Chapter

Introductory Chapter: From Measuring Serotonin Neurotransmission to Evaluating Serotonin Post-Receptor Signaling Transduction

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1. Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a well-established monoamine neurotransmitter in the central nervous system (CNS). The discovery of 5-HT dates as far back as 1868 and can be traced to its presence in the blood and in the gastrointestinal tract [1]. Its well-known biological functions include modulating cognition, sleep, emotion, learning, memory, and numerous physiological processes. 5-HT is primarily found in the enteric nervous system located in the gastrointestinal tract [2], where it

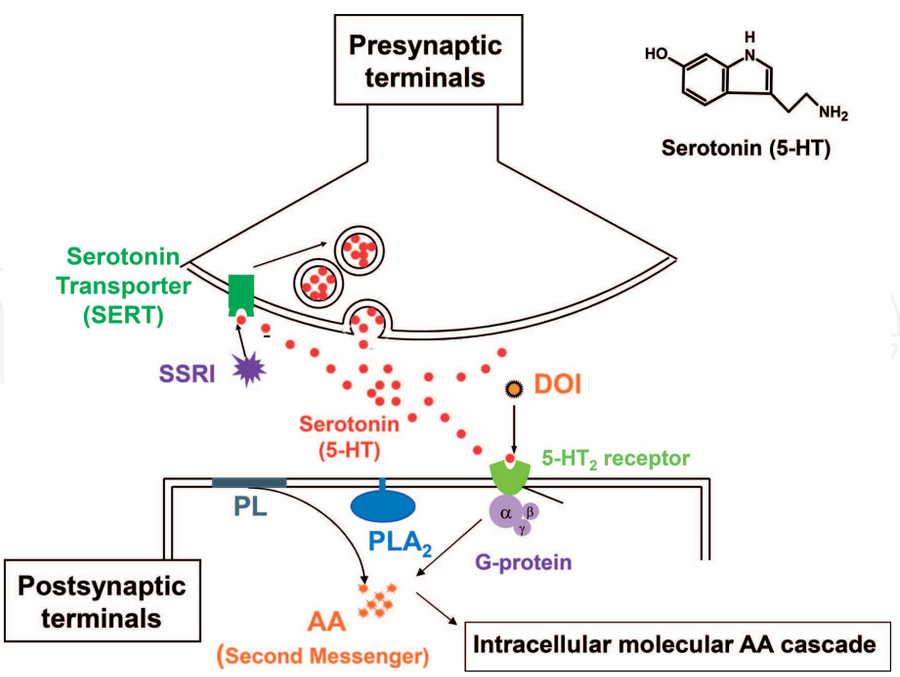

Figure 1.
Model explaining PLA_2 activation in response to serotonergic drugs. Under normal conditions, the 5-HT that is released from presynaptic vesicles into the synaptic cleft binds to postsynaptic 5-HT receptors coupled via a G-protein to PLA_2, thus hydrolyzing arachidonic acid (AA) from membrane phospholipids (PL). Administration serotonergic drugs activate PLA and increase incorporation of AA by different routes. (1) 5-$HT_{2A/2C}$ agonist, DOI directly binds to 5-HT_2 receptors to activate this signal; (2) fluoxetine (SSRI) inhibits 5-HT uptake, thus increasing 5-HT in the synaptic cleft so as to increase PLA activation and AA release. This figure adapted from [23].

regulates intestinal movements [2], and the remainder is synthesized in the serotonergic neurons of the CNS, where it has various functions such as the regulation of mood, appetite, and sleep. Modulation of 5-HT at synapses is thought to be a major action of several classes of pharmacological antidepressants. Among these, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and citalopram, are the most important class of antidepressant in the treatment of major depressive disorder (MDD) and anxiety disorders [3]. The exact mechanism of action of SSRIs is not fully revealed. SSRIs are able to increase the extracellular level of the neurotransmitter 5-HT by inhibiting its reuptake into the presynaptic terminal, increasing the level of 5-HT in the synaptic cleft available to bind to the postsynaptic 5-HT receptor (as shown in **Figure 1**). SSRIs have different degrees of selectivity for the other monoamine transporters, and the most selective SSRI has weak affinity for the norepinephrine and dopamine transporters. They are the most widely prescribed antidepressants in many countries, and their efficacy in mild or moderate cases of depression has been disputed [4] and may be outweighed by side effects [3]. I have been involved in 5-HT research for two decades. This chapter summarized my research on 5-HT-related projects from measuring 5-HT concentration, attempting to discover a new generation of SSRIs to investigate 5-HT-regulated post-receptor signaling transduction. This chapter also discusses some perspectives research that is important for SSRI and depression treatment.

2. Measuring serotonin in CNS system

In the early 1990s, liquid chromatography (LC) with an electrochemical detector (ED) had been widely used for the measurement of neurochemicals [5]. The first 5-HT project that I worked with was to develop a method for measuring 5-HT concentration in chicken brain tissue [6]. An isocratic LC-ED for the determination of L-3,4-dihydroxyphenylalanine, dopamine, norepinephrine, epinephrine, 5-HT, and their major metabolites, 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid, and 5-hydroxyindole-3-acetic acid in chicken brain tissue was developed in our lab. The method was applied to study the influence of food restriction on the concentration of 5-HT and other monoamine neurotransmitters in different brain areas, known to be involved in the feeding and reproductive behavior of female broiler chickens. In the experiment, two to six micropunches from 20 different brain areas on 300 μm cryostat brain section were punched out and expelled into Eppendorf for homogenization and extraction. Supernatant was injected onto LC-ED, and over 1000 micro-punched tissue samples from ad libitum fed and food-restricted female broiler chickens were analyzed. Tissue pellets were dissolved in PBS buffer for protein content determination to express the results as pg monoamine/μg protein. Although the concentration of monoamines in the brain is not high, multiple tissue micropunches made enough amount of monoamine and 5-HT to match the sensitivity of the assay. Our results provided a possible role for catecholamines and indolamines in the altered feeding and reproductive behavior of the broiler chicken [6]. To finish my Ph.D. thesis, I modified this method to measure 5-HT and other monoamine neurotransmitters in cat visual cortex [7]. The role of monoaminergic neuromodulators in the reorganization of cortical topography following limited sensory deprivation in the adult cat was investigated in this study [8]. The total concentrations of dopamine, noradrenaline, 5-HT, and their major metabolites were measured in the visual cortex of both control and experimental animals using this microbore LC-ED method. The sensory deprivation cats were subjected to a binocular retinal lesion corresponding to the central 10 degrees of vision and sacrificed 2 weeks post-lesion. The deprivation was confirmed in area 17 by measuring immediate-early gene if-268 messenger RNA expression. The total concentration

