SEROTONIN, RECEPTORS AND TRANSPORTERS: EXPLORING NEW AND KNOWN SIGNALING PATHWAYS TO IMPROVE THE EFFICACY OF ANTIDEPRESSANT TREATMENT

EDITED BY: Thorsten Lau, Patrick Schloss, Nasser Haddjeri and Guillaume Lucas

PUBLISHED IN: Frontiers in Neuroscience

frontiers Research Topics

frontiers

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88963-889-5
DOI 10.3389/978-2-88963-889-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

SEROTONIN, RECEPTORS AND TRANSPORTERS: EXPLORING NEW AND KNOWN SIGNALING PATHWAYS TO IMPROVE THE EFFICACY OF ANTIDEPRESSANT TREATMENT

Topic Editors:
Thorsten Lau, University of Heidelberg, Germany
Patrick Schloss, University of Heidelberg, Germany
Nasser Haddjeri, Institut National de la Santé et de la Recherche Médicale (INSERM), France
Guillaume Lucas, INSERM U1215 Neurocentre Magendie, France

Citation: Lau, T., Schloss, P., Haddjeri, N., Lucas, G., eds. (2020). Serotonin, Receptors and Transporters: Exploring New and Known Signaling Pathways to Improve the Efficacy of Antidepressant Treatment. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-889-5

Table of Contents

frontiers in Neuroscience

REVIEW
published: 20 December 2018
doi: 10.3389/fnins.2018.00982

Somato-Dendritic Regulation of Raphe Serotonin Neurons; A Key to Antidepressant Action

Emily Quentin[1,2,3], *Arnauld Belmer*[1,2,3†] *and Luc Maroteaux*[1,2,3*]

[1] INSERM UMR-S 839, Institut du Fer à Moulin, Paris, France, [2] Sorbonne Universités, UPMC University Paris 6, Paris, France, [3] Institut du Fer à Moulin, Paris, France

OPEN ACCESS

Edited by:
Thorsten Lau,
Central Institute of Mental Health, Germany

Reviewed by:
Alfredo Meneses,
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), Mexico
Patrick Schloss,
Central Institute of Mental Health, Germany
Andrzej Pilc,
Institute of Pharmacology (PAN), Poland

***Correspondence:**
Luc Maroteaux
luc.maroteaux@upmc.fr

†Present address:
Arnauld Belmer,
Translational Research Institute, Queensland University of Technology, Brisbane, QLD, Australia

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: 27 September 2018
Accepted: 07 December 2018
Published: 20 December 2018

Citation:
Quentin E, Belmer A and Maroteaux L (2018) Somato-Dendritic Regulation of Raphe Serotonin Neurons; A Key to Antidepressant Action. Front. Neurosci. 12:982. doi: 10.3389/fnins.2018.00982

Several lines of evidence implicate serotonin (5-hydroxytryptamine, 5-HT)in regulating personality traits and mood control. Serotonergic neurons are classically thought to be tonic regular-firing, "clock-like" neurons. Neurotransmission by serotonin is tightly regulated by the serotonin transporter (SERT) and by autoreceptors (serotonin receptors expressed by serotonin neurons) through negative feedback inhibition at the cell bodies and dendrites (5-HT_{1A} receptors) of the dorsal raphe nuclei or at the axon terminals (5-HT_{1B} receptors). In dorsal raphe neurons, the release of serotonin from vesicles in the soma, dendrites, and/or axonal varicosities is independent of classical synapses and can be induced by neuron depolarization, by the stimulation of L-type calcium channels, by activation of glutamatergic receptors, and/or by activation of 5-HT_2 receptors. The resulting serotonin release displays a slow kinetic and a large diffusion. This process called volume transmission may ultimately affect the rate of discharge of serotonergic neurons, and their tonic activity. The therapeutic effects induced by serotonin-selective reuptake inhibitor (SSRI) antidepressants are initially triggered by blocking SERT but rely on consequences of chronic exposure, i.e., a selective desensitization of somatodendritic 5-HT_{1A} autoreceptors. Agonist stimulation of 5-HT_{2B} receptors mimicked behavioral and neurogenic SSRI actions, and increased extracellular serotonin in dorsal raphe. By contrast, a lack of effects of SSRIs was observed in the absence of 5-HT_{2B} receptors (knockout-KO), even restricted to serotonergic neurons (*Htr2b*$^{5-HTKO}$ mice). The absence of 5-HT_{2B} receptors in serotonergic neurons is associated with a higher 5-HT_{1A}-autoreceptor reactivity and thus a lower firing activity of these neurons. In agreement, mice with overexpression of 5-HT_{1A} autoreceptor show decreased neuronal activity and increased depression-like behavior that is resistant to SSRI treatment. We propose thus that the serotonergic tone results from the opposite control exerted by somatodendritic (Gi-coupled) 5-HT_{1A} and (Gq-coupled) 5-HT_{2B} receptors on dorsal raphe neurons. Therefore, 5-HT_{2B} receptors may contribute to SSRI therapeutic effects by their positive regulation of adult raphe serotonergic neurons. Deciphering the molecular mechanism controlling extrasynaptic release of serotonin, and how autoreceptors interact in regulating the tonic activity of serotonergic neurons, is critical to fully understand the therapeutic effect of SSRIs.

Keywords: serotonin receptors, somatodendritic release, volume transmission, antidepressants, autoreceptors

INTRODUCTION

In any given year, nearly 40% of the population in European countries is affected, directly or indirectly, by mental illness (Insel and Sahakian, 2012). Mental illness or psychiatric diseases are heterogeneous pathologies and much effort remains necessary to improve diagnosis and therapies. For example, 30–40% of patients with major depression do not respond to current treatments, which suggests that ontogeny of the disease may vary among individuals, and that novel pathways and therapeutic targets have to be identified. Serotonin (5-hydroxytryptamine, 5-HT) is implicated in the processing of perception, emotion, and cognitions and has been involved in various psychiatric disorders (Krishnan and Nestler, 2008). Several lines of evidence implicate serotonin in regulating personality traits and mood control. Indeed, serotonin has also been implicated in the etiology of several mood disorders, including autism spectrum disorders (ASD), major depressive disorder (MDD), schizophrenia or bipolar disorder (BD) (Vadodaria et al., 2018). Accordingly, a growing interest in understanding the molecular and cellular effect of many therapeutic compounds has emerged: serotonin transporter (SERT) is the main target of serotonin selective reuptake inhibitor (SSRI) antidepressants, and 5-HT_2 receptors are targets of atypical antipsychotics.

Variations in serotonin levels may affect mood and motivation but functions of endogenous serotonin remain controversial. It has been recently suggested that serotonin enables organisms to adapt to dynamic environments by controlling neuronal plasticity and behavior (Matias et al., 2017). Therefore, the clinical benefits of improving serotonin function would stem from facilitating adaptive changes to negative affects rather than positively modulating the emotional states (Branchi, 2011). Serotonergic neurons are classically thought to display regular tonic firing, or "clock-like," neurons (Jacobs and Azmitia, 1992), whereas phasic firing in bursts is associated with specific behaviors. Phasic and tonic firing of serotonergic neurons have also been proposed to have opposite functions. However, the respective contribution of serotonergic mode of firing to behavior remains unclear. Tonic firing of serotonin neuron population activity seems related to the extra-synaptic tonic serotonin levels and burst firing to the rapid, high-amplitude, and intra-synaptic phasic serotonin release.

However, how the positive modulation of serotonin tone translates into raised mood or decreased anxiety is not yet understood and the precise relationship between certain behaviors and brain serotonin levels remains unclear. For instance, anxiolysis as a result of reducing brain serotonin is well established, suggesting that serotonin increases anxiety. However, anxiety is often paired with depression, which is classically associated with low serotonin levels (Jennings et al., 2010). Also, SSRIs are effective in treating both disorders, but only in a fraction of patients. Therefore, the precise relationship between serotonin levels and behavior is still to be established. Studies to date have not provided a sufficiently detailed understanding of how tonic serotonin neuron activity can be related to serotonin levels. In this review, we will summarize the known molecular mechanisms controlling tonic release of serotonin, in which autoreceptors (serotonin receptors expressed by serotonin neurons) and SERT participate in regulating the excitability of serotonergic neurons. An understanding of the detailed dynamics of serotonin dendritic release might clarify how serotonin governs behavior, which is critical to fully understand the therapeutic effect of SSRIs.

THE TWO MODES OF MONOAMINE AND SEROTONIN TRANSMISSION

In the brain, neuronal communication is mediated by two major modes of chemical transmission. In the presynaptic terminal, neurotransmitters are released rapidly and locally, and signal to post-synaptic partners for synaptic transmission. In "non-synaptic" transmission, by contrast, neuromodulators diffuse over a large area to stimulate surrounding cells including glial cells (Agnati et al., 1995). In fast neurotransmission, the active zone, which is formed by defined and ordered protein network and docks synaptic vesicles, releases neurotransmitters in millisecond timing. By enhancing their release probability, this neurotransmission allows ordered vesicles to fuse in front of post-synaptic neurotransmitter receptors (Südhof, 2012). The non-synaptic mode of transmission does not take place between two pre- and post-synaptic elements as described above, and neuromodulators are released in a pseudo-open space. Thus, non-synaptic transmission is defined as "volume transmission" (Agnati et al., 1995; Zoli et al., 1999) and lasts for seconds. Precise organization of secretion is not necessary for volume transmission. This signal, which is slow and diffuses in a space larger than the synaptic cleft, involves a low concentration of neurotransmitters.

Monoamine (including serotonin) release has been subdivided into tonic and phasic modes. Tonic release controls the large variation in extracellular monoamine through basal and non-synchronous firing of neurons; by contrast, in phasic release, synchronized burst firing results in a fast, large, and transient neuromodulator increase (Grace, 2016). These neurochemical findings correspond to different neuronal activities. For example, the tonic activity of serotonin neurons can be related to extra-synaptic serotonin-containing vesicle release; the burst firing can be related to the rapid, high-amplitude, intra-synaptic phasic serotonin-containing vesicle release. Tonic firing is characterized by low frequency (0.1–3 Hz), and is classically defined as having clock-like, pace-maker regularity. Phasic firing characterized with burst of higher firing rates (up to 17 Hz) has indeed been reported in serotonin neurons (Allers and Sharp, 2003; Kocsis et al., 2006; Hajós et al., 2007). The precise control of neuronal activity that differentiates these two modes of release is not yet well understood.

The existence of serotonin volume transmission has been supported by several observations, (1) the distribution of serotonergic receptors and transporter not facing post-synaptic densities suggests that they detect serotonin released extrasynaptically (Ridet et al., 1994; Bunin and Wightman, 1999); this is notably the case for the 5-HT_{1A} receptor, which is known to play an autoreceptor function in the dorsal raphe

(Kia et al., 1996; Riad et al., 2000); (2) serotonin- and vesicular transporter (VMAT2)-positive vesicles are found not only in axonal varicosities, but also in the soma and dendrites; these VMAT2-positive vesicles are located independently of post-synaptic elements (Chazal and Ralston, 1987; Descarries and Mechawar, 2000), suggesting that non-synaptic vesicular storage and release can also occur in the somatodendritic compartment; (3) finally, it has been shown that similar amount of serotonin can be found at the somatic or dendritic level compared to axonal terminals (Bruns et al., 2000; Kaushalya et al., 2008b); in addition, extracellular concentrations of serotonin can increase in response to single stimulation pulses (Bunin and Wightman, 1999). Extrasynaptic release mechanisms likely occur by regulated exocytosis of vesicles (Trueta and De-Miguel, 2012) leading a widespread release in the extracellular space.

In axons, serotonin can be released from presynaptic terminals, but also from extra-synaptic sites (varicosities). In axonal varicosities, in dendrites and in soma, serotonin is released via volume transmission. The tonic activity of serotonin neurons being related to extra-synaptic serotonin release is likely to use volume transmission. However, the vesicular release machinery for this mode of transmission may be different from that used for synaptic transmission.

VESICULAR COMPLEXES INVOLVED IN SEROTONIN RELEASE BY VOLUME TRANSMISSION

Members of the family of soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptors (SNAREs) are involved in intracellular vesicular trafficking. The association of SNARE proteins expressed by interacting membranes triggers exocytosis by forming complexes through four coiled-coil SNARE motifs (Jahn and Scheller, 2006). Evoked synaptic vesicle release needs the canonical SNARE proteins, including the vesicle-associated SNAREs (v-SNAREs) synaptobrevin 2 that interacts with target membrane SNAREs (t-SNAREs) syntaxin 1 and SNAP-25 that are required for vesicle fusion (**Figure 1** and **Table 1**).

