT at such a low concentration. It should be noted that 5HT<sub>1A</sub>Rs are densely located on the somatodendritic sites of serotonergic neurons in the raphe (Li *et al.*, 1997) and their high binding affinity to 5HT is physiologically important for their role in negative feedback regulation.

5HT<sub>1A</sub>Rs are also distributed on many types of postsynaptic neurons (Bert *et al.*, 2006), particularly glutamatergic and GABAergic neurons in the cortices (de Almeida *et al.*, 2008; Martin-Ruiz *et al.*, 2001). It has often been shown that these postsynaptic 5HT<sub>1A</sub>Rs can be activated by 5HT at a relatively high concentration between 1-10  $\mu\text{M}$  (Goodfellow *et al.*, 2009; Schmitz *et al.*, 1998), controlling the functional balance between glutamate and GABA transmissions.

Thus, since 5HT<sub>ext</sub> in response to most psychoactive drugs is normally in a nanomolar range (Gardier *et al.*, 2003), these postsynaptic receptors are unlikely to be able to be activated. In the case of 5HT<sub>ext</sub> exceeding maximum tolerable limits, there is a global activation of postsynaptic 5HT<sub>1A</sub>Rs, causing fluctuation of GABA and glutamate, two functionally opposite neurotransmitters. It is likely that this fluctuation in the CNS is responsible for neuromuscular hyperactivity (Paterson *et al.*, 2009), but verification of this hypothesis

related to the serotonin syndrome involving head weaving, tremor and forepaw treading awaits for further investigation.

5HT <sub>1A</sub> R	9.1 - 9.7
5HT <sub>7</sub> R	8.1 - 9.6
5HT <sub>1D</sub> R	8.0 - 9.0
5ht <sub>1e</sub> R	8.0 - 8.2
5HT <sub>2B</sub> R	7.9 - 8.4
5HT <sub>1F</sub> R	7.7 - 8.0
5HT <sub>1B</sub> R	7.4 - 9.0
5HT <sub>2C</sub> R	6.8 - 8.6
5HT <sub>6</sub> R	6.8 - 7.5
5ht <sub>5a</sub> R	6.7 - 6.9
5HT <sub>3A</sub> R	6.5 - 6.9
5HT <sub>2A</sub> R	6.0 - 8.4
5HT <sub>3AB</sub> R	6.0
5HT <sub>4</sub> R	5.9 - 7.0

Data from the database provided by the International Union of Basic and Clinical Pharmacology or IUPHAR (<http://www.iuphar-db.org/DATABASE/ObjectDisplayForward?familyId=1&objectId=1>)

Table 1. 5HT binding affinity (pK<sub>i</sub>)

Based on 5HT<sub>ext</sub> concentration, we suggest that the 5HT subtypes can be hypothetically classified into 5 groups: sub-nM, nM, high nM, sub-μM and μM. As elucidated in Table 2, more and more subtypes in the brain are involved when the concentration is increased. It is likely that, as 5HT<sub>ext</sub> exceeds its upper tolerable level, lower affinity subtypes are activated along with higher affinity ones.

5HT <sub>ext</sub> levels	Sub-nM	nM	High nM	Sub-μM	μM
<b>Affected receptors</b>	5HT <sub>1A</sub> R	5HT <sub>1A</sub> R	5HT <sub>1A</sub> R	5HT <sub>1A</sub> R (pre-synaptic)	5HT <sub>1A</sub> R (pre-synaptic)
	5HT <sub>7</sub> R	5HT <sub>7</sub> R	5HT <sub>7</sub> R	5HT <sub>7</sub> R	HT <sub>7</sub> R
		5HT <sub>2B</sub> R	5HT <sub>2B</sub> R	5HT <sub>2B</sub> R	5HT <sub>2B</sub> R
		5ht <sub>1e</sub> R	5ht <sub>1e</sub> R	5ht <sub>1e</sub> R	5ht <sub>1e</sub> R
		5HT <sub>1D</sub> R	5HT <sub>1D</sub> R	5HT <sub>1D</sub> R	5HT <sub>1D</sub> R
			5HT <sub>1B</sub> R	5HT <sub>1B</sub> R	5HT <sub>1B</sub> R
			5HT <sub>1F</sub> R	5HT <sub>1F</sub> R	5HT <sub>1F</sub> R
				5HT <sub>2C</sub> R	5HT <sub>2C</sub> R
				5HT <sub>6</sub> R	5HT <sub>6</sub> R
				5ht <sub>5a</sub> R	5ht <sub>5a</sub> R
				5HT <sub>3A</sub> R	5HT <sub>3A</sub> R
					5HT <sub>2A</sub> R
					5HT <sub>4</sub> R
					5HT <sub>3AB</sub> R

Table 2. Neurological relationship between subtype activation and increased 5HT<sub>ext</sub>. More and more subtypes are activated as 5HT<sub>ext</sub> levels are increased from sub-nM to μM