of 5-HT was significantly lower in the deprived cortex, and the metabolite of 5-HT, 5-hydroxyindole-3-acetic acid, was significantly higher in the nondeprived cortex than in deprived cortex and normal cortex. The levels of noradrenaline and dopamine were significantly higher in the nondeprived cortex of retinal lesion cats than in the deprived cortex of retinal lesion cats and the cortex of normal animals. This pattern follows the release of the excitatory neurotransmitter glutamate under the same conditions. These results suggest that the modulation of 5-HT, noradrenaline, and dopamine is regulated by visual afferent activity [8].

To switch my scientific career to the pharmaceutical industry, I joined the CNS drug discovery team for making a new generation dual function SSRI [9] for depression treatment. Fluoxetine (Prozac) [10] is the first SSRI and widely used for the treatment of depression which was used as reference compounds for new SSRI discovery. Fluoxetine exerts its behavioral and clinical therapeutic effect by blocking the transport of 5-HT at the serotonin reuptake transporter (SERT), thereby increasing extracellular level of 5-HT in the serotonergic synaptic cleft of many brain regions as shown in **Figure 1**. In vivo microdialysis has been extensively used to document the changes of extracellular level of 5-HT in the rat brain after administration of fluoxetine [11]. Therefore, we designed a 21-hour in vivo microdialysis experiment and the effect of acute systemic administration of fluoxetine (3 and 10 mg/kg s.c.) on extracellular level of 5-HT in the frontal cortex of freely moving rats was analyzed by LC with ESA CoulArray coulometric detector (an electrochemical detector) [9, 12]. In this experiment, the guide cannula was implanted on rats' brain by surgery and secured in place with skull screws and dental cement. Animals were allowed at least 3 days to recover from surgery prior to experimentation. Dialysis probes were perfused with artificial cerebral spinal fluid (aCSF, 47 mM NaCl, 4 mM KCl, 0.85 mM $MgCl_2$, 2.3 mM $CaCl_2$, pH 7.4) at a flow rate of 1 μL/min. Samples were collected every 60 min. Microdialysates were analyzed by LC-ED. Separation was performed on a C18 column. All values for microdialysis studies were calculated as percentage change at each time point compared with the average of three baseline values. Due to the limitation of low recovery of microdialysis probe (less than 20% in average) and low concentration of 5-HT in the frontal cortex of rat brain (about 100 fg/μL in this microdialysates), high sensitivity analytical tool is required. LC-ED was the most popular method to measure 5-HT. In recent years, liquid chromatography with tandem mass spectrometry (LC-MS/MS) was also used for this purpose [13].

Pharmacokinetic (PK) characterization and in vivo pharmacological properties of new chemical entities are important components during lead compound selection and optimization in the drug discovery process. Accordingly, reliable techniques are needed that can generate the requisite pharmacokinetic/pharmacodynamic (PK/PD) information for an increased number of compounds. When dealing with compounds targeting the central nervous system (CNS), biophase PK may differ significantly from plasma PK, because blood-brain barrier (BBB) transport and brain distribution often do not occur instantaneously and to a full extent. In vivo microdialysis technique can be used to collect not only the extracellular endogenous substances but also the extracellular free drug in the same local interstitial environment, which may reflect the amount of drug available at the pharmacological target. However, the application of this technique was highly limited by the lack of the proper sensitive analytical methods to determine the endogenous substance and exogenous drug. LC-MS/MS technique improvement provides a direct, structural-specific measurement of individual components with very high sensitivity. The mass spectrometer has minimal baseline drift and can be equilibrated very rapidly. For this purpose, we have developed a series of LC-MS/MS methods, which enable us to monitor drug, citalopram, and 5-HT in the same

microdialysis samples [13]. These applications demonstrated in vivo microdialysis coupled with LC-MS/MS is a very important tool to evaluate the PK/PD relationship by comparing the time course of free drug versus biomarker. LC-MS/MS method measuring 5-HT concentration in the brain is possible, but not widely applied [13].