Volume transmission likely involves a particular vesicular machinery. Vesicular transporters traffic to synaptic vesicles as well as large dense core vesicles (Fei et al., 2008). It has been shown that, in transfected neurons, VMAT-2 is spontaneously targeted to the regulated secretory pathway and is sufficient to drive regulated exocytotic release of monoamine (Li et al., 2005). In midbrain, it has been recently reported that axons of dopamine neurons contain non-synaptic release sites (varicosities) that are required for action potential-triggered dopamine release in 30% of dopamine vesicle clusters, leading to the conclusion that a large proportion dopamine varicosities release dopamine independently of action potentials and thus use a different exocytotic release machinery (Liu et al., 2018).

If synaptic transmission mechanisms are well described, volume transmission mechanisms remain to be precisely investigated. Vesicles exocytosis might use similar machinery to the evoked transmitter-release exocytosis of neurons and neurosecretory cells. Regulated release likely uses the non-canonical SNARE proteins, present in serotonergic neurons (Okaty et al., 2015) and listed in **Table 1** including VAMP4, VAMP7 (Raingo et al., 2012; Bal et al., 2013), Vti1a or Vti1b (Kunwar et al., 2011; Ramirez et al., 2012), see for reviews (Burré, 2007; Ramirez and Kavalali, 2012). Whether volume transmission uses a mechanism more closely related to regulated vesicular release rather than classical synaptic release has to be further investigated.

TABLE 1 | Vesicles-associated molecules and mRNA expression in serotonergic neurons.

Molecule	Type	Expression in 5-HT Neurons[D]
Vesicular SNAREs (v-SNAREs)[B,R]		
Synaptobrevin 1/VAMP1	NC	++
Synaptobrevin 2/VAMP2	C	++++
Vamp3	NC	+
Vamp4	NC	++
Vamp7	NC	+
Vti1a	NC	+
Vti1b	NC	++
Target membrane SNAREs (t-SNAREs)[B,R]		
Syntaxin Stx1a	C	+
Stx1b	C	+++
Stx2	NC	+
Stx3	NC	+
Stx4a	NC	++
Stx5a	NC	+
Stx6	NC	+
Stx7	NC	++
Stx8	NC	+
Stx12	NC	+++
Stx16	NC	++
Stx17	NC	+
Stx18	NC	+
SNAP-25	C	+++++
SNAP-29	NC	+
Calcium sensors[B,R]		
Synaptotagmin Syt1		++++
Syt2		+
Syt3		+
Syt4		+++
Syt5		++
Syt6		+
Syt7		+
Syt9		++
Syt11		+++
Syt12		+
Syt13		+++
Syt16		+
Syt17		+

C, Canonical SNAREs; NC, non-canonical SNAREs; data are from [D](Okaty et al., 2015), [R](Ramirez and Kavalali, 2012), and [B](Burré, 2007).

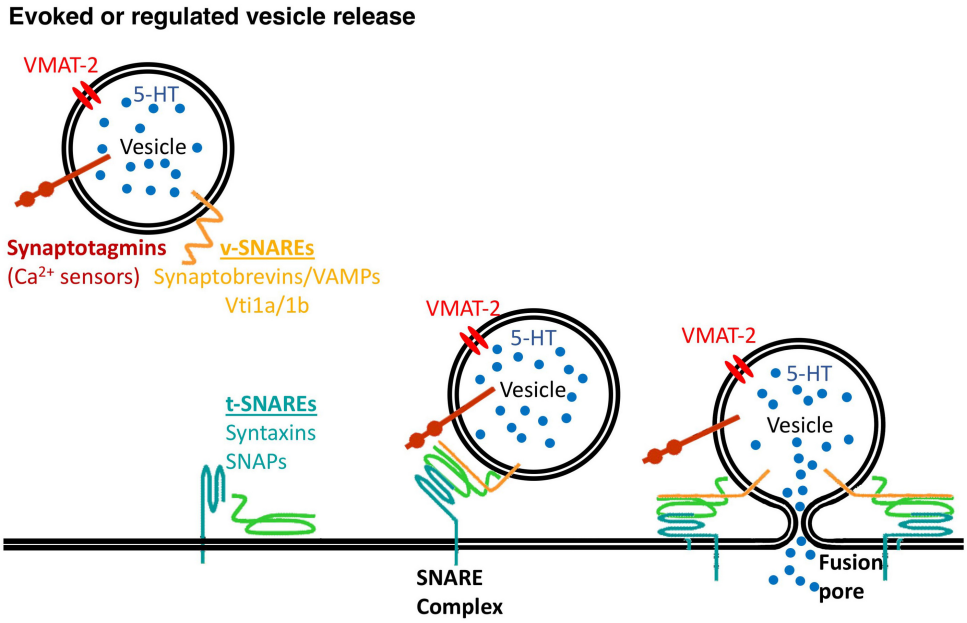


FIGURE 1 | Vesicle release needs the SNARE proteins. v-SNARE proteins, synaptobrevins/VAMPs, Vti1a/1b and t-SNARE proteins, syntaxins and synaptosomal-associated proteins (SNAPs) mediate synaptic vesicles fusion to the plasma membrane with a contribution of calcium sensors, synaptotagmins.

MODELS OF SOMATODENDRITIC SEROTONIN RELEASE

The mechanisms of non-synaptic serotonin release are difficult to study in physiological situations. Therefore, only few models of non-synaptic serotonin release have been described. Serotonin can be non-synaptically released at somatodendritic, pure somatic and/or pure dendritic compartments, with different control mechanisms (de Kock et al., 2006; Kaushalya et al., 2008a; Colgan et al., 2009; Leon-Pinzon et al., 2014).

In Leeches

One of the best described model is the leech Retzius giant serotonergic neurons, in which low electrical stimulation (induced by a single action potential) causes the somatodendritic release of serotonin as evaluated by amperometry (Bruns et al., 2000). This release lasts several seconds following initial stimulation (Trueta et al., 2003), allowing serotonin to spread to several micrometers. The initial stimulation triggers the opening of L-type calcium channels (Trueta et al., 2003), the release of serotonin from few serotonin-containing vesicles, which then via 5-HT_2-receptor activation produces a Ca^{2+} release from intracellular calcium stocks amplifying the release of serotonin from serotonin-containing vesicles (Trueta et al., 2004; Trueta and De-Miguel, 2012; Leon-Pinzon et al., 2014). In summary, somatodendritic release/exocytosis of serotonin occurs following low electrical stimulation and opening the L-type calcium channels. Ca^{2+}-induced Ca^{2+} release is reinforced by activation of 5-HT_2 receptors, which, by their coupling to the PLC pathway, amplify the serotonin release in a feed-forward manner (Leon-Pinzon et al., 2014; **Figure 2**). The resulting positive feedback loop maintains exocytosis for the following several seconds until the last vesicles in the cluster have fused (Trueta and De-Miguel, 2012; Leon-Pinzon et al., 2014). Taking into account the fact that some serotonergic neurons are capable of releasing glutamate, the co-release of this neuromodulator by simultaneous stimulation of the 5-HT_2 receptors and NMDA receptors would induce a stronger signal and thus a rapid and strong reinforcement of serotonin transmission.

In Rats

At somatodendritic level of dorsal raphe neurons, the presence of VMAT2 allows the accumulation of serotonin in vesicles (Chazal and Ralston, 1987). As shown by amperometry and 2-Photon calcium imaging, the non-synaptic somatodendritic release of serotonin-containing vesicles can be induced by the stimulation of calcium channels, or by activation of glutamatergic NMDA receptors in the absence of action potentials (de Kock et al., 2006). Using 3-Photon microscopy in living rat brain slices along with immunofluorescence and electron microscopy, vesicular serotonin release from soma and dendrites in the dorsal raphe was visualized for the first time (Kaushalya et al., 2008a; Colgan et al., 2012). These authors clearly established that punctate fluorescence does represent serotonin based on properties of multiphoton wavelength excitation, its detection in microdialysis serotonergic neurons, and its depletion upon exposure to serotonin synthesis inhibitors. Moreover, the presence of clusters of serotonin vesicles in dendrites was confirmed by (i) the immunolocalization of VMAT2 and the dendritic marker MAP2 with serotonin, (ii) the localization of VMAT2 vesicle clusters by electron microscopy in dendrites of serotonergic neuron, (iii) the size of dendritic serotonin/VMAT2 clusters comparable to

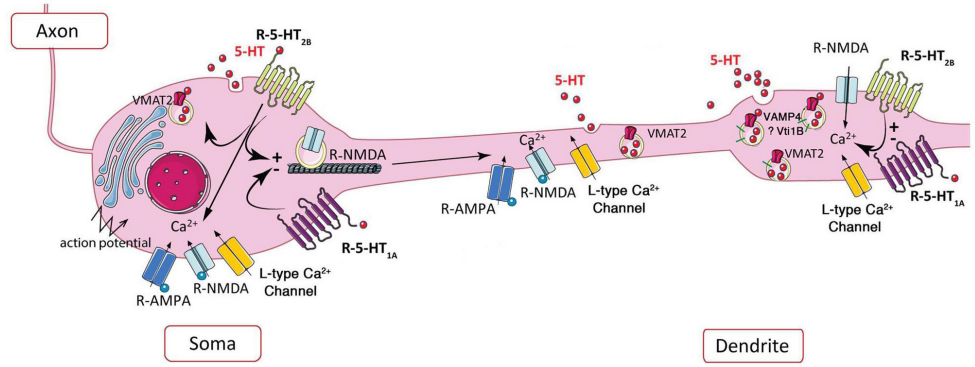


FIGURE 2 | Schematic representation of the potential role of 5-HT_{2B} receptor in extrasynaptic release of serotonin. Left, in soma, the somatic release of serotonin depends on AMPA and NMDA receptors, L-type calcium channels, and action potentials, 5-HT_{1A} and potentially 5-HT_{2B} receptor via its coupling to PKC (Trueta et al., 2003, 2004; de Kock et al., 2006; Colgan et al., 2012; Leon-Pinzon et al., 2014). Activation of 5-HT_{1A} and 5-HT_{2B} receptors may decrease or increase the membrane expression of NMDA receptors, respectively (Yuen et al., 2005, 2008). Right, the NMDA receptor-dependent dendritic release is controlled by L-type calcium channels (Colgan et al., 2012), negatively by 5-HT_{1A} and positively by 5-HT_{2B} receptors at dendritic "puncta" independently of action potential (Colgan et al., 2012).

the size of dendritic puncta and larger than terminal boutons, and (iv) the serotonin release from dendritic vesicles upon electrical stimulation or exposure to glutamate agonists, which requires extracellular Ca^{2+} and is blocked by the VMAT2 inhibitors. In the soma of serotonergic neurons, calcium channel- and NMDA receptor-activation by action potentials increases serotonin release (**Figure 2-left**); in proximal dendrites, both AMPA and NMDA receptor activation by back propagating action potentials may facilitate serotonin release; in contrast to standard release from axon terminals triggered by glutamate receptors, dendritic release of serotonin is independent of action potentials and requires L-type Ca^{2+} channels, but not sodium channels (Colgan et al., 2012; **Figure 2-right**).

Thus, unlike synaptic dendritic release in other spiking neurons, the dendritic release/exocytosis of serotonin is based on dendritic glutamatergic excitation without requirement for back-propagating action potentials, and is characterized by its sensitivity to NMDA, L-type Ca^{2+} channel blocker nimodipine. Furthermore, it was reported that upon electrical stimulation, the serotonin releasable pool is 300 times lower in comparison with dopamine despite comparable tissue content. Serotonin may be stored in vesicles or other compartments that do not exocytose consistent with a small quantity of serotonin available for release (Hashemi et al., 2012; Jennings, 2013). Hence, dorsal raphe dendrites release serotonin, and this function is physiologically and pharmacologically unique, although the molecular effectors and regulators of these dendritic non-synaptic events remain to be described in details.

SEROTONIN TONE AND SEROTONERGIC AUTORECEPTORS

5-HT_1 Receptors

Neurotransmission by serotonin is tightly regulated by autoreceptors through negative feedback inhibition at somatodendritic levels (5-HT_{1A} receptors) of the raphe nuclei or at axonal levels (5-HT_{1B} receptors). The 5-HT_{1A} autoreceptor is found in the soma and dendrites of serotonergic neurons of raphe (Kia et al., 1996; Riad et al., 2000). In the raphe, the 5-HT_{1A} autoreceptor-mediated inhibition was for long time believed to be the only homeostatic feedback mechanism controlling the tonic firing rate, pacemaker-like, of serotonergic neurons, mainly based on *in vitro* data, for review see (Piñeyro and Blier, 1999; Vizi et al., 2010). However, accumulating results are weakening the traditional model postulating that serotonin neuron autoinhibition is mediated exclusively by the hyperpolarizing 5-HT_{1A} autoreceptor and that is the main factor controlling the pacemaker-like firing rate of serotonergic neurons, for review see (Andrade et al., 2015).