Using a radioligand receptor binding assay, studies have shown that 5HT<sub>2A</sub>Rs have low affinity and are not easily bound to 5HT (Peroutka *et al.*, 1981; Peroutka *et al.*, 1983). The significance of this finding has not been recognized simply because the threshold level of 5HT<sub>ext</sub> concentration in maintaining mental health is not fully appreciated. 5HT<sub>2A</sub>Rs are mainly (but not exclusively) distributed on glutamatergic neurons in the cortices (de Almeida *et al.*, 2007) and are critical for regulating the interconnection between the cortex and raphe. It has been widely documented in *in vitro* studies that 5HT<sub>2A</sub>Rs cannot be activated until the 5HT concentration reaches 20-50  $\mu$ M (Aghajanian *et al.*, 1997; Zhou *et al.*, 1999), suggesting that their activation threshold is much higher than 5HT<sub>1A</sub>Rs. Importantly, the answer to whether 5HT<sub>2A</sub>Rs are involved in the syndrome is a life-or-death issue. This is mainly because 5HT<sub>2A</sub>Rs are the major receptor strongly associated with hyperthermia and other autonomic hyperactivity (Mazzola-Pomietto *et al.*, 1995; Zhang *et al.*, 2011). Hyperthermia is believed to be the major cause for severe brain injury as demonstrated by MDMA studies (Malberg *et al.*, 1998). Collectively, the serotonin syndrome resulting from activation of 5HT<sub>1A</sub>Rs and 5HT<sub>2A</sub>Rs is associated with excessive 5HT<sub>ext</sub> up to a single to double-digit  $\mu$ M concentration at synapses. This suggests that 5HT<sub>ext</sub> in the synapse has arisen by 1000 times while it usually remains at a low nanomolar level. The effect is likely to correspond to a double-digit fold increase in the cerebrospinal fluid measured by *in vivo* microdialysis (Zhang *et al.*, 2009).

Most recent data have revealed that the functional activity of some 5HTR subtypes can be altered by environmental factors (Krishnamoorthy *et al.*, 2010; Nicholas *et al.*, 2003; Zhang *et al.*, 2011). For instance, Nicholas *et al.* demonstrated that, compared to a normal experimental condition, 5HT<sub>1A</sub>R activity was markedly reduced in animals examined in warm ambient temperatures. On the other hand, the responsivity of 5HT<sub>2A</sub>Rs could be markedly enhanced in warmer environments (Zhang *et al.*, 2011). Similarly, it has been found in an *in vitro* binding kinetic assay that 5HT affinity to 5HT<sub>2A</sub>Rs was strongly increased in a temperature-dependent manner (Dalpiaz *et al.*, 1995). Taken together, 5HT<sub>ext</sub> concentration required for activation of 5HTRs may vary. Indeed, it has been observed that serotonin syndrome evoked by MDMA and other 5HT-promoting drugs is severely augmented in hot and crowded conditions at raves and dance clubs (Parrott, 2002) but ameliorated in a cooling environment (Krishnamoorthy *et al.*, 2010).

#### 4. Summary

The aim of this review is to elucidate possible ranges of brain 5HT<sub>ext</sub> in association with therapeutic benefit, tolerable side effects and the development of serotonin syndrome. 5HT<sub>ext</sub> is derived from either spontaneous release or active stimulation, which initially presents at synapses and subsequently diffuses into the cerebrospinal fluid. In contrast to 5HT at synapses, 5HT<sub>ext</sub> in the cerebrospinal fluid can be easily determined by microdialysis. Physiologically, a low nanomolar concentration (nM) is crucial for normal function of maintaining constant activation of postsynaptic 5HTRs. In the case of depression, 5HT<sub>ext</sub> is at a lower level, resulting in reduced activity of 5HTRs that can affect mood and behaviors. An appropriate elevation of 5HT<sub>ext</sub> promotes a positive mood and an energized sense of physical well-being. Therapeutically, 5HT<sub>ext</sub> elevated by 5HT-promoting drugs such as SRIs or MAOIs up to 5-fold in the cerebrospinal fluid is sufficient to improve the mood.

Unfortunately, MDMA ("Ecstasy") hijacks such positive aspects of the serotonergic effect, causing drug abuse. Although generally safe, 5HT-promoting drugs could evoke an increase in 5HT<sub>ext</sub> in the cerebrospinal fluid exceeding 10-fold. When it occurs, the 5HT<sub>ext</sub> level in the synapses most likely reaches a single to double-digit  $\mu\text{M}$  concentration, which would globally activate 5HT<sub>1A</sub>Rs and/or 5HT<sub>2A</sub>Rs on glutamatergic and GABAergic neurons. Such global activation involved in both glutamate and GABA transmission leads to neurological disorders, which are manifested as serotonin syndrome. Importantly, the actions of 5HT<sub>ext</sub> on 5HTRs are highly variable, and strongly depend on behavioral states as well as external environments. Thus, the severity of the syndrome can vary from mild, to moderate, to life-threatening (Krishnamoorthy *et al.*, 2010; Parrott, 2002).

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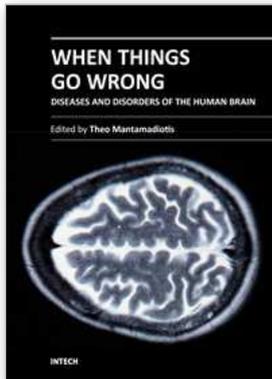
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## **When Things Go Wrong - Diseases and Disorders of the Human Brain**

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In this book we have experts writing on various neuroscience topics ranging from mental illness, syndromes, compulsive disorders, brain cancer and advances in therapies and imaging techniques. Although diverse, the topics provide an overview of an array of diseases and their underlying causes, as well as advances in the treatment of these ailments. This book includes three chapters dedicated to neurodegenerative diseases, undoubtedly a group of diseases of huge socio-economic importance due to the number of people currently suffering from this type of disease but also the prediction of a huge increase in the number of people becoming afflicted. The book also includes a chapter on the molecular and cellular aspects of brain cancer, a disease which is still amongst the least treatable of cancers.

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