3. Evaluating PK/PD profile of the dual function SSRI

The World Health Organization (WHO) estimates that more than 300 million individuals of all ages suffer from depression [14]. SSRIs have been the drugs for depression treatment. These drugs increase 5-HT levels in the synaptic cleft by inhibiting its reuptake into the presynaptic neuron through blockade of the SERT. Although many patients experience relief after treatment with one of the many marketed SSRIs, efficacy is noticeable only after weeks of treatment. Many physicians are reported to co-prescribe stimulants with SSRI to provide subjective relief during the beginning weeks of antidepressant therapy [15]. Most of these stimulants are increased dopamine release and produced robust behavioral activation, which had the risk of allowing patients to act on their suicidal ideation. It is very important to choose other classes of molecules that have been shown to produce wakefulness in animals without releasing dopamine or producing behavioral activation. Wake-promoting agents such as modafinil are used in the clinic as adjuncts to antidepressant therapy in order to alleviate lethargy. Histamine H_3 receptor antagonist has been demonstrated having the wake-promoting action in numerous animal studies and may therefore be a viable strategy for use as an antidepressant therapy in conjunction with SSRIs. Therefore, some potential antidepressant molecules were created, which combined the wake-promoting effect of a histamine H_3 receptor antagonist with 5-HT reuptake blockage effects of SERT inhibitor [9]. The synthetic approach and structure-activity relationships associated with this effort have been studied [16–18]. In vivo microdialysis experiments were used to examine whether a compound was capable of inducing a robust and persistent increase in 5-HT level over baseline. One of these molecules, JNJ-28583867 (2-methyl-4-(4-methylsulfanyl-phenyl)-7-(3-morpholin-4-yl-propoxy)-1,2,3,4-tetrahydro-isoquinoline), is a selective and potent histamine H_3 receptor antagonist (Ki = 10.6 nM) and inhibitor of the SERT (Ki = 3.7 nM), with 30-fold selectivity for SERT over the dopamine and norepinephrine transporters [9]. After subcutaneous administration, JNJ-28583867 significantly increased cortical extracellular levels of 5-HT as shown in **Figure 2A**. Baseline measurements of 5-HT levels were performed for 4 h prior to administration of JNJ-28583867. At all doses, 5-HT levels remained elevated for the duration of the experiment up to 18 h after dosing. JNJ-28583867 was also tested in a classical test of antidepressant activity, the mouse tail suspension model. As was expected based on the neurochemical profile of JNJ-28583867, an increase in struggling time was observed. Some PK characterization of JNJ-28583867 was carried out in the rat. The behavioral experiments had indicated good oral bioavailability and this was confirmed. The half-life correlates well with the observation that effects could be observed up to 24 h after a single oral dose, as was the case in the head twitch test. The plasma and brain levels of JNJ-28583867 are sustained and correlated reasonably well with efficacy for an extended period of time as shown in **Figure 2B** [9]. Similar PK/PD profiles were observed from norfluoxetine, which is the metabolite of reference SSRI, fluoxetine [12]. Norfluoxetine is the most important active metabolite of the widely used antidepressant fluoxetine. Following subcutaneous administration of fluoxetine in rats, plasma, and brain PK of fluoxetine and norfluoxetine were monitored, respectively, by LC-MS/MS. The extracellular level of 5-HT in the frontal cortex was measured by microdialysis as a PD endpoint. Norfluoxetine when directly

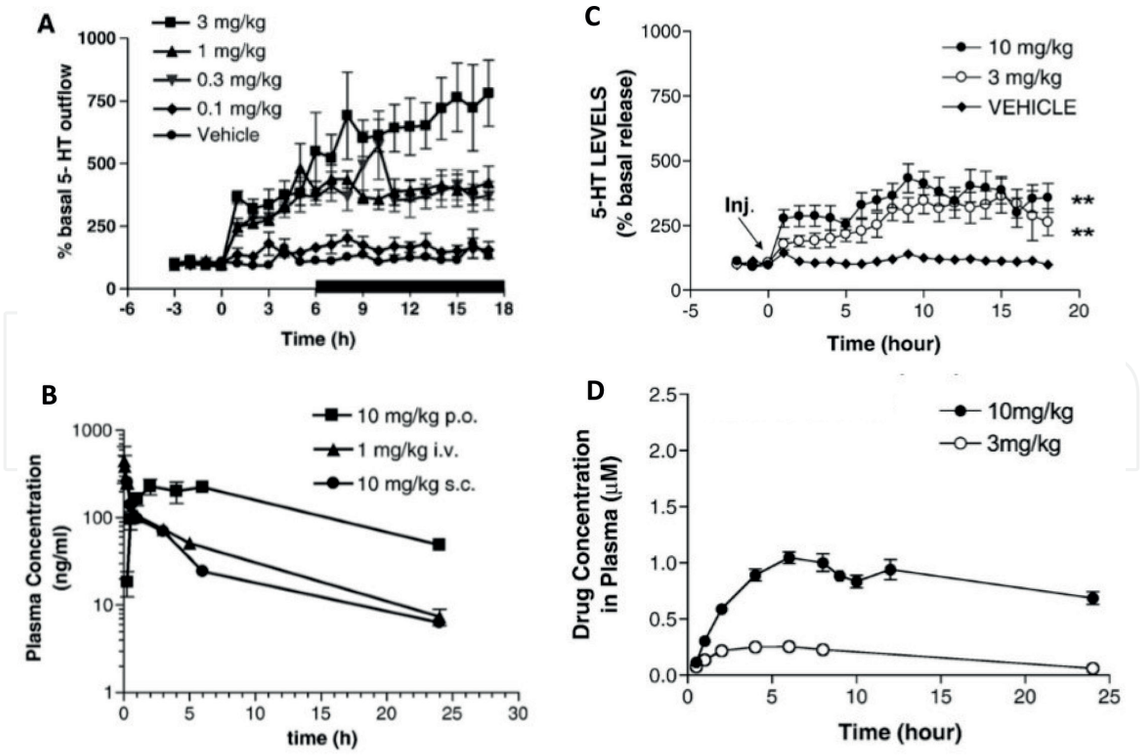

Figure 2.
*(A). Effect of JNJ-28583867, administered s.c., on extracellular 5-HT levels in the frontal cortex of male Sprague-Dawley rats. Microdialysis time course. Results are expressed as the average ± S.E.M. of n = 3–6 rats per group. (B). Plasma levels of JNJ-28583867 after oral (10 mg/kg, square), intravenous (1 mg/kg, triangle), and subcutaneous (10 mg/kg, circle) administration to the rat. Results are shown as the average ± S.D. of n = 2–3 samples. (C). Effect of norfluoxetine on the extracellular level of 5-HT in the frontal cortex of free moving rat. Values are mean ± S.E.M. of extracellular 5-HT levels and expressed as a percentage of the average of three baseline samples (defined as 100%). Two-way ANOVA-post-hoc Duncan's multiple range tests were used for comparison. Control (n = 5), 3 mg/kg (n = 6), and 10 mg/kg (n = 6) norfluoxetine were subcutaneously administrated. Asterisks indicate significance of overall effect of drug treatment versus vehicle, P < 0.01. (D). Time course of plasma concentrations of fluoxetine and norfluoxetine. Plasma concentrations (mean ± S.E., n = 10) of norfluoxetine were measured following subcutaneous administration of 3 or 10 mg/kg fluoxetine. **Figure 4A** and **B** was adapted from [9]; **Figure 4C** and **D** was adapted from [12].*