At somatodendritic levels, a reduction of expression of 5-HT_{1A} autoreceptors produces strong antidepressant effects, probably due to a reduction of the negative feedback on serotonergic neuron activity (Bortolozzi et al., 2012). Moreover, the genetic suppression of 5-HT_{1A} autoreceptors causes an anxiety-like behavior in the basal state, and a higher increase in serotonin release compared to wild-type mice in response to stress (Richardson-Jones et al., 2010). Deletion of either 5-HT_{1A} or 5-HT_{1B} autoreceptors (somatodendritic and axonal, respectively) does not modify brain serotonergic tone as assessed by microdialysis (Guilloux et al., 2011). Moreover, while complete deletion of both receptors in *Htr1a/1b*$^{-/-}$ mice affected the acute response to SSRIs in the forced swim test, the chronic effects of SSRIs were still observed in anxiety test (Guilloux et al., 2011). In mice with overexpression of 5-HT_{1A} autoreceptor, hypothermic response is increased, and both serotonin content and neuronal activity are decreased in the dorsal raphe. These mice display increased anxiety- and depression-like behaviors that are resistant to chronic antidepressant treatment (Vahid-Ansari et al., 2017). In addition, blockade of 5-HT_{1A} autoreceptors in dorsal raphe brain slices was found to have surprisingly no effect on the firing of the serotonergic neurons

as reviewed in Liu et al. (2005). There is thus a discrepancy in 5-HT_{1A} receptors acting as a regulator of pace-maker homeostasis of serotonergic neurons between *in vivo* and *in vitro* studies.

Other studies showed that serotonergic cell groups can be interconnected, the dorsal raphe in particular receiving serotonergic inputs from the caudal raphe (Bang et al., 2012), which may implicate different types of serotonergic neurons. 5-HT_{1A} receptors participate in serotonergic neurons with different electrophysiological profiles, the inhibitory effect of 5-HT_{1A} receptors being superior in dorsal raphe than in median raphe neurons, suggesting greater negative feedback in the dorsal raphe (Beck et al., 2004). Similarly, Teissier et al. (2015) identified opposed consequences of dorsal vs. median raphe serotonergic neuron inhibition, suggesting that median raphe hyperactivity increases anxiety, whereas low dorsal over median raphe serotonergic activity ratio increases depression-like behavior. These observations suggest a heterogeneity of serotonergic neurons, which are interconnected but not necessarily located in the same serotonergic nucleus. It will thus be worth testing the effect of altering volume transmission in various raphe nuclei.

5-HT_2 Receptors

On dorsal raphe slices, most serotonin neurons are hyperpolarized following the opening of GIRK channels by the application of a 5-HT_{1A} receptor agonist. In the presence of 5-HT_{1A}-receptor antagonists, it has been reported that serotonin induces a depolarization, which can be blocked by different antagonists specific of Gq-coupled 5-HT_2 receptors (Craven et al., 2001). In another study using rat brain slices, the stimulation of 5-HT_{1A} receptors also hyperpolarized most serotonin neurons, and about half of these neurons show also a depolarization in response to 5-HT_2 receptor agonists (Marinelli et al., 2004). These data suggest that 5-HT_2 receptors expressed by subsets of serotonergic neurons could participate in serotonin somatodendritic volume transmission. Local agonist stimulation of 5-HT_{2B} receptors in dorsal raphe increased extracellular serotonin, supporting an excitatory effect of this receptor on serotonergic neuron activity (Doly et al., 2008). Furthermore, a fraction of raphe serotonergic neurons coexpress both 5-HT_{1A} and 5-HT_{2B} receptors (Diaz et al., 2012). These observations confirmed that serotonergic neurons are heterogeneous by expressing different serotonin receptors and that both 5-HT_{1A} and 5-HT_2 receptors could participate in serotonin tone regulation.

Putative positive regulation of dorsal raphe by 5-HT_{2B} receptors has been proposed (McDevitt and Neumaier, 2011). Strikingly, acute and long-term effects of SSRIs both in behavior and neurogenesis were eliminated after genetic ablation of 5-HT_{2B} receptors or upon selective antagonist treatment (Diaz et al., 2012). Conversely, pharmacological experiments indicated that acute agonist stimulation of 5-HT_{2B} receptors mimicked acute SSRI action (Diaz and Maroteaux, 2011) and that chronic agonist stimulation of 5-HT_{2B} receptors mimicked chronic SSRI action on behavior and neurogenesis, which were abolished in mice knocked-out (KO) for the 5-HT_{2B} receptor gene (*Htr2b*$^{-/-}$) (Diaz et al., 2012). Accordingly, conditional KO mice for 5-HT_{2B} receptors only in serotonergic neurons (*Htr2b-cKO*$^{5-HT}$ mice), reproduced the lack of SSRI effects; these mice also displayed a reduced tonic firing frequency of dorsal raphe serotonin neurons, and a stronger hypothermic effect of 5-HT_{1A}-autoreceptor stimulation (Belmer et al., 2018). The increased excitability of serotonergic neurons observed upon selective 5-HT_{2B}-receptor overexpression in raphe serotonergic neurons confirmed the cell autonomous effect of this receptor. The excess of inhibitory control exerted by 5-HT_{1A} receptors in *Htr2b-cKO*$^{5-HT}$ mice may thus explain the lack of response to chronic SSRI in these mice. Conversely, the raphe neurons from mice expressing reduced amount of 5-HT_{1A} receptors (5-HT_{1A}-Low) are more likely to fire at higher rates than control mice, consistent with decreased autoinhibition (Richardson-Jones et al., 2010). In parallel, Philippe et al. (2018) showed that an increased 5-HT_{1A}-autoreceptor binding and function led to reduced serotonergic tone, increased anxiety-depression-like behaviors, and induced mice to be resistant to chronic fluoxetine. A higher 5-HT_{1A}-autoreceptor reactivity and a lower firing activity of these neurons was observed in *Htr2b-cKO*$^{5-HT}$ mice (Belmer et al., 2018). Confirmation of these findings have been obtained in mice expressing the activator Gq-coupled DREADDS hM3Dq (similar to 5-HT_{2B} receptor's coupling) in serotonergic neurons, which demonstrates, upon stimulation, an increase in serotonergic neurons firing rates (Teissier et al., 2015) and an antidepressant-like behavioral response (Urban et al., 2016). On the contrary, mice expressing the inhibitory Gi-coupled DREADDS hM4Di (similar to 5-HT_{1A} receptor's coupling) in serotonergic neurons display, upon stimulation, a decrease in serotonin neuronal firing rates (Teissier et al., 2015). The serotonergic tone may thus result from the opposite control exerted by cross-regulation between Gi-coupled 5-HT_{1A} and Gq-coupled 5-HT_{2B} receptors on serotonergic neurons (Belmer and Maroteaux, 2018).

Interestingly, frog motor neurons showed potentiation of NMDA-induced depolarization by serotonin. The underlying mechanism involves: (1) activation of 5-HT_{2B} receptors; (2) activation of a Gq-protein; (3) a transduction mechanism causing an influx of extracellular Ca^{2+} through L-type calcium channels; (4) binding of Ca^{2+} to calmodulin; and (5) reduction of the open-channel block of the NMDA receptor produced by physiological concentration of Mg^{2+} ions (Holohean and Hackman, 2004). Furthermore, Bigford et al. (2012), showed that either 5-HT_{2B} or 5-HT_{2C} receptor antiserum immunoprecipitated GluN1 subunit of NMDA receptors, suggesting that these receptor subtypes are able to interact in complexes with NMDA receptors and our unpublished data confirmed a 5-HT_{2B}- and GluN1-receptor association. Independently, the 5-HT_{2B} receptor, which is expressed in stomach and cardiomyocytes, has been reported to act via L-type calcium channels in both tissues (Cox and Cohen, 1996; Bai et al., 2010) and activation of 5-HT_{2B} receptors triggered also intracellular calcium release from ryanodine-sensitive stores as shown in the leech somatodendritic release of serotonin (Leon-Pinzon et al., 2014). Together, these data indicate that somatodendritic release of serotonin is a model in which 5-HT_{2B} receptors could participate and regulate the excitability of serotonergic neurons together with 5-HT_{1A} receptors.

The mechanism by which these two receptors interact remains to be described as well as the associated partners and intracellular pathways involved in the regulation of serotonergic tone at the level of serotonin neurons themselves.

VOLUME TRANSMISSION, SERT, AND SSRI ANTIDEPRESSANTS

The serotonin transporter SERT by regulating extracellular levels of serotonin is a major partner in the regulation of serotonin tone (Piñeyro and Blier, 1999; Vizi et al., 2010). Under normal conditions, evoked extracellular serotonin concentration shows strong firing frequency-dependence. Mice lacking SERT (KO mice) or treated with SSRIs display extracellular serotonin concentrations evoked by stimulation that tend to similar high levels at all frequencies, while in SERT overexpressing mice, evoked extracellular serotonin concentrations tend to equal low levels (Jennings et al., 2010). These findings, therefore, indicate that SERT plays a role of a frequency pass filter in regulating extracellular serotonin concentrations evoked by stimulation. The role of SERT in setting basal extracellular serotonin concentrations and detailed contribution to serotonergic tonic and volume transmission has yet to be investigated *in vivo*.

The therapeutic effects of SSRIs are initially triggered by blocking SERT. Microdialysis experiments have shown that acute SSRI injections increase extracellular levels of serotonin by approximately 400% in the dorsal raphe and nearly 200% in forebrain terminal regions (Invernizzi et al., 1992, 1997). The SSRI-dependent increases in extracellular serotonin concentration require Ca^{2+}-dependent vesicular release, which should induce somatodendritic 5-HT_{1A} autoreceptor-mediated decreases in spontaneous release of serotonergic neurons (Gartside et al., 1995; Hajos et al., 1995). Following SSRI injection, although basal serotonergic firing rates should decrease, the tonic activity increases extracellular serotonin levels (Dankoski et al., 2016). Interestingly, local infusion in the dorsal raphe of a 5-HT_{2B} receptor agonist through the microdialysis probe produced an increase in extracellular serotonin concentration that could be blocked by 5-HT_{2B} receptor antagonist (Doly et al., 2008) and mimicked the SSRIs effects. These data support a contribution of this receptor subtype in carrier-dependent serotonin accumulation.

One mechanism by which SERT can contribute to the enhancement of extracellular serotonin includes reversed transport, i.e., by carrier-mediated efflux (Forrest et al., 2008; Sitte and Freissmuth, 2015). The "club drug" 3,4-methylenedioxy methamphetamine (MDMA, ecstasy) binds preferentially to and reverses the activity of SERT, by causing release of serotonin from vesicles. Acute pharmacological inhibition or genetic ablation of 5-HT_{2B} receptors in KO mice completely abolished MDMA-induced hyperlocomotion, sensitization, and serotonin release. Furthermore, the 5-HT_{2B} receptor dependence of MDMA-stimulated release of endogenous serotonin relies on its expression in serotonergic neurons as recently demonstrated in mice lacking 5-HT_{2B} receptors only in serotonergic neurons (*Htr2b-cKO*$^{5-HT}$ mice) (Belmer et al., 2018). These data support also a contribution of this receptor subtype in carrier-dependent serotonin efflux. Unlike serotonin release in soma or terminals, dendritic serotonin release in response to AMPA or NMDA receptor stimulation requires L-type Ca^{2+} channels. AMPA-evoked serotonin release measured with varying fluoxetine concentrations showed that somatic serotonin release has fivefold greater sensitivity to fluoxetine than responses from dendritic puncta (Colgan et al., 2012). Differences in SERT regulation, localization and/or function may explain this difference, since SERT immunoreactivity has been mainly found at the plasma membrane in extrasynaptic location including axonal varicosities, whereas in soma and dendrites it was mainly observed intracellularly (Vizi et al., 2010; Belmer et al., 2017).

The therapeutic effects induced by SSRIs rely on long-term neuroadaptations. Since the activation of 5-HT_{1A} autoreceptor decreases the activity of serotonin neurons (Commons, 2008), more than 2 weeks of SSRI treatment are necessary to observe a decreased expression of 5-HT_{1A} receptors in serotonergic neurons (Popa et al., 2010). This decrease in expression of 5-HT_{1A} receptors, which is followed by an increase in the firing of serotonergic neurons, has been proposed to explain the clinical delay of the antidepressant effect of SSRIs (Adell et al., 2002; Santarelli et al., 2003; Richardson-Jones et al., 2010; Rainer et al., 2012). In SERT KO mice, 5-HT_{1A} autoreceptors are desensitized in raphe nuclei, while they remain intact in post-synaptic neurons (Fabre et al., 2000). This desensitization of 5-HT_{1A} autoreceptors in the raphe is thought to be due to the chronic accumulation of extracellular serotonin in the absence of uptake (Soiza-Reilly et al., 2015). The lack of acute and chronic SSRI efficacy observed in *Htr2b-cKO*$^{5-HT}$ mice is associated with a reduced tonic firing frequency of dorsal raphe serotonin neurons, whereas the selective 5-HT_{2B}-receptor overexpression in raphe serotonergic neurons increases the excitability of these neurons (Belmer et al., 2018). Together with the observation that agonist stimulation of 5-HT_{2B} receptors is sufficient to reproduce SSRI effects including raphe serotonin accumulation, these results support that the reduction in 5-HT_{1A} receptor activity drives the antidepressant efficacy that may involve SERT regulation.