administrated to rats caused a significant increase in the extracellular level of 5-HT in the frontal cortex and maintained for 18 hours as shown in **Figure 2C**. This result is correlated well with higher plasma and brain concentration and longer plasma and brain retention time of norfluoxetine (as shown in **Figure 2D**) [12]. In summary, these studies have shown that the combination of histamine H_3 receptor antagonism with SSRI activity in a single molecule results in a pharmacology consistent with the combination of either class of molecule alone. JNJ-28583867 can be a prototype of such a compound to improve current SSRI efficacy and safety profiles [9].

4. Serotonin-mediated post-receptor signaling transduction

Although antidepressants are generally effective in the treatment of MDD, side effects still exist. Serotonin syndrome is a potentially life-threatening adverse drug reaction that results from therapeutic drug use and a predictable consequence of excess serotonergic agonism of CNS and peripheral serotonergic receptors [19]. In 2002, the Toxic Exposure Surveillance System, which receives case descriptions from office-based practices, inpatients settings, and emergency department, reported 26,733 incidences of exposure to SSRIs that caused significant toxic effects in 7349 persons and resulted in 93 deaths [19, 20]. The development mechanism of serotonin syndrome is unknown. It is hypothesized that the level of 5-HT elevation

in blood plasma has to be 10–15% above the baseline levels to result in 5-HT toxicity [21]. Several lines of evidence converge to suggest that agonism of 5-HT_{2A} receptors contributes substantially to the condition [22].

To address this question, we studied 5-HT-mediated post-receptor signaling transduction [23]. The 5-HT_2 receptor is G protein-coupled receptor and is recognized to be coupled to the phospholipase A_2 (PLA_2) signaling pathway, stimulating the release of the second messenger, arachidonic acid (AA). This signaling pathway is illustrated in **Figure 1**. PLA_2 activation can be initiated by serotonergic 5-HT_2 receptors via a G-protein. The in vivo fatty acid methods were developed in our lab to measure regional brain incorporation of a radiolabeled fatty acid, including [5,6,8,9,11,12,14,15-^{3}H] arachidonic acid (^{3}H-AA) in conscious rats. Tracer incorporation, represented as the incorporation coefficient k*, reflects PLA_2-mediated AA release. Activation of PLA_2 in the brain is revealed as increments in k* in different receptors or to change serotonergic neurotransmission (**Figure 1**). The fatty acid method can be used to evaluate serotonergic neurotransmission mediated by PLA_2 in awake rats. It can quantify and localize brain PLA_2 signaling in response to different drugs administered acutely or chronically.

In rats, 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), which is a 5-$HT_{2A/2C}$ receptor agonist, provokes head twitches, skin jerks, and forepaw tapping, behaviors that are considered part of a "5-HT syndrome" [24]. The responses usually appear at a dose of 1.0 mg/kg and peak at 2.5 mg/kg. In one of our studies, DOI, when administered to unanesthetized rats, produced widespread and significant increases, of the order of 60%, in k* for arachidonate, particularly in neocortical brain regions reported to have high densities of 5-HT_{2A} receptors [25]. The increases could be entirely blocked by chronic pretreatment with mianserin, a 5-HT_2 receptor antagonist, which is an atypical antidepressant [25]. The results suggest that the 5-HT_2 syndrome involves widespread brain activation of PLA_2 via 5-HT_{2A} receptors, leading to the release of the second messenger, arachidonic acid. Chronic mianserin, a 5-HT_2 antagonist, prevents this activation [25]. In another study, brain PLA_2-mediated signal transduction in response to acute fluoxetine administration in unanesthetized rats had been imaged [26]. By inhibiting presynaptic 5-HT reuptake, fluoxetine is thought to act by increasing 5-HT in the synaptic cleft, thus 5-HT binding to postsynaptic 5-$HT_{2A/2C}$ receptors, activates PLA_2 pathway, and releases the second messenger AA from synaptic membrane phospholipids. To image this activation, fluoxetine (10 mg/kg) or saline vehicle was administered i.p. to unanesthetized rats, and regional brain incorporation coefficients k* of intravenously injected radiolabeled AA were measured after 30 min. Compared with vehicle, fluoxetine significantly increased k* in prefrontal, motor, somatosensory, and olfactory cortex, as well as in the basal ganglia, hippocampus, and thalamus. Many of these regions demonstrate high densities of the SERT and of 5-$HT_{2A/2C}$ receptors. The brain stem, spinal cord, and cerebellum, which showed no significant response to fluoxetine, have low densities of the transporters and receptors. The results show that it is possible to image quantitatively PLA_2-mediated signal transduction in vivo in response to fluoxetine [26]. Fluoxetine's therapeutic action when chronically administered has been ascribed to desensitization of pre-synaptic 5-HT_{1A} and 5-HT_{1B} auto-receptors, further augmenting extracellular 5-HT [27]. We thereby conducted a study to see if this signaling process in rat brain would be altered by chronic administration of fluoxetine followed by 3 days of washout of this SSRI [28]. [^{3}H] AA was intravenously injected in unanesthetized rats and used quantitative autoradiography to determine the incorporation coefficient k* for AA (regional brain radioactivity/integrated plasma radioactivity), a marker of PLA_2 activation, in each of 86 brain regions. k* was measured following acute i.p. saline or DOI (1.0 mg/kg i.p.), in rats injected for 21 days with 10 mg/kg i.p. fluoxetine or saline daily, followed by 3 days without injection. As shown in **Figure 3**,