Colgan et al. (2012) proposed that the differential regulation between somatic vs. dendritic serotonin release may explain the antidepressant effects of inhibitors of NMDA receptors like ketamine (Machado-Vieira et al., 2009; Casamassima et al., 2010). Ketamine, has recently been shown to increase serotonin in prefrontal cortex, which correlates with antidepressant-like activity in the forced swimming test; its antidepressant-like activity requires activation of raphe AMPA receptors that recruits the prefrontal cortex neural circuit (Pham et al., 2017). Furthermore, AMPA receptor-dependent serotonin release and subsequent 5-HT_{1A} receptor stimulation may be involved in the actions of an mGlu2/3 receptor antagonist and ketamine in the NSF test (Fukumoto et al., 2014). However, it has been reported that a direct activation of AMPA receptors by ketamine metabolites and mTOR signaling is sufficient to increase synaptogenesis in prefrontal cortical pyramidal neurons and to enhance serotonergic neurotransmission via descending inputs to the raphe nuclei or even by a direct inhibition of NMDA receptors localized on GABAergic interneurons, for reviews see

(Artigas et al., 2018; Zanos and Gould, 2018). It is therefore unlikely that the rapid antidepressant effects of NMDA receptor inhibitors act through a control of serotonergic tone, which would require time to be efficient, but through a direct control of upstream targets.

GENETIC VARIANTS OF MOLECULES PUTATIVELY ASSOCIATED TO VOLUME TRANSMISSION

Interestingly, human polymorphisms associated to psychiatric diseases have been found in genes encoding molecules putatively involved in somatodendritic release, including voltage-gated L-type calcium channel subunit, 5-HT_{2B} receptor, 5-HT_{1A} receptor, VMAT-2, or SERT. Single-nucleotide polymorphisms (SNPs) in the α1 subunit (*CACNA1C*) of the L-type calcium channels Cav1.2 rank among the most consistent and replicable genetics findings in psychiatry and have been associated with schizophrenia, bipolar disorder and major depression (Casamassima et al., 2010; Dedic et al., 2018) and more recently with treatment resistant depression (Fabbri et al., 2018). In humans, a loss-of-function SNP of 5-HT_{2B} receptors is associated with serotonin-dependent phenotypes, including impulsivity and suicidality (Bevilacqua et al., 2010). Association studies with the functional 5-HT_{1A} receptor promoter SNP rs6295 showed that patients present early deficits in cognitive, fear and stress reactivity that may lead to depression (Albert and Fiori, 2014). A specific haplotype in SLC18A2, the gene encoding VMAT-2, was significantly associated with depression symptoms in men (Christiansen et al., 2007). Furthermore, a significant association was found between post-traumatic stress disorder (diagnosis) and SNPs in SLC18A2 (Solovieff et al., 2014). Carriers of the short allele of the promoter polymorphism of SERT gene (5-HTTPR) have increased anxiety-related traits and elevated risk of depression (Pezawas et al., 2005). Evidence points to a lower response to SSRIs among Caucasian patients with the 5-HTTPR short genotype and among (Asian) patients with the STin2 10/12 genotype (Smits et al., 2008). However, humans carrying the short variant of the 5-HTTPR outperform subjects carrying the long allele in an array of cognitive and social tasks (Homberg and Lesch, 2011). So, one has to be careful in interpreting data from human gene polymorphism, without extensive characterization of their physiological consequences. These human polymorphisms that are associated to psychiatric diseases have then to be validated in models of serotonin somatodendritic release.

CONCLUSION

Our understanding of serotonin transmission has been limited by technical problems. This review has summarized different mode of serotonin transmission and how they could impact behavioral and antidepressant efficacy. A better description of the molecular mechanisms involved in regulating serotonin somatodendritic release *in vivo*, using for example 3-Photons microscopy, is necessary to identify the impact of various modes of serotonin release and to unravel the mechanisms of tonic serotonin level regulation. These data should indicate if different modes of serotonin release mediate distinct behavioral effects. Understanding whether and how serotonin tone is controlled may also increase our understanding how its impact on behavior. By deciphering the molecular mechanisms of serotonin release that regulate firing patterns we should be able to increase our knowledge of serotonin function in physiological and pathophysiological situations. This should ultimately allow us to improve treatment of psychiatric disorders involving serotonin, such as depression.

AUTHOR CONTRIBUTIONS

All authors collected references, wrote the manuscript, and prepared the figures.

FUNDING

Research in the Maroteaux laboratory has been supported in part by funds from the *Centre National de la Recherche Scientifique*, the *Institut National de la Santé et de la Recherche Médicale*, the *Sorbonne Université Sciences – Pierre et Marie Curie*, and by grants from the *Fondation pour la Recherche sur le Cerveau*, the *Fondation de France*, the *Fondation pour la Recherche Médicale* "Equipe FRM DEQ2014039529," the French Ministry of Research (Agence Nationale pour la Recherche ANR-17-CE16-0008 and the Investissements d'Avenir programme ANR-11-IDEX-0004-02) and the DIM Cerveau et Pensée from Region Ile de France. LM's team is part of the École des Neurosciences de Paris Ile-de-France network and of the Bio-Psy Labex and as such this work was supported by French state funds managed by the ANR within the Investissements d'Avenir programme under reference ANR-11-IDEX-0004-02.

REFERENCES

Adell, A., Celada, P., Abellan, M. T., and Artigas, F. (2002). Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res. Brain Res. Rev.* 39, 154–180. doi: 10.1016/S0165-0173(02)00182-0

Agnati, L. F., Zoli, M., Strömberg, I., and Fuxe, K. (1995). Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* 69, 711–726. doi: 10.1016/0306-4522(95)00308-6

Albert, P. R., and Fiori, L. M. (2014). Transcriptional dys-regulation in anxiety and major depression: 5-HT1A gene promoter architecture as a therapeutic opportunity. *Curr. Pharm. Des.* 20, 3738–3750. doi: 10.2174/13816128113196660740

Allers, K. A., and Sharp, T. (2003). Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience* 122, 193–204. doi: 10.1016/S0306-4522(03)00518-9

Andrade, R., Huereca, D., Lyons, J. G., Andrade, E. M., and McGregor, K. M. (2015). 5-HT1A receptor-mediated autoinhibition and the control of serotonergic cell firing. *ACS Chem. Neurosci.* 6, 1110–1115. doi: 10.1021/acschemneuro.5b00034

Artigas, F., Celada, P., and Bortolozzi, A. (2018). Can we increase the speed and efficacy of antidepressant treatments? Part II. Glutamatergic and RNA interference strategies. *Eur. Neuropsychopharm.* 28, 457–482. doi: 10.1016/j.euroneuro.2018.01.005

Bai, C.-F., Liu, J.-C., Zhao, R., Cao, W., Liu, S.-B., Zhang, X.-N., et al. (2010). Role of 5-HT2B receptor in cardiomyocyte apoptosis of norepinephrine-induced cardiomyopathy model in rats. *Clin. Exp. Pharmacol. Physiol.* 37, e145–e151. doi: 10.1111/j.1440-1681.2010.05388.x

Bal, M., Leitz, J., Reese, A. L., Ramirez, D. M. O., Durakoglugil, M., Herz, J., et al. (2013). Reelin mobilizes a VAMP7-dependent synaptic vesicle pool and selectively augments spontaneous neurotransmission. *Neuron* 80, 934–946. doi: 10.1016/j.neuron.2013.08.024

Bang, S. J., Jensen, P., Dymecki, S. M., and Commons, K. G. (2012). Projections and interconnections of genetically defined serotonin neurons in mice. *Eur. J. Neurosci.* 35, 85–96. doi: 10.1111/j.1460-9568.2011.07936.x

Beck, S. G., Pan, Y.-Z., Akanwa, A. C., and Kirby, L. G. (2004). Median and dorsal raphe neurons are not electrophysiologically identical. *J. Neurophysiol.* 91, 994–1005. doi: 10.1152/jn.00744.2003

Belmer, A., Klenowski, P. M., Patkar, O. L., and Bartlett, S. E. (2017). Mapping the connectivity of serotonin transporter immunoreactive axons to excitatory and inhibitory neurochemical synapses in the mouse limbic brain. *Brain Struct. Funct.* 222, 1297–1314. doi: 10.1007/s00429-016-1278-x

Belmer, A., and Maroteaux, L. (2018). Regulation of raphe serotonin neurons by serotonin 1A and 2B receptors. *Neuropsychopharmacology* 44, 218–219. doi: 10.1038/s41386-018-0214-6

Belmer, A., Quentin, E., Diaz, S. L., Guiard, B. P., Fernandez, S. P., Doly, S., et al. (2018). Positive regulation of raphe serotonin neurons by serotonin 2B receptors. *Neuropsychopharmacology* 43, 1623–1632. doi: 10.1038/s41386-018-0013-0

Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., et al. (2010). A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* 468, 1061–1066. doi: 10.1038/nature09629

Bigford, G. E., Chaudhry, N. S., Keane, R. W., and Holohean, A. M. (2012). 5-Hydroxytryptamine 5HT2C receptors form a protein complex with N-methyl-D-aspartate GluN2A subunits and activate phosphorylation of Src protein to modulate motoneuronal depolarization. *J. Biol. Chem.* 287, 11049–11059. doi: 10.1074/jbc.M111.277806

Bortolozzi, A., Castañé, A., Semakova, J., Santana, N., Alvarado, G., Cortés, R., et al. (2012). Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. *Mol. Psychiatry* 17, 612–623. doi: 10.1038/mp.2011.92

Branchi, I. (2011). The double edged sword of neural plasticity: increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover. *Psychoneuroendocrinology* 36, 339–351. doi: 10.1016/j.psyneuen.2010.08.011

Bruns, D., Riedel, D., Klingauf, J., and Jahn, R. (2000). Quantal release of serotonin. *Neuron* 28, 205–220. doi: 10.1016/S0896-6273(00)00097-0

Bunin, M. A., and Wightman, R. M. (1999). Paracrine neurotransmission in the CNS: involvement of 5-HT. *Trends Neurosci.* 22, 377–382. doi: 10.1016/S0166-2236(99)01410-1

Burré, J., and Volknandt, W. (2007). The synaptic vesicle proteome. *J. Neurochem.* 101, 1448–1462. doi: 10.1111/j.1471-4159.2007.04453.x

Casamassima, F., Hay, A. C., Benedetti, A., Lattanzi, L., Cassano, G. B., and Perlis, R. H. (2010). L-type calcium channels and psychiatric disorders: a brief review. *Am. J. Med. Genet. B* 153B, 1373–1390. doi: 10.1002/ajmg.b.31122

Chazal, G., and Ralston, H. J. (1987). Serotonin-containing structures in the nucleus raphe dorsalis of the cat: an ultrastructural analysis of dendrites, presynaptic dendrites, and axon terminals. *J. Comp. Neurol.* 259, 317–329. doi: 10.1002/cne.902590302

Christiansen, L., Tan, Q., Iachina, M., Bathum, L., Kruse, T. A., McGue, M., et al. (2007). Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomatology in an elderly population. *Biol. Psychiatry* 61, 223–230. doi: 10.1016/j.biopsych.2006.03.046

Colgan, L. A., Cavolo, S. L., Commons, K. G., and Levitan, E. S. (2012). Action potential-independent and pharmacologically unique vesicular serotonin release from dendrites. *J. Neurosci.* 32, 15737–15746. doi: 10.1523/JNEUROSCI.0020-12.2012

Colgan, L. A., Putzier, I., and Levitan, E. S. (2009). Activity-dependent vesicular monoamine transporter-mediated depletion of the nucleus supports somatic release by serotonin neurons. *J. Neurosci.* 29, 15878–15887. doi: 10.1523/JNEUROSCI.4210-09.2009

Commons, K. G. (2008). Evidence for topographically organized endogenous 5-HT-1A receptor-dependent feedback inhibition of the ascending serotonin system. *Eur. J. Neurosci.* 27, 2611–2618. doi: 10.1111/j.1460-9568.2008.06235.x

Cox, D. A., and Cohen, M. L. (1996). 5-HT2B receptor signaling in the rat stomach fundus: dependence on calcium influx, calcium release and protein kinase C. *Behav. Brain Res.* 73, 289–292. doi: 10.1016/0166-4328(96)00125-8

Craven, R. M., Grahame-Smith, D. G., and Newberry, N. R. (2001). 5-HT1A and 5-HT2 receptors differentially regulate the excitability of 5-HT-containing neurones of the guinea pig dorsal raphe nucleus in vitro. *Brain Res.* 899, 159–168. doi: 10.1016/S0006-8993(01)02221-1

Dankoski, E. C., Carroll, S., and Wightman, R. M. (2016). Acute selective serotonin reuptake inhibitors regulate the dorsal raphe nucleus causing amplification of terminal serotonin release. *J. Neurochem.* 136, 1131–1141. doi: 10.1111/jnc.13528

de Kock, C. P. J., Cornelisse, L. N., Burnashev, N., Lodder, J. C., Timmerman, A. J., Couey, J. J., et al. (2006). NMDA receptors trigger neurosecretion of 5-HT within dorsal raphe nucleus of the rat in the absence of action potential firing. *J. Physiol.* 577, 891–905. doi: 10.1113/jphysiol.2006.115311

Dedic, N., Pöhlmann, M. L., Richter, J. S., Mehta, D., Czamara, D., Metzger, M. W., et al. (2018). Cross-disorder risk gene CACNA1C differentially modulates susceptibility to psychiatric disorders during development and adulthood. *Mol. Psychiatry* 23, 533–543. doi: 10.1038/mp.2017.133

Descarries, L., and Mechawar, N. (2000). Ultrastructural evidence for diffuse transmission by monoamine and acetylcholine neurons of the central nervous system. *Prog. Brain Res.* 125, 27–47. doi: 10.1016/S0079-6123(00)25005-X

Diaz, S. L., Doly, S., Narboux-Neme, N., Fernandez, S., Mazot, P., Banas, S., et al. (2012). 5-HT2B receptors are required for serotonin-selective antidepressant actions. *Mol. Psychiatry* 17, 154–163. doi: 10.1038/mp.2011.159

Diaz, S. L., and Maroteaux, L. (2011). Implication of 5-HT2B receptors in the serotonin syndrome. *Neuropharmacology* 61, 495–502. doi: 10.1016/j.neuropharm.2011.01.025

Doly, S., Valjent, E., Setola, V., Callebert, J., Herve, D., Launay, J. M., et al. (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J. Neurosci.* 28, 2933–2940. doi: 10.1523/JNEUROSCI.5723-07.2008

Fabbri, C., Corponi, F., Albani, D., Raimondi, I., Forloni, G., Schruers, K., et al. (2018). Pleiotropic genes in psychiatry: calcium channels and the stress-related FKBP5 gene in antidepressant resistance. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 81, 203–210. doi: 10.1016/j.pnpbp.2017.10.005

Fabre, V., Beaufour, C., Evrard, A., Rioux, A., Hanoun, N., Lesch, K. P., et al. (2000). Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur. J. Neurosci.* 12, 2299–2310. doi: 10.1046/j.1460-9568.2000.00126.x

Fei, H., Grygoruk, A., Brooks, E. S., Chen, A., and Krantz, D. E. (2008). Trafficking of vesicular neurotransmitter transporters. *Traffic* 9, 1425–1436. doi: 10.1111/j.1600-0854.2008.00771.x

Forrest, L. R., Zhang, Y. W., Jacobs, M. T., Gesmonde, J., Xie, L., Honig, B. H., et al. (2008). Mechanism for alternating access in neurotransmitter transporters. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10338–10343. doi: 10.1073/pnas.0804659105

Fukumoto, K., Iijima, M., and Chaki, S. (2014). Serotonin-1A receptor stimulation mediates effects of a metabotropic glutamate 2/3 receptor antagonist, 2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495), and an N-methyl-D-aspartate receptor antagonist, ketamine, in the novelty-suppressed feeding test. *Psychopharmacology* 231, 2291–2298. doi: 10.1007/s00213-013-3378-0

Gartside, S. E., Umbers, V., Hajós, M., and Sharp, T. (1995). Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br. J. Pharmacol.* 115, 1064–1070. doi: 10.1111/j.1476-5381.1995.tb15919.x

Grace, A. A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nat. Rev. Neurosci.* 17, 524–532. doi: 10.1038/nrn.2016.57

Guilloux, J.-P., David, D. J. P., Xia, L., Nguyen, H. T., Rainer, Q., Guiard, B. P., et al. (2011). Characterization of 5-HT(1A/1B)-/- mice: an animal model sensitive to anxiolytic treatments. *Neuropharmacology* 61, 478–488. doi: 10.1016/j.neuropharm.2011.02.009

Hajós, M., Allers, K. A., Jennings, K., Sharp, T., Charette, G., Sík, A., et al. (2007). Neurochemical identification of stereotypic burst-firing neurons in the rat dorsal raphe nucleus using juxtacellular labelling methods. *Eur. J. Neurosci.* 25, 119–126. doi: 10.1111/j.1460-9568.2006.05276.x

Hajos, M., Gartside, S. E., Villa, A. E., and Sharp, T. (1995). Evidence for a repetitive (burst) firing pattern in a sub-population of 5-hydroxytryptamine neurons in the dorsal and median raphe nuclei of the rat. *Neuroscience* 69, 189–197. doi: 10.1016/0306-4522(95)00227-A

Hashemi, P., Dankoski, E. C., Lama, R., Wood, K. M., Takmakov, P., and Wightman, R. M. (2012). Brain dopamine and serotonin differ in regulation and its consequences. *Proc. Natl. Acad. Sci. U.S.A.* 109, 11510–11515. doi: 10.1073/pnas.1201547109

Holohean, A. M., and Hackman, J. C. (2004). Mechanisms intrinsic to 5-HT2B receptor-induced potentiation of NMDA receptor responses in frog motoneurones. *Br. J. Pharmacol.* 143, 351–360. doi: 10.1038/sj.bjp.0705935

Homberg, J. R., and Lesch, K.-P. (2011). Looking on the bright side of serotonin transporter gene variation. *Biol. Psychiatry* 69, 513–519. doi: 10.1016/j.biopsych.2010.09.024

Insel, T. R., and Sahakian, B. J. (2012). Drug research: a plan for mental illness. *Nature* 483:269. doi: 10.1038/483269a

Invernizzi, R., Belli, S., and Samanin, R. (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. *Brain Res.* 584, 322–324. doi: 10.1016/0006-8993(92)90914-U

Invernizzi, R., Velasco, C., Bramante, M., Longo, A., and Samanin, R. (1997). Effect of 5-HT1A receptor antagonists on citalopram-induced increase in extracellular serotonin in the frontal cortex, striatum and dorsal hippocampus. *Neuropharmacology* 36, 467–473. doi: 10.1016/S0028-3908(97)00060-9

Jacobs, B. L., and Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiol. Rev.* 72, 165–229. doi: 10.1152/physrev.1992.72.1.165

Jahn, R., and Scheller, R. H. (2006). SNAREs–engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* 7, 631–643. doi: 10.1038/nrm2002

Jennings, K. A. (2013). A comparison of the subsecond dynamics of neurotransmission of dopamine and serotonin. *ACS Chem. Neurosci.* 4, 704–714. doi: 10.1021/cn4000605

Jennings, K. A., Lesch, K.-P., Sharp, T., and Cragg, S. J. (2010). Non-linear relationship between 5-HT transporter gene expression and frequency sensitivity of 5-HT signals. *J. Neurochem.* 115, 965–973. doi: 10.1111/j.1471-4159.2010.07001.x

Kaushalya, S. K., Desai, R., Arumugam, S., Ghosh, H., Balaji, J., and Maiti, S. (2008a). Three-photon microscopy shows that somatic release can be a quantitatively significant component of serotonergic neurotransmission in the mammalian brain. *J. Neurosci. Res.* 86, 3469–3480. doi: 10.1002/jnr.21794

Kaushalya, S. K., Nag, S., Ghosh, H., Arumugam, S., and Maiti, S. (2008b). A high-resolution large area serotonin map of a live rat brain section. *Neuroreport* 19, 717–721. doi: 10.1097/WNR.0b013e3282fd6946

Kia, H. K., Miquel, M. C., Brisorgueil, M. J., Daval, G., Riad, M., El Mestikawy, S., et al. (1996). Immunocytochemical localization of serotonin1A receptors in the rat central nervous system. *J. Comp. Neurol.* 365, 289–305. doi: 10.1002/(SICI)1096-9861(19960205)365:2<289::AID-CNE7>3.0.CO;2-1

Kocsis, B., Varga, V., Dahan, L., and Sik, A. (2006). Serotonergic neuron diversity: identification of raphe neurons with discharges time-locked to the hippocampal theta rhythm. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1059–1064. doi: 10.1073/pnas.0508360103

Krishnan, V., and Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature* 455, 894–902. doi: 10.1038/nature07455

Kunwar, A. J., Rickmann, M., Backofen, B., Browski, S. M., Rosenbusch, J., Schöning, S., et al. (2011). Lack of the endosomal SNAREs vti1a and vti1b led to significant impairments in neuronal development. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2575–2580. doi: 10.1073/pnas.1013891108

Leon-Pinzon, C., Cercós, M. G., Noguez, P., Trueta, C., and De-Miguel, F. F. (2014). Exocytosis of serotonin from the neuronal soma is sustained by a serotonin and calcium-dependent feedback loop. *Front. Cell. Neurosci.* 8:169. doi: 10.3389/fncel.2014.00169

Li, H., Waites, C. L., Staal, R. G., Dobryy, Y., Park, J., Sulzer, D. L., et al. (2005). Sorting of vesicular monoamine transporter 2 to the regulated secretory pathway confers the somatodendritic exocytosis of monoamines. *Neuron* 48, 619–633. doi: 10.1016/j.neuron.2005.09.033

Liu, C., Kershberg, L., Wang, J., Schneeberger, S., and Kaeser, P. S. (2018). Dopamine Secretion is mediated by sparse active zone-like release sites. *Cell* 172, 706–718.e15. doi: 10.1016/j.cell.2018.01.008

Liu, R.-J., Lambe, E. K., and Aghajanian, G. K. (2005). Somatodendritic autoreceptor regulation of serotonergic neurons: dependence on L-tryptophan and tryptophan hydroxylase-activating kinases. *Eur. J. Neurosci.* 21, 945–958. doi: 10.1111/j.1460-9568.2005.03930.x

Machado-Vieira, R., Salvadore, G., Diazgranados, N., and Zarate, C. A. (2009). Ketamine and the next generation of antidepressants with a rapid onset of action. *Pharmacol. Ther.* 123, 143–150. doi: 10.1016/j.pharmthera.2009.02.010

Marinelli, S., Schnell, S. A., Hack, S. P., Christie, M. J., Wessendorf, M. W., and Vaughan, C. W. (2004). Serotonergic and nonserotonergic dorsal raphe neurons are pharmacologically and electrophysiologically heterogeneous. *J. Neurophysiol.* 92, 3532–3537. doi: 10.1152/jn.00437.2004

Matias, S., Lottem, E., Dugué, G. P., and Mainen, Z. F. (2017). Activity patterns of serotonin neurons underlying cognitive flexibility. *eLife* 6:e20552. doi: 10.7554/eLife.20552

McDevitt, R. A., and Neumaier, J. F. (2011). Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J. Chem. Neuroanat.* 41, 234–246. doi: 10.1016/j.jchemneu.2011.05.001

Okaty, B. W., Freret, M. E., Rood, B. D., Brust, R. D., Hennessy, M. L., Debairos, D., et al. (2015). Multi-scale molecular deconstruction of the serotonin neuron system. *Neuron* 88, 774–791. doi: 10.1016/j.neuron.2015.10.007

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., et al. (2005). 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nat. Neurosci.* 8, 828–834. doi: 10.1038/nn1463

Pham, T. H., Mendez-David, I., Defaix, C., Guiard, B. P., Tritschler, L., David, D. J., et al. (2017). Ketamine treatment involves medial prefrontal cortex serotonin to induce a rapid antidepressant-like activity in BALB/cJ mice. *Neuropharmacology* 112, 198–209. doi: 10.1016/j.neuropharm.2016.05.010

Philippe, T. J., Vahid-Ansari, F., Donaldson, Z. R., Le François, B., Zahrai, A., Turcotte-Cardin, V., et al. (2018). Loss of MeCP2 in adult 5-HT neurons induces 5-HT1A autoreceptors, with opposite sex-dependent anxiety and depression phenotypes. *Sci. Rep.* 8:5788. doi: 10.1038/s41598-018-24167-8

Piñeyro, G., and Blier, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol. Rev.* 51, 533–591.