acute DOI produced statistically significant increments in k* in brain regions with high densities of 5-$HT_{2A/2C}$ receptors, but the increments did not differ significantly between the chronic fluoxetine- and saline-treated rats. Additionally, chronic fluoxetine is compared with saline widely and significantly increased baseline values of k*. These results suggest that 5-$HT_{2A/2C}$ receptor-initiated AA signaling is unaffected by chronic fluoxetine plus 3 days of washout in the rat, but that baseline AA signaling is nevertheless upregulated. This upregulation likely occurs because of significant active drug in the brain, considering the long brain half-lives of its metabolite, norfluoxetine [12]. To further understand SERT regulate brain serotonergic transmission and its mediated signaling transduction, we measured PLA_2 activation in SERT knockout mice (SERT−/−) and their littermate controls (SERT+/+). Following administration of 1.5 mg/kg s.c. DOI to unanesthetized mice injected intravenously with radiolabeled AA, PLA_2 activation, represented as the regional incorporation coefficient k* of AA, was determined with quantitative autoradiography in each of 71 brain regions. As shown in **Figure 4**, in SERT+/+ mice, DOI significantly increased k* in 27 regions known to have 5-$HT_{2A/2C}$ receptors, including the frontal, motor, somatosensory, pyriform and cingulate cortex, white matter, nucleus accumbens, caudate putamen, septum, CA1 of the hippocampus, thalamus, and hypothalamus. In contrast, DOI did not increase k* significantly in any brain region of SERT−/− mice. Head twitches following DOI, which also were measured, were robust in SERT+/+ mice but were markedly attenuated in SERT−/− mice. These results show that a lifelong elevation of the synaptic 5-HT concentration in SERT−/− mice leads to downregulation of 5-$HT_{2A/2C}$ receptor-mediated PLA_2 signaling via AA and of head twitches, in response to DOI. Compared with wild-type mice, DOI-induced k* increments were reduced in

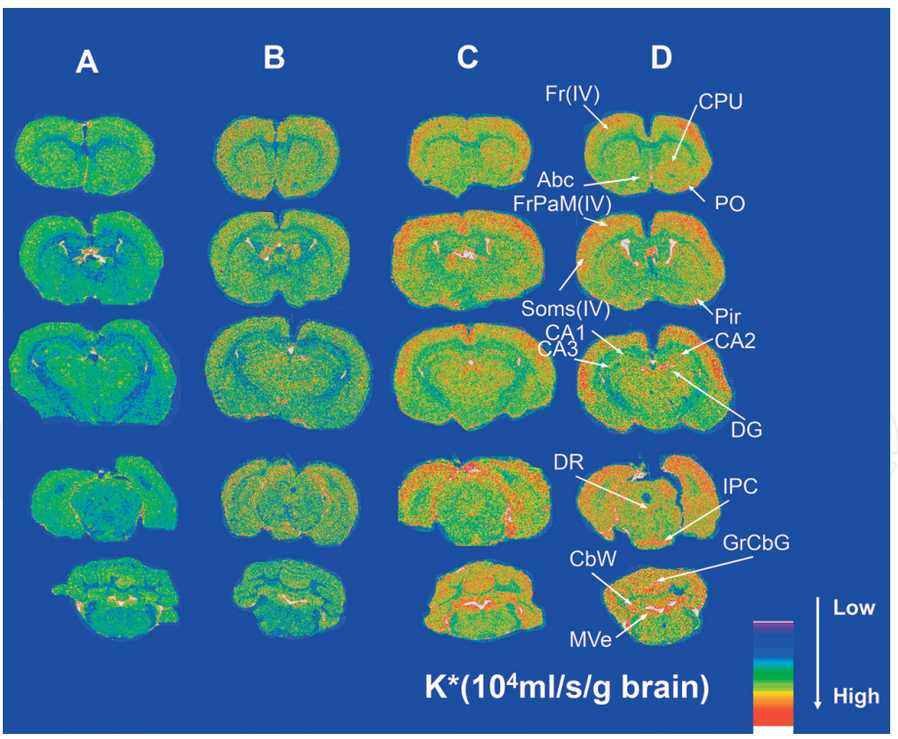

Figure 3.
Coronal autoradiographs demonstrating arachidonic acid incorporation coefficients k. Brain of (A) control rat given acute saline 3 days after receiving i.p. saline for 21 days; (B) control rat given acute DOI (1.0 mg/kg i.p.), 3 days after receiving i.p. saline for 21 days; (C) rat given fluoxetine (10 mg/kg i.p. daily) for 21 days, followed by 3 day washout, and then i.p. Saline on day 24; (D) rat given fluoxetine (10 mg/kg i.p. daily) for 21 days, followed by 3 day washout, and then acute DOI (1.0 mg/kg i.p.). k is color-coded. Abbreviations: Fr (IV), frontal cortex, layer IV; FrPaM (IV), frontal motor (layer IV); Soms, somatosensory cortex; IPC, interpeduncular nucleus; CPU, caudate putamen; CA1, CA2, CA3, DG, regions of the hippocampus; Pir, pyriform cortex; PO, olfactory cortex; GrCbG, granular layer, cerebellar gray; CbW, cerebellar white; DR, dorsal raphe; MVe, medial vestibular nucleus; Abc, nucleus accumbens. This figure adapted from [28].

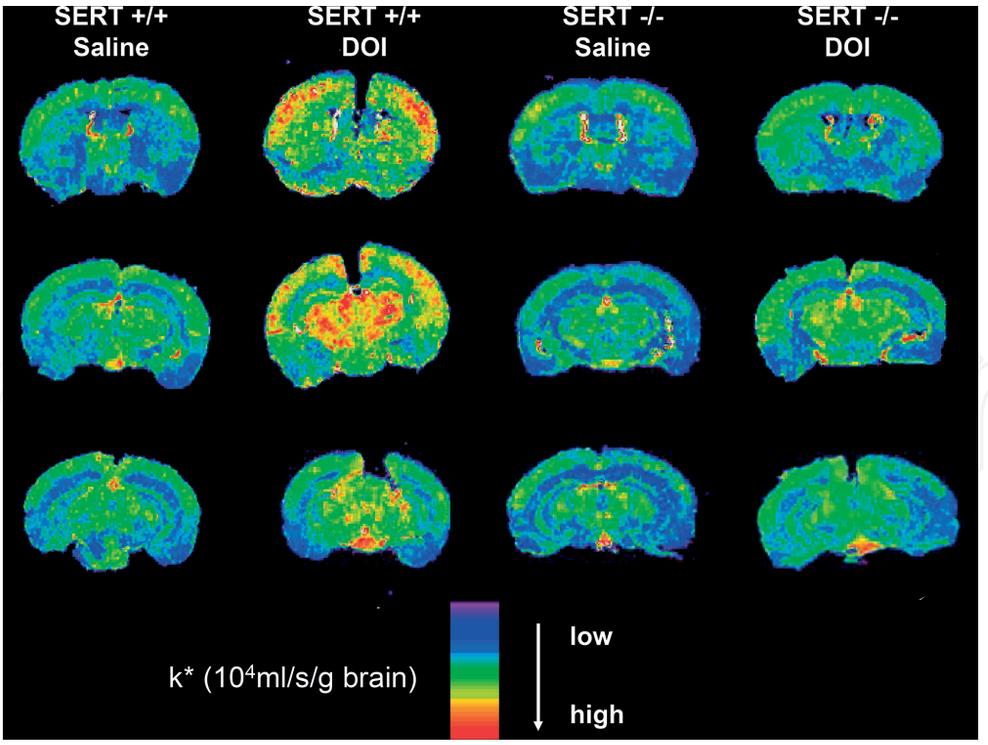

Figure 4.
Coronal autoradiographs demonstrating incorporation coefficients k for arachidonic acid, from brain of SERT+/+ mouse given saline s.c.; SERT +/+ mouse given DOI (1.5 mg/kg s.c.); SERT−/− mouse given saline; SERT+/+ mouse given DOI. k* is color coded. This figure adapted from [29].*

SERT knock out mice [29], but there was no significant effect of 3 weeks of fluoxetine plus washout on DOI-induced k* increments in compared with baseline of chronic fluoxetine treated rats. The difference suggests that a life-long, but not a 3-week, elevation of synaptic 5-HT will downregulate 5-$HT_{2A/2C}$ receptor signaling involving PLA_2.

In summary, these studies suggest that labeled AA can be used to examine in vivo brain PLA_2 signaling initiated by a serotonergic drug. Eventually, brain 5-$HT_{2A/2C}$-mediated signaling coupled to PLA_2 might be imaged in such subjects with positron emission tomography [30].

5. Monitoring therapeutic SSRI in patients

Depression is among the most prevalent psychiatric disorders with a highly variable treatment response and up to one-third of patients not achieving response [31]. SSRIs are the most commonly prescribed antidepressants and the best overall treatments for depression patients. However, therapeutic outcomes of SSRIs are often far from satisfactory for both patients and prescribing physicians [32]. Therefore, after having focused clinical research on the development of new drugs, growing evidence suggests that an improved application of available drug may still bring substantial benefit to patients [33, 34]. Moreover, there is a gap between the available pharmacological knowledge and its utilization in health care. The newest initiative to bridge this gap is "Precision Medicine." It considers individual variability to build the evidence base needed to guide clinical practice [35]. Therapeutic drug monitoring (TDM) is a patient management tool for precision medicine [36]. It enables tailoring the dosage of the medications to the individual patient by combining the quantification of drug concentration in blood, information on drug properties, and patient characteristics [37]. Because patients differ in their ability

to absorb, distribute, metabolize, and excrete drug due to concurrent disease, age, concomitant medication or genetic abnormalities, the drug's steady-state concentration in the body may have a more than 20-fold interindividual variation when the same dose of drug is administrated [38, 39]. TDM quantifies the drug's concentration in plasma or serum to adjust the dosage of individual patients, which increases probability of response and decreases risk of adverse drug reactions/toxicity [40, 41]. Moreover, TDM has the potential to enhance the cost-effectiveness of antidepressant therapy [42–44]. The benefits of TDM for optimization of pharmacotherapy, however, can only be obtained when the method is adequately integrated into the clinical treatment process. Current TDM use in depression care is often suboptimal as demonstrated by systematic studies [45–47]. The suboptimal use of TDM wastes laboratory resources and bears the risk of misleading results that will adversely influence clinical decision making. Studies on TDM for antidepressant will further specify the information on the imperfect use of TDM [48].

Among SSRIs, citalopram is the most SSRI [13], and some studies reported that it is more effective and better tolerated than other drugs for depression but has been associated with suicidality and worsening depression especially in adolescents and young adults [49]. Citalopram is strongly recommended for TDM by the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AFNP) guidelines and was recently upgraded into the level 1 recommendation drug [37, 50]. Its reported therapeutic reference ranges (50–110 ng/mL) are established and have been quantified. Controlled clinical trials have known beneficial effects of TDM, reports on decreased tolerability or intoxications [50]. Fluoxetine strongly inhibits 5-HT uptake with minimal effects on other neurotransmitter uptake system [51]. Norfluoxetine, an active metabolite of fluoxetine, contributes to the long elimination half-life (3-15 days) and overall clinical effect of fluoxetine [12]. TDM of fluoxetine is listed as "useful" AFNP guidelines [37, 50]. The therapeutic reference range of 120–500 ng/mL includes the quantification of fluoxetine and its long-lasting active metabolite, norfluoxetine. The total concentration of fluoxetine and norfluoxetine in plasma is needed to be determined. Thus, there is a clinical demand for the detection of fluoxetine and norfluoxetine when patients are receiving fluoxetine. The clinical service for TDM of antidepressants needs to be established.