Popa, D., Cerdan, J., Reperant, C., Guiard, B. P., Guilloux, J. P., David, D. J., et al. (2010). A longitudinal study of 5-HT outflow during chronic fluoxetine treatment using a new technique of chronic microdialysis in a highly emotional mouse strain. *Eur. J. Pharmacol.* 628, 83–90. doi: 10.1016/j.ejphar.2009.11.037

Rainer, Q., Nguyen, H. T., Quesseveur, G., Gardier, A. M., David, D. J., and Guiard, B. P. (2012). Functional status of somatodendritic serotonin 1A autoreceptor after long-term treatment with fluoxetine in a mouse model of anxiety/depression based on repeated corticosterone administration. *Mol. Pharmacol.* 81, 106–112. doi: 10.1124/mol.111.075796

Raingo, J., Khvotchev, M., Liu, P., Darios, F., Li, Y. C., Ramirez, D. M. O., et al. (2012). VAMP4 directs synaptic vesicles to a pool that selectively maintains asynchronous neurotransmission. *Nat. Neurosci.* 15, 738–745. doi: 10.1038/nn.3067

Ramirez, D. M. O., and Kavalali, E. T. (2012). The role of non-canonical SNAREs in synaptic vesicle recycling. *Cell. Logist.* 2, 20–27. doi: 10.4161/cl.20114

Ramirez, D. M. O., Khvotchev, M., Trauterman, B., and Kavalali, E. T. (2012). Vti1a identifies a vesicle pool that preferentially recycles at rest and maintains

spontaneous neurotransmission. *Neuron* 73, 121–134. doi: 10.1016/j.neuron.2011.10.034

Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, E., Langlois, X., et al. (2000). Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J. Comp. Neurol.* 417, 181–194. doi: 10.1002/(SICI)1096-9861(20000207)417:2<181::AID-CNE4>3.0.CO;2-A

Richardson-Jones, J. W., Craige, C. P., Guiard, B. P., Stephen, A., Metzger, K. L., Kung, H. F., et al. (2010). 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* 65, 40–52. doi: 10.1016/j.neuron.2009.12.003

Ridet, J. L., Tamir, H., and Privat, A. (1994). Direct immunocytochemical localization of 5-hydroxytryptamine receptors in the adult rat spinal cord: a light and electron microscopic study using an anti-idiotypic antiserum. *J. Neurosci. Res.* 38, 109–121. doi: 10.1002/jnr.490380114

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809. doi: 10.1126/science.1083328

Sitte, H. H., and Freissmuth, M. (2015). Amphetamines, new psychoactive drugs and the monoamine transporter cycle. *Trends Pharmacol. Sci.* 36, 41–50. doi: 10.1016/j.tips.2014.11.006

Smits, K. M., Smits, L. J., Peeters, F. P., Schouten, J. S., Janssen, R. G., Smeets, H. J., et al. (2008). The influence of 5-HTTLPR and STin2 polymorphisms in the serotonin transporter gene on treatment effect of selective serotonin reuptake inhibitors in depressive patients. *Psychiatr. Genet.* 18, 184–190. doi: 10.1097/YPG.0b013e3283050aca

Soiza-Reilly, M., Goodfellow, N. M., Lambe, E. K., and Commons, K. G. (2015). Enhanced 5-HT1A receptor-dependent feedback control over dorsal raphe serotonin neurons in the SERT knockout mouse. *Neuropharmacology* 89, 185–192. doi: 10.1016/j.neuropharm.2014.09.017

Solovieff, N., Roberts, A. L., Ratanatharathorn, A., Haloosim, M., De Vivo, I., King, A. P., et al. (2014). Genetic association analysis of 300 genes identifies a risk haplotype in SLC18A2 for post-traumatic stress disorder in two independent samples. *Neuropsychopharmacology* 39, 1872–1879. doi: 10.1038/npp.2014.34

Südhof, T. C. (2012). The presynaptic active zone. *Neuron* 75, 11–25. doi: 10.1016/j.neuron.2012.06.012

Teissier, A., Chemiakine, A., Inbar, B., Bagchi, S., Ray, R. S., Palmiter, R. D., et al. (2015). Activity of raphé serotonergic neurons controls emotional behaviors. *Cell Rep.* 13, 1965–1976. doi: 10.1016/j.celrep.2015.10.061

Trueta, C., and De-Miguel, F. F. (2012). Extrasynaptic exocytosis and its mechanisms: a source of molecules mediating volume transmission in the nervous system. *Front. Physiol.* 3:319. doi: 10.3389/fphys.2012.00319

Trueta, C., Méndez, B., and De-Miguel, F. F. (2003). Somatic exocytosis of serotonin mediated by L-type calcium channels in cultured leech neurones. *J. Physiol.* 547, 405–416. doi: 10.1113/jphysiol.2002.030684

Trueta, C., Sánchez-Armass, S., Morales, M. A., and De-Miguel, F. F. (2004). Calcium-induced calcium release contributes to somatic secretion of serotonin in leech retzius neurons. *J. Neurobiol.* 61, 309–316. doi: 10.1002/neu.20055

Urban, D. J., Zhu, H., Marcinkiewcz, C. A., Michaelides, M., Oshibuchi, H., Rhea, D., et al. (2016). Elucidation of the behavioral program and neuronal network encoded by dorsal raphe serotonergic neurons. *Neuropsychopharmacology* 41, 1404–1415. doi: 10.1038/npp.2015.293

Vadodaria, K. C., Stern, S., Marchetto, M. C., and Gage, F. H. (2018). Serotonin in psychiatry: in vitro disease modeling using patient-derived neurons. *Cell Tissue Res.* 371, 161–170. doi: 10.1007/s00441-017-2670-4

Vahid-Ansari, F., Daigle, M., Manzini, M. C., Tanaka, K. F., Hen, R., Geddes, S. D., et al. (2017). Abrogated Freud-1/Cc2d1a repression of 5-HT1A autoreceptors induces fluoxetine-resistant anxiety/depression-like behavior. *J. Neurosci.* 37, 11967–11978. doi: 10.1523/JNEUROSCI.1668-17.2017

Vizi, E. S., Fekete, A., Karoly, R., and Mike, A. (2010). Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. *Br. J. Pharmacol.* 160, 785–809. doi: 10.1111/j.1476-5381.2009.00624.x

Yuen, E. Y., Jiang, Q., Chen, P., Feng, J., and Yan, Z. (2008). Activation of 5-HT2A/C receptors counteracts 5-HT1A regulation of n-methyl-D-aspartate receptor channels in pyramidal neurons of prefrontal cortex. *J. Biol. Chem.* 283, 17194–17204. doi: 10.1074/jbc.M801713200

Yuen, E. Y., Jiang, Q., Chen, P., Gu, Z., Feng, J., and Yan, Z. (2005). Serotonin 5-HT1A receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. *J. Neurosci.* 25, 5488–5501. doi: 10.1523/JNEUROSCI.1187-05.2005

Zanos, P., and Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Mol. Psychiatry* 23, 801–811. doi: 10.1038/mp.2017.255

Zoli, M., Jansson, A., Syková, E., Agnati, L. F., and Fuxe, K. (1999). Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol. Sci.* 20, 142–150. doi: 10.1016/S0165-6147(99)01343-7

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Quentin, Belmer and Maroteaux. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 21 December 2018
doi: 10.3389/fnins.2018.00974

Anxiolytic- and Antidepressant-Like Effects of Fish Oil-Enriched Diet in Brain-Derived Neurotrophic Factor Deficient Mice

*Juliane Zemdegs[1,2,3], Quentin Rainer[2], Cindy P. Grossmann[3], Delphine Rousseau-Ralliard[4,5], Alain Grynberg[5], Eliane Ribeiro[1] and Bruno P. Guiard[2,3]**

[1] Department of Physiology, Discipline of Nutrition Physiology, Universidade Federal de São Paulo, São Paulo, Brazil, [2] Faculté de Pharmacie, Université Paris Sud, Université Paris-Saclay, Chatenay-Malabry, France, [3] Centre de Recherches sur la Cognition Animale (CRCA), Centre de Biologie Intégrative (CBI), Centre National de la Recherche Scientifique, Université de Toulouse, Toulouse, France, [4] INRA, Unité Mixte de Recherche BDR, ENVA, Université Paris Saclay, Jouy-en-Josas, France, [5] INRA, Unité Mixte de Recherche 1154, Laboratoire Lipides Membranaires et Régulations Fonctionnelles du Coeur et des Vaisseaux, Jouy-en-Josas, France

OPEN ACCESS

Edited by:
Nasser Haddjeri,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Amandine Gautier-Stein,
INRA – Centre Auvergne-Rhône-Alpes, France
Eliyahu Dremencov,
Slovak Academy of Sciences, Slovakia

***Correspondence:**
Bruno P. Guiard
bruno.guiard@univ-tlse3.fr;
bruno.guiard@u-psud.fr

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: 28 September 2018
Accepted: 05 December 2018
Published: 21 December 2018

Citation:
Zemdegs J, Rainer Q, Grossmann CP, Rousseau-Ralliard D, Grynberg A, Ribeiro E and Guiard BP (2018) Anxiolytic- and Antidepressant-Like Effects of Fish Oil-Enriched Diet in Brain-Derived Neurotrophic Factor Deficient Mice. Front. Neurosci. 12:974. doi: 10.3389/fnins.2018.00974

Despite significant advances in the understanding of the therapeutic activity of antidepressant drugs, treatment-resistant depression is a public health issue prompting research to identify new therapeutic strategies. Evidence strongly suggests that nutrition might exert a significant impact on the onset, the duration and the severity of major depression. Accordingly, preclinical and clinical investigations demonstrated the beneficial effects of omega-3 fatty acids in anxiety and mood disorders. Although the neurobiological substrates of its action remain poorly documented, basic research has shown that omega-3 increases brain-derived neurotrophic factor (BDNF) levels in brain regions associated with depression, as antidepressant drugs do. In contrast, low BDNF levels and hippocampal atrophy were observed in animal models of depression. In this context, the present study compared the effects of long-lasting fish oil-enriched diet, an important source of omega-3 fatty acids, between heterozygous BDNF$^{+/-}$ mice and their wild-type littermates. Our results demonstrated lower activation of Erk in BDNF$^{+/-}$ mice whereas this deficit was rescued by fish oil-enriched diet. In parallel, BDNF$^{+/-}$ mice displayed elevated hippocampal extracellular 5-HT levels in relation with a local decreased serotonin transporter protein level. Fish oil-enriched diet restored normal serotonergic tone by increasing the protein levels of serotonin transporter. At the cellular level, fish oil-enriched diet increased the pool of immature neurons in the dentate gyrus of BDNF$^{+/-}$ mice and the latter observations coincide with its ability to promote anxiolytic- and antidepressant-like response in these mutants. Collectively, our results demonstrate that the beneficial effects of long-term exposure to fish oil-enriched diet in behavioral paradigms known to recapitulate diverse abnormalities related to the depressive state specifically in mice with a partial loss of BDNF. These findings contrast with the mechanism of action of currently available antidepressant drugs for which the full manifestation of their therapeutic activity depends on the

enhancement of serotoninergic and BDNF signaling. Further studies are warranted to determine whether fish oil supplementation could be used as an add-on strategy to conventional pharmacological interventions in treatment-resistant patients and relevant animal models.

Keywords: brain-derived neurotrophic factor (BDNF), neurobehavior, antidepressant, anxiolytic fish oil (n-3) fatty acids, serotonin

INTRODUCTION

Major depressive disorder (MDD) is an important public health concern worldwide. The lifetime prevalence of MDD is nowadays 15–20% of the population, and is expected to become the second most prevalent cause of illness-induced disability by 2020 (Lecrubier, 2001). These epidemiological data prompt research to identify the cellular and molecular mechanisms underpinning these mental disorders and to develop innovative treatments with better therapeutic effects than currently available medications. Indeed, despite their therapeutic activity, antidepressant drugs, including selective serotonin reuptake inhibitors (SSRIs), alleviate depression symptoms in only a limited percentage of patients, and remain insufficiently effective in treatment responders (Hamon and Blier, 2013).

Omega-3 polyunsaturated fatty acids (PUFAs) deficiency has been associated with several pathologies such as mood disorders, cardiovascular diseases, and stroke (Hibbeln et al., 2006). Mammals are unable to synthesize omega-3 and its supply depends on dietary intake. Fish oils represent the main source of omega-3 PUFAs [(i.e., eicosapentaenoic (EPA) and docosahexaenoic (DHA)] (Calder, 1998). Interestingly, it has been reported that depressed patients display low plasma and brain levels of omega-3 PUFAs (McNamara et al., 2007; Lin et al., 2010). Such deficits were also found in other populations with mental disorders: e.g., lower DHA and total omega-3 PUFAs in postpartum depression (De Vriese et al., 2003) and lower DHA in bipolar disorders (Chiu et al., 2003). Conversely, multiple sources of evidence suggested that consumption of omega-3 PUFAs produces antidepressant activity in patients with MDD (Peet et al., 1998; Marangell et al., 2003; Silvers et al., 2005; Freeman et al., 2006; Lin and Su, 2007; Owen et al., 2008; Su et al., 2008) or bipolar disorders (Montgomery and Richardson, 2008). A recent meta-analysis also revealed a beneficial overall effect of omega-3 PUFAs in patients under antidepressant drugs treatment (Mocking et al., 2016), suggesting that supplementation with these fatty acids could be used as an "add-on" strategy to reduce treatment resistance, and potentiate treatment response (Peet and Horrobin, 2002; Jazayeri et al., 2008; Gertsik et al., 2012). Consistent with these clinical studies, research in rodents showed that omega-3 PUFAs elicits a robust anxiolytic-like activity in the elevated plus maze (EPM) (Pérez et al., 2013) and an antidepressant-like activity in the forced swim and tail suspension tests (Blondeau et al., 2009; Venna et al., 2009; Moranis et al., 2012; Park et al., 2012; Vines et al., 2012). Moreover, omega-3 PUFAs were shown to improve anxiety-like and depressive-like phenotypes in various animal models of depression (Pérez et al., 2013; Pudell et al., 2014; Tang et al., 2015; Wu et al., 2016) and their combination with SSRIs appeared to be more effective than antidepressant drugs alone for reducing depression-like behaviors (Lakhwani et al., 2007; Laino et al., 2010; Able et al., 2014).

Antidepressant drugs activity is associated with the stimulation of brain serotonergic neurotransmission (Gardier et al., 1996) accompanied with an enhancement of adult hippocampal neurogenesis. On the contrary, disruption of hippocampal neurogenecis prevents the behavioral effects of various classes of antidepressant in mice (Schmidt and Duman, 2007). A number of factors have been proposed to participate in adult hippocampal neurogenesis and SSRI response including Brain-Derived Neurotrophic Factor (BDNF) (Nibuya et al., 1995). A single bilateral infusion of BDNF into the dentate gyrus of hippocampus produced antidepressant-like effects in naive mice (Deltheil et al., 2009) or in animal models of depression such as the learned helplessness (Shirayama et al., 2002). Interestingly, in heterozygous $BDNF^{+/-}$ mice or in inducible BDNF KO lines of mice, deletion of BDNF in adults does not impact on depression-like behavior evaluated in the forced swim test (FST) (MacQueen et al., 2001; Saarelainen et al., 2003; Monteggia et al., 2007). However, these mutants display signs of antidepressant drugs resistance, notably at the behavioral and neurochemical levels (Saarelainen et al., 2003; Monteggia et al., 2004; Daws et al., 2007; Monteggia et al., 2007; Guiard et al., 2008; Ibarguen-Vargas et al., 2009). In an attempt to clarify the relationship between BDNF and the serotonergic system, alterations in behaviors regulated by serotonin such as hyperphagia and weight gain were demonstrated in $BDNF^{+/-}$ mice (Lyons et al., 1999). $BDNF^{+/-}$ mice also exhibit accelerated age-related loss of serotonergic innervation to the hippocampus (Lyons et al., 1999; Luellen et al., 2007) and increased expression of 5-HT transporter (Guiard et al., 2008). The latter effects likely contribute to dampen serotonergic neurotransmission (Siuciak et al., 1996; Mamounas et al., 2000) and strongly suggest that normal BDNF signaling is essential for antidepressant efficacy in mice.

Interestingly, the time course of omega-3 PUFAs-induced antidepressant-like effects in rodents is compatible with molecular and morphological changes taking place in the hippocampus. In particular, it has been reported that prolonged omega-3 PUFAs exposure stimulated BDNF expression and adult hippocampal neurogenesis in mice (Wu et al., 2004; Rao et al., 2007; Blondeau et al., 2009; Venna et al., 2009). In this context, the present study was designed to determine to what extent fish oil-enriched diet containing omega-3 PUFAs influence serotonergic tone and markers of hippocampal plasticity in $BDNF^{+/-}$ mice and their wild-type littermates. Using behavioral

paradigms assessing anxiolytic/antidepressant-like activities, we also examined whether fish oil-enriched diet represents an alternative therapeutic strategy to currently available antidepressant drugs in BDNF$^{+/-}$ mice.

MATERIALS AND METHODS

Animals and Dietary Treatment

Experiments were performed in accordance with the European Union (86/609/EEC) and the French National Committee of Ethics (87/848) policies regarding the care and use of laboratory animals. BDNF$^{+/-}$ mice and their wild-type littermates initially bred on a mixed S129/Sv x C57BL/6 genetic background (Korte et al., 1995) were backcrossed to 129Sv strain, mated and raised at the animal facility of the *Université Paris-Sud* (Châtenay-Malabry, France) or at the *Universidade Federal de São Paulo* (Sao Paulo, Brazil). One-month-old male mice were genotyped by polymerase chain reaction and were randomly assigned to receive either a control diet or a fish oil-enriched diet for 12 weeks. Diets were prepared according to the recommendations of the American Institute of Nutrition (AIN-93) for rodents (Reeves, 1997) and were isocaloric and normolipidic, i.e., diets had identical energy and lipid content. The source of fat was soybean oil in the control diet and fish oil (Sigma-Aldrich, St. Louis, MO, United States). Both diets met the minimum suggested requirement for rodents of 2 g/kg diet of alpha-linolenic acid (ALA). The fatty acids composition of diets is depicted in **Supplementary Table S1**. Animals were housed in groups of five mice per cage under standard conditions (12:12 h light-dark cycle, 22 ± 1°C ambient temperature, 60% relative humidity), with *ad libitum* access to food and water. Experimental timeline is depicted in **Figure 1**. Procedures were conducted in conformity with the institutional guidelines in compliance with national and policy (Council directive #87–848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permission #92.196).

Fatty Acids Analysis in the Hippocampus

Mice were killed by cervical dislocation. The brains were withdrawn and rinsed in saline (NaCl 0.9%). The hippocampi were finely dissected, weighed and stored at −80°C in CHCl3-MeOH (v/v, 2/1) to further determine its fatty acids profile. The lipids were extracted with chloroform/methanol (2/1), according to an adaptation of the method previously described (Rousseau et al., 2003). The phospholipids (PL) were separated from non-phosphorous lipids on silica acid cartridges. After the separation, the phospholipid fractions, mostly representative of the membranes, were transmethylated with boron trifluoride methanol 7% (Sigma-Aldrich, Saint Quentin Fallavier, France). The methyl esters of phospholipid fatty acids were analyzed

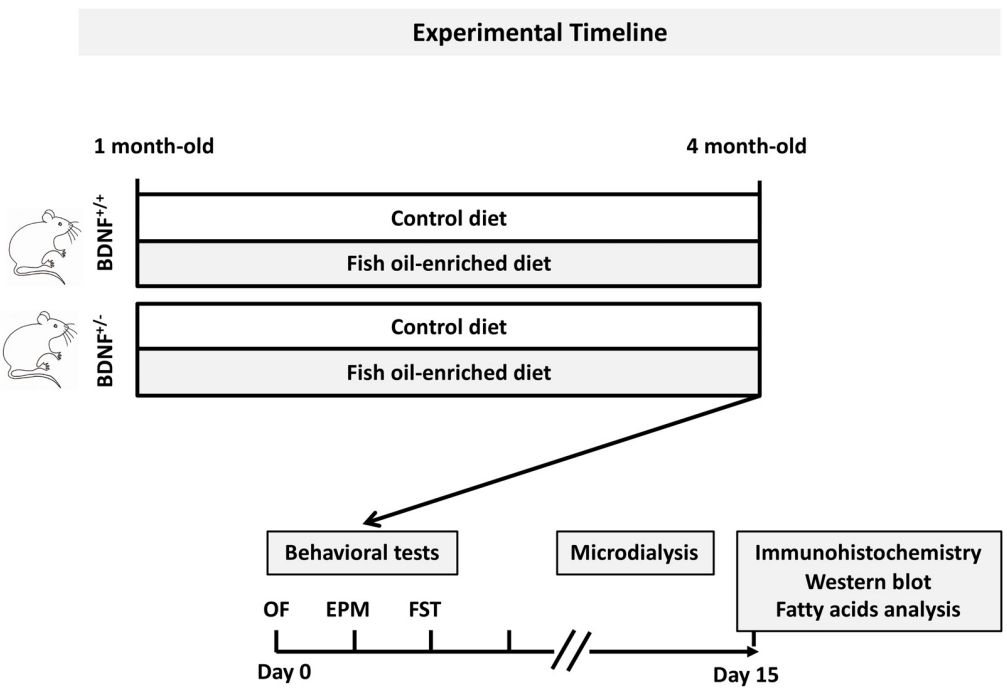


FIGURE 1 | General procedure and experimental groups. Mice from each genotype were fed a control or fish oil-enriched diet for 12 weeks. *In vivo* procedures (i.e., behavioral and microdialysis experiments) were conducted while the animals were maintained under their respective diets. Given that it has been demonstrated that the interval between behavioral tests could be as little as 1 day, with a weak effect on overall performance (Paylor et al., 2006), in the present study, a 2-day recovery period between each test was provided. Moreover, to avoid the interference of behavioral testing on microdialysis experiments and notably the fact that the FST represents a strong stressor for the animals, the collection of dialysate samples was conducted 10 days after the behavioral assessment. The next day, animals were euthanized for immunohistochemistry, Western-blot and biochemical analyses.

by gas chromatography coupled to FID (Auto Sampling 8410 Gas Chromatograph 3900; Varian, Les Ulis, France) on an Econo-Cap EC-WAX capillary column (30-m, 0.32-mm internal diameter, 0.25-μm Film, ref 19654, ALLTECH Associates Inc., Templemars, France), using heptadecanoic acid (margaric acid, C17:0) as internal standard. Fatty acid composition was expressed as the percentage of total fatty acid weight. Desaturase activity was estimated according to Warensjo et al. (2009).

Intracerebral Microdialysis for the Determination of Hippocampal Extracellular Serotonin (5-HT) Levels

Under anesthesia (chloral hydrate, 400 mg/kg, i.p.), mice were stereotaxically implanted with concentric microdialysis probes (active membrane length: 2.0 mm, molecular weight cut-off: 4.5 kD) in the ventral hippocampus (coordinates in mm from bregma: AP: −3.4, L: ± 3.4, V: 4.0). The next day, mice were connected to a swivel system and the probes were connected to a microinjection pump, allowing a continuous perfusion of artificial cerebrospinal fluid (composition: NaCl 147 mM, KCl 3.5 mM, CaCl2 1.26 mM, MgCl2 1.2 mM, NaH2PO4 1.0 mM, NaHCO3 25.0 mM; pH 7.4 ± 0.2) at a flow rate of 1.5 μl/min. A 2 h-perfusion was performed to allow stabilization of 5-HT concentrations and microdialysis samples were then collected every 15 min. Microdialysates were kept at −80°C until analysis of 5-HT content by high performance liquid chromatography (HPLC) coupled to an amperometric detector (VT03; Antec Leyden, Netherlands). The amounts of 5-HT in microdialysates (19 μl) were calculated by measurement of peak heights relative to external standards. The limit of sensitivity for 5-HT was ∼ 0.5 fmol/sample (signal-to-noise ratio = 2).

Western-Blot Analyses of Serotonin Transporter (SERT) and TrkB-BDNF Signaling Pathway

The hippocampi were homogenized in lysis buffer (1% Triton X-100, 100 mM Tris-HCl pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 2.0 mM phenylmethylsulfonyl fluoride and 0.1 mg aprotinin/ml). Protein concentrations were determined using a commercial kit (BioAgency, Brazil). Equal amounts of proteins (50 μg) were loaded and separated on 10% SDS polyacrylamide gels and transferred onto nitrocellulose membranes (Amersham Biosciences, GE Healthcare, United States). Membranes were saturated with a blocking solution containing 1% BSA in TPBS (10 mM Tris, 150 mM NaCl and 0.02% Tween 20). Protein blots were incubated in 1% BSA in TPBS, overnight with the following primary antibodies: anti-phospho-Akt, anti-phospho-p44/p42 Erk, anti-SERT, and anti-alpha-tubulin. Primary antibodies were purchased from Cell Signaling (anti-phospho-Akt, anti phospho-p44/p42 Erk) or Santa Cruz Biotechnology (St. Louis, MO, United States) (serotoninergic and alpha-tubulin). After washing, membranes were incubated with the appropriate HRP-conjugated secondary antibodies (Sigma-Aldrich, St. Louis, MO, United States). Staining was revealed using the ECL-Plus Western blotting detection system (Thermo Scientific, Rockford IL, United States). Chemiluminescence was quantified by Scion Image software. After each revelation, membranes were incubated in stripping solution (62.6 mM Tris-HCl, 2% SDS, 100 mM *b*-mercaptoethanol, pH 6.8) for 30 min at 45°C and reblotted. Results are presented as the ratio of the protein, or phosphoprotein levels, to alpha-tubulin and are expressed as a percentage of the controls (wild-type under the control diet).

Immunohistochemistry to Assess Adult Neurogenesis

5-Bromo-2-Deoxyuridine (BrdU) Injection and Brain Preparation

A new cohort of mice was used for adult neurogenesis experiments. Just before the beginning of the dietary treatment, mice received i.p. injections of 5-bromo-2-deoxyuridine BrdU (150 mg/kg; 2 times/day) dissolved in saline (0.9% NaCl) for 4 days. After 12 weeks of dietary treatment, mice were deeply anesthetized with ketamine and transcardially perfused with 0.9% sodium chloride followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate saline buffer (PBS). Brains were removed and postfixed overnight in 4% PFA at 4°C. Brains equilibrated in 30% sucrose 0.1 M phosphate buffer were embedded in Tissue-Tek OCT (Sakura, United States) and frozen. Coronal 40 μm-thick sections were obtained with a cryostat (Leica, Bensheim, Germany) and stored in cryoprotectant at −20°C until use.