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References

[1] Feldman R, Meyer J, Quenzer L. Principles of Neuropsychopharmacology. Sunderland, MA, USA: Sinauer Associates, Inc.; 1997

[2] Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annual Review of Medicine. 2009;**60**:355-366

[3] Jakobsen JC, Katakam KK, Schou A, Hellmuth SG, Stallknecht SE, Leth-Moller K, et al. Selective serotonin reuptake inhibitors versus placebo in patients with major depressive disorder. A systematic review with meta-analysis and trial sequential analysis. BMC Psychiatry. 2017;**17**:58

[4] Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, Shelton RC, et al. Antidepressant drug effects and depression severity: A patient-level meta-analysis. JAMA. 2010;**303**:47-53

[5] Duda CT, Kissinger PT. Methods in Neurotransmitter and Neuropeptide Research. Amsterdam: Elsevier; 1993

[6] Qu Y, Moons L, Vandesande F. Determination of serotonin, catecholamines and their metabolites by direct injection of supernatants from chicken brain tissue homogenate using liquid chromatography with electrochemical detection. Journal of Chromatography. B, Biomedical Sciences and Applications. 1997;**704**:351-358

[7] Qu Y, Vandesande F, Arckens L. Identification and quantification of monoaminergic neuromodulators in the sub-cortical region of cat visual cortex by microbore HPLC-ED and protein assay. Journal of Liquid Chromatography and Related Technologies. 2001;**24**:2087-2100

[8] Qu Y, Eysel UT, Vandesande F, Arckens L. Effect of partial sensory deprivation on monoaminergic neuromodulators in striate cortex of adult cat. Neuroscience. 2000;**101**:863-868

[9] Barbier AJ, Aluisio L, Lord B, Qu Y, Wilson SJ, Boggs JD, et al. Pharmacological characterization of JNJ-28583867, a histamine H(3) receptor antagonist and serotonin reuptake inhibitor. European Journal of Pharmacology. 2007;**576**:43-54

[10] Fuller RW. Serotonin uptake inhibitors: Uses in clinical therapy and in laboratory research. Progress in Drug Research. 1995;**45**:167-204

[11] Koch S, Perry KW, Nelson DL, Conway RG, Threlkeld PG, Bymaster FP. R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: An in vivo microdialysis and receptor binding study. Neuropsychopharmacology. 2002;**27**:949-959

[12] Qu Y, Aluisio L, Lord B, Boggs J, Hoey K, Mazur C, et al. Pharmacokinetics and pharmacodynamics of norfluoxetine in rats: Increasing extracellular serotonin level in the frontal cortex. Pharmacology, Biochemistry, and Behavior. 2009;**92**:469-473

[13] Qu Y, Olson L, Jiang XH, Aluisio L, King C, Jones EB, et al. Evaluating PK/PD relationship of CNS drug by using liquid chromatography/ tandem mass spectrometry coupled to in vivo microdialysis. In: Prasain JK, editor. Tandem Mass Spectrometry - Applications and Principles. London, UK: IntechOpen; 2012. pp. 421-440

[14] World Health Organization. Depression key fact. 2018. Available from: http://www.who.int/news-room/fact-sheets/detail/depression

[15] Menza MA, Kaufman KR, Castellanos A. Modafinil augmentation

of antidepressant treatment in depression. The Journal of Clinical Psychiatry. 2000;**61**:378-381

[16] Keith JM, Gomez LA, Letavic MA, Ly KS, Jablonowski JA, Seierstad M, et al. Dual serotonin transporter/histamine H3 ligands: Optimization of the H3 pharmacophore. Bioorganic & Medicinal Chemistry Letters. 2007;**17**:702-706

[17] Letavic MA, Keith JM, Jablonowski JA, Stocking EM, Gomez LA, Ly KS, et al. Novel tetrahydroisoquinolines are histamine H3 antagonists and serotonin reuptake inhibitors. Bioorganic & Medicinal Chemistry Letters. 2007;**17**:1047-1051

[18] Letavic MA, Keith JM, Ly KS, Barbier AJ, Boggs JD, Wilson SJ, et al. Novel naphthyridines are histamine H3 antagonists and serotonin reuptake transporter inhibitors. Bioorganic & Medicinal Chemistry Letters. 2007;**17**:2566-2569

[19] Boyer EW, Shannon M. The serotonin syndrome. The New England Journal of Medicine. 2005;**352**:1112-1120

[20] Isbister GK, Bowe SJ, Dawson A, Whyte IM. Relative toxicity of selective serotonin reuptake inhibitors (SSRIs) in overdose. Journal of Toxicology. Clinical Toxicology. 2004;**42**:277-285

[21] Domino FJ, Baldor RA. The 5-Minute Clinical Consult 2014. Philadelphia, US: Lippincott Williams & Wilkins; 2013

[22] Nisijima K, Yoshino T, Yui K, Katoh S. Potent serotonin (5-HT)(2A) receptor antagonists completely prevent the development of hyperthermia in an animal model of the 5-HT syndrome. Brain Research. 2001;**890**:23-31

[23] Qu Y, Chang L, Klaff J, Seeman R, Balbo A, Rapoport SI. Imaging of brain serotonergic neurotransmission involving phospholipase A2 activation and arachidonic acid release in unanesthetized rats. Brain Research. Brain Research Protocols. 2003;**12**:16-25

[24] Wettstein JG, Host M, Hitchcock JM. Selectivity of action of typical and atypical anti-psychotic drugs as antagonists of the behavioral effects of 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI). Progress in Neuro-Psychopharmacology & Biological Psychiatry. 1999;**23**:533-544