BrdU Immunohistochemistry

One in six series of coronal sections (spaced 240 μm) throughout the rostrocaudal extent of the hippocampus was used for BrdU staining to evaluate new cell survival. Free-floating brain sections were rinsed in PB containing 0.9% NaCl and 0.25% Triton X-100 (PBST) before inactivation of endogenous peroxidases with 3% H_2O_2 in 10% methanol in PBS. Sections were incubated in 2N HCl in PBST for 50 min to denature DNA and then neutralized in 0.1M borate buffer (pH 8.5). Sections were then blocked in PBST containing 5% normal goat serum for 60 min, followed by overnight incubation in primary antibody monoclonal rat anti-BrdU (1:400; OBT-0030, Harlan Seralab, Loughborough, United Kingdom) in PBST with 0.1% sodium azide containing 5% normal goat serum. After incubation in goat anti-rat-biotinylated antibody (1:100, BA9400 Vector) for 1 h at room temperature, sections were incubated in the avidin-biotin complex (1:400 in PBS-T; Vector Laboratories ABC Elite Kit) and staining was visualized with DAB-Ni.

Doublecortin Immunohistochemistry

One in twelve series of coronal sections (spaced 480 μm) of the rostrocaudal extent of the hippocampus was used for doublecortin (DCX) staining to evaluate maturation of newborn neurons. Sections were incubated in 0.1M phosphate buffered saline with 0.5% Triton X-100 and 10% normal donkey serum

(NDS), followed by goat anti-doublecortin primary antibody (1:500; Santa Cruz Biotechnology, SC8066, Santa Cruz, CA, United States) in TBS/Tx/NDS for 24 h at 4°C. Sections were then incubated in biotinylated donkey anti-goat secondary antibody (1:500; Jackson ImmunoResearch, West Grove, PA, United States) in TBS/NDS for 1 h at room temperature, followed by a 1 h amplification step using an avidin-biotin complex (Vector Laboratories ABC Elite Kit) and diaminobenzidine (DAB; Vectastain DAB Kit) as previously described (Quesseveur et al., 2013).

Quantification of Immunoreactive Cells

Slides were coded before analysis; the experimenter was blind to genotype and diet until all samples were counted. Quantification of BrdU-immunoreactive (BrdU+) and DCX-immunoreactive (DCX+) cells was conducted using Olympus BX51 microscope (Olympus Deutschland GmbH, Hamburg, Germany). The corresponding surface area of the granule cell layer (GCL) sampled for counting was measured using the Mercator stereology system (Explora Nova, La Rochelle, France). Density of positive cells was then calculated by dividing the number of positive cells by the GCL area sampled. Results were expressed as the number of positive cells/mm^2.

Behavioral Tests

Behavioral tests were performed between 9:00 and 11:00 am in a low light condition. Studies in animals are reported in accordance with the ARRIVE guidelines (McGrath et al., 2010). Thus, each mouse was subjected the open-field (OF), the EPM and the FST. This sequence was applied to minimize the impact of stress across tests and a 2-day recovery period between each test was provided (**Figure 1**). It is noteworthy that reducing the inter-test interval reduces the possible effect of dietary administration on tests.

Open Field was performed in Plexiglas setups (MED Associates, France) during a 30-min session. Entries count and total time in the center were measured by an automated system (MED Associated, France). Total ambulatory distance was also measured to ensure the absence of any locomotor effect of genotypes and/or diets.

Elevated Plus Maze was performed in a Plexiglas apparatus (MED Associates, France) during a 5-min session. Mice were placed in the center of the EPM facing an open arm and entries as well as time spent in the open and closed arms were measured by an automated system (ANY-maze, Stoelting Co., Wood Dale, IL, United States).

Forced Swim Test was performed in plastic buckets (20 cm diameter, 23 cm deep) filled up to two thirds with water at 23–25°C. FST was videotaped for a 6-min session period and the last 4 min were scored for active (climbing and swimming) and passive (immobility) behaviors by an experimenter blind to both genotypes and diets.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (Version 5, San Diego, CA, United States). Comparisons between groups were made using an analysis of variance (ANOVA), followed by Tukey's *post hoc* analysis when warranted. Significance was set at $p < 0.05$.

RESULTS

BDNF$^{+/-}$ Mice and Their Wild-Type Littermates Fed a Fish Oil-Enriched Diet Similarly Incorporate Omega-3 PUFAs in Their Hippocampus

In the present study, two diets equivalent in total fat, protein, carbohydrate and caloric content were formulated. The control diet contained the fatty acid ALA, the precursor of omega-3 PUFAs, and the fish oil-enriched diet presented higher levels of EPA and DHA, two omega-3 PUFAs components (**Supplementary Table S1**). The fish oil-enriched diet increased levels of omega-3 PUFAs [$F_{(3,10)} = 83.1$; $p < 0.001$] and decreased the ratio omega-6 to omega-3 [$F_{(3,10)} = 568.2$; $p < 0.001$] in the hippocampus of both wild-type and BDNF$^{+/-}$ mice. Hence, the partial genetic inactivation of BDNF did not prevent the incorporation of omega-3 fatty acids into hippocampal phospholipid membranes (**Table 1**).

Erk Phosphorylation Is Reduced in the Hippocampus of BDNF$^{+/-}$ Mice and Can Be Rescued by Fish Oil-Enriched Diet

Because initial studies demonstrated that forebrain BDNF mRNA and protein levels in BDNF$^{+/-}$ mice were ≈50% of the wild-type (Ernfors et al., 1994; Korte et al., 1995; Kolbeck et al., 1999), the activation of ErK and Akt in the hippocampus was used in the present study as an indirect marker of changes in BDNF signaling (Schmidt and Duman, 2010; Quesseveur et al., 2013; Lepack et al., 2016). Under control diet, a significant reduction in p-Erk protein levels was unveiled in BDNF$^{+/-}$ mice compared to wild-type littermates ($p = 0.04$, **Figure 2A**). Fish oil restored p-Erk protein to normal levels ($p = 0.04$) in BDNF$^{+/-}$ mice, whereas it had no impact on p-Erk in wild-type animals ($p = 0.7$; **Figure 2A**). We also monitored the expression of p-Akt and found that partial BDNF depletion had no impact this parameter. Moreover, no significant effects were detected in either wild-type nor BDNF$^{+/-}$ mice fed a fish oil-enriched diet [$F_{(3,16)} = 0.9$; $p = 0.4$; **Figure 2B**]. Altogether, these data support the fact that the partial BDNF depletion leads to impairment of Erk signaling pathway, a deficit which can be rescued by fish oil-enriched diet.

Fish Oil-Enriched Diet Thwarts the Perturbation of Serotoninergic Neurotransmission Induced by BDNF Depletion in the Hippocampus

We then sought to determine whether fish oil-enriched diet influenced hippocampal serotonergic tone by first assessing the expression of the 5-HT transporter SERT in the hippocampus

TABLE 1 | Fatty acids profile (% of total fatty acids) of hippocamrpus membrane phospholipids of wild-type and BDNF$^{+/-}$ mice treated with control or fish oil-enriched diet.

	Wild-type mice		BDNF$^{+/-}$mice	
	Control diet	**Fish oil-enriched diet**	**Control diet**	**Fish oil-enriched d**
Fatty acids				
C14:0	0.83 ± 0.02	0.95 ± 0.02	0.98 ± 0.21	0.79 ± 0.03
C16:0	23.17 ± 0.05	23.06 ± 0.12	15.34 ± 5.27	22.79 ± 0.17
C18:0	21.27 ± 0.05	20.78 ± 0.15	24.38 ± 1.87	20.60 ± 0.21
Σ SFA	**46.03 ± 0.01**	**45.58 ± 0.23**	**41.45 ± 3.27**	**45.02 ± 0.41**
C16:1n-7	0.57 ± 0.00	0.76 ± 0.034	0.933 ± 0.354	0.756 ± 0.046
C16:1cis-9	0.57 ± 0.02	0.64 ± 0.02	0.65 ± 0.12	0.62 ± 0.01
C18:1cis-9	14.41 ± 0.12	16.27 ± 0.16	15.87 ± 1.22	16.64 ± 0.13
Σ MUFA	**20.90 ± 0.19**	**22.93 ± 0.26**	**23.43 ± 2.08**	**23.34 ± 0.19**
C18:2 n-6 (LA)	1.44 ± 0.11	0.88 ± 0.03***	1.65 ± 0.1	0.68 ± 0.02***
C20:4 n-6 (AA)	12.20 ± 0.00	8.62 ± 0.10***	12.91 ± 0.57	8.45 ± 0.07***
C18:3 n-3 (ALA)	0.04 ± 0.00	0.17 ± 0.04	0.04 ± 0.01	0.04 ± 0.00
	0.03 ± 0.00	0.57 ± 0.05***	0.03 ± 0.01	0.63 ± 0.09***
C20:5 n-3 (DPA)				
	0.24 ± 0.02	0.67 ± 0.02***	0.23 ± 0.02	0.74 ± 0.02***
C22:5 n-3 (EPA)				
	14.49 ± 0.10	17.33 ± 0.24***	15.33 ± 0.30	17.62 ± 0.20***
C22:6 n-3 (DHA)				
Σ PUFAs	**32.91 ± 0.04**	**30.95 ± 0.35*** **	**34.36 ± 1.78**	**30.96 ± 0.29*** **
	17.42 ± 0.04	11.77 ± 0.13***	18.50 ± 0.87	11.58 ± 0.07***
Σ PUFAs n-6				
Σ PUFAs n-3	15.13 ± 0.15	19.18 ± 0.21***	16.10 ± 0.30	19.38 ± 0.28***
	1.15 ± 0.01	0.61 ± 0.0***	1.15 ± 0.03	0.60 ± 0.01***
n-6/n-3				
Desaturase activity				
Δ9 16:1/16:0	0.025 ± 0.00	0.033 ± 0.00	1.244 ± 1.219	0.033 ± 0.002
Δ9 18:1/18:0	0.673 ± 0.008	0.783 ± 0.012***	0.654 ± 0.02	0.808 ± 0.016**
Δ6 20:3/18:2	0.343 ± 0.01	0.433 ± 0.027***	0.299 ± 0.021	0.519 ± 0.019**
Δ5 20:4/20:3	25.55 ± 0.43	22.847 ± 0.48	26.13 ± 0.99	24.21 ± 0.81

*SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahesaenoic acid; DPA, docosapentaenoic acid. Σ SFA: sum of C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C22:0 + C23:0 + C24:0. Σ MUFA: sum of C14:1 n-9 + C15:1 n-9 + C16:1 n-7 + C16:1 n-9 + C16:1 n-11 + C18:1 n-7 + C18:1n-9 + C20:1 n-7 + C20:1 n-9 + C20:1 n-11 + C22:1 n-9. Σ PUFAs: sum of C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:4 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3 + C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:2 n-6 + C22:3 n-6 + C22:4 n-6 + C22:5 n-6. Σ n-3: sum of C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:4 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3. Σ n-6: sum of C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n6 + C22:4 n-6 + C22:2 n-6 + C22:3 n-6 + C22:4 n-6 + C22:5 n-6. **p < 0.01 and ***p < 0.001: significantly different from the corresponding group of mice fed a control diet (n = 3/4 mice/group).*

of wild-type and BDNF$^{+/-}$ mice. Under control diet, a significant decrease in SERT protein levels was observed in BDNF$^{+/-}$ compared to wild-type mice (p = 0.015). Although fish oil had no effect on SERT protein levels in wild-type mice (p = 0.7), this diet rescued SERT protein to control levels in BDNF$^{+/-}$ mice (p = 0.02; **Figure 3A**). In light of these findings, we tested the possibility that serotonergic tone might be differentially modified in wild-type and BDNF$^{+/-}$ mice. Accordingly, under control diet a significant increase in extracellular 5-HT levels ([5-HT]$_{ext}$) was detected in the ventral hippocampus of BDNF$^{+/-}$ mice compared to their wild-type littermates (p < 0.001). Fish oil-enriched diet normalized this parameter in BDNF$^{+/-}$ mice (p < 0.001; **Figure 3B**). These results indicate that the partial BDNF depletion is responsible for an enhancement of serotonergic tone in the hippocampus whereas fish oil-enriched diet restored normal [5-HT]$_{ext}$ levels in BDNF$^{+/-}$ mice by heightening SERT protein expression.

Fish Oil-Enriched Diet Increases Densities of Immature Adult-Born Neurons in the Dentate Gyrus of BDNF$^{+/-}$ Mice

Because previous reports pointed out a role for 5-HT and BDNF signaling in the control of hippocampal plasticity, particularly regarding its ability to influence new cell survival and neuronal differentiation (Nibuya et al., 1995; Quesseveur et al., 2013), we examined the effects of fish oil-enriched diet on these parameters in wild-type and BDNF$^{+/-}$ mice. To this end, we quantified