[25] Qu Y, Chang L, Klaff J, Balbo A, Rapoport SI. Imaging brain phospholipase A2 activation in awake rats in response to the 5-HT2A/2C agonist (+/−)2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI). Neuropsychopharmacology. 2003;**28**:244-252

[26] Qu Y, Chang L, Klaff J, Seemann R, Rapoport SI. Imaging brain phospholipase A2-mediated signal transduction in response to acute fluoxetine administration in unanesthetized rats. Neuropsychopharmacology. 2003;**28**:1219-1226

[27] Blier P, de Montigny C. Current advances and trends in the treatment of depression. Trends in Pharmacological Sciences. 1994;**15**:220-226

[28] Qu Y, Chang L, Klaff J, Seemann R, Greenstein D, Rapoport SI. Chronic fluoxetine upregulates arachidonic acid incorporation into the brain of unanesthetized rats. European Neuropsychopharmacology. 2006;**16**:561-571

[29] Qu Y, Villacreses N, Murphy DL, Rapoport SI. 5-HT2A/2C receptor signaling via phospholipase A2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. Psychopharmacology. 2005;**180**:12-20

[30] Giovacchini G, Chang MC, Channing MA, Toczek M, Mason A,

Bokde AL, et al. Brain incorporation of [11C]arachidonic acid in young healthy humans measured with positron emission tomography. Journal of Cerebral Blood Flow and Metabolism. 2002;**22**:1453-1462

[31] Bergfeld IO, Mantione M, Figee M, Schuurman PR, Lok A, Denys D. Treatment-resistant depression and suicidality. Journal of Affective Disorders. 2018;**235**:362-367

[32] Adli M, Baethge C, Heinz A, Langlitz N, Bauer M. Is dose escalation of antidepressants a rational strategy after a medium-dose treatment has failed? A systematic review. European Archives of Psychiatry and Clinical Neuroscience. 2005;**255**:387-400

[33] Ceskova E. The need to improve current psychopharmacotherapy before developing new drugs. Expert Opinion on Pharmacotherapy. 2014;**15**:1969-1973

[34] Shin C, Han C, Pae CU, Patkar AA. Precision medicine for psychopharmacology: A general introduction. Expert Review of Neurotherapeutics. 2016;**16**:831-839

[35] Collins FS, Varmus H. A new initiative on precision medicine. The New England Journal of Medicine. 2015;**372**:793-795

[36] Jang SH, Yan Z, Lazor JA. Therapeutic drug monitoring: A patient management tool for precision medicine. Clinical Pharmacology and Therapeutics. 2016;**99**:148-150

[37] Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K, et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: Update 2017. Pharmacopsychiatry. 2018;**51**:9-62

[38] Egberts KM, Mehler-Wex C, Gerlach M. Therapeutic drug monitoring in child and adolescent psychiatry. Pharmacopsychiatry. 2011;**44**:249-253

[39] Hermann M, Waade RB, Molden E. Therapeutic drug monitoring of selective serotonin reuptake inhibitors in elderly patients. Therapeutic Drug Monitoring. 2015;**37**:546-549

[40] Qu Y, Brady K, Apilado R, O'Malley T, Reddy S, Chitkara P, et al. Capillary blood collected on volumetric absorptive microsampling (VAMS) device for monitoring hydroxychloroquine in rheumatoid arthritis patients. Journal of Pharmaceutical and Biomedical Analysis. 2017;**140**:334-341

[41] Qu Y, Noe G, Breaud AR, Vidal M, Clarke WA, Zahr N, et al. Development and validation of a clinical HPLC method for the quantification of hydroxychloroquine and its metabolites in whole blood. Future Science OA. 2015;**1**:FSO26

[42] Ostad Haji E, Mann K, Dragicevic A, Muller MJ, Boland K, Rao ML, et al. Potential cost-effectiveness of therapeutic drug monitoring for depressed patients treated with citalopram. Therapeutic Drug Monitoring. 2013;**35**:396-401

[43] Akerblad AC, Bengtsson F, von Knorring L, Ekselius L. Response, remission and relapse in relation to adherence in primary care treatment of depression: A 2-year outcome study. International Clinical Psychopharmacology. 2006;**21**:117-124

[44] von Knorring L, Akerblad AC, Bengtsson F, Carlsson A, Ekselius L. Cost of depression: Effect of adherence and treatment response. European Psychiatry. 2006;**21**:349-354

[45] Loayza N, Crettol S, Riquier F, Eap CB. Adherence to antidepressant

treatment: What the doctor thinks and what the patient says. Pharmacopsychiatry. 2012;**45**:204-207

[46] Sharma S, Joshi S, Mukherji S, Bala K, Tripathi CB. Therapeutic drug monitoring: Appropriateness and clinical utility in neuropsychiatry practice. American Journal of Therapeutics. 2009;**16**:11-16

[47] Vuille F, Amey M, Baumann P. Use of plasma level monitoring of antidepressants in clinical practice. Towards an analysis of clinical utility. Pharmacopsychiatry. 1991;**24**:190-195

[48] Mann K, Hiemke C, Schmidt LG, Bates DW. Appropriateness of therapeutic drug monitoring for antidepressants in routine psychiatric inpatient care. Therapeutic Drug Monitoring. 2006;**28**:83-88

[49] Sharbaf Shoar N, Padhy RK. Citalopram. Treasure Island (FL): StatPearls; 2018

[50] Schoretsanitis G, Paulzen M, Unterecker S, Schwarz M, Conca A, Zernig G, et al. TDM in psychiatry and neurology: A comprehensive summary of the consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology, update 2017. The World Journal of Biological Psychiatry. 2018;**19**:162-174

[51] Beasley CM, Masica DN, Potvin JH. Fluoxetine: A review of receptor and functional effects and their clinical implications. Psychopharmacology. 1992;**107**:1-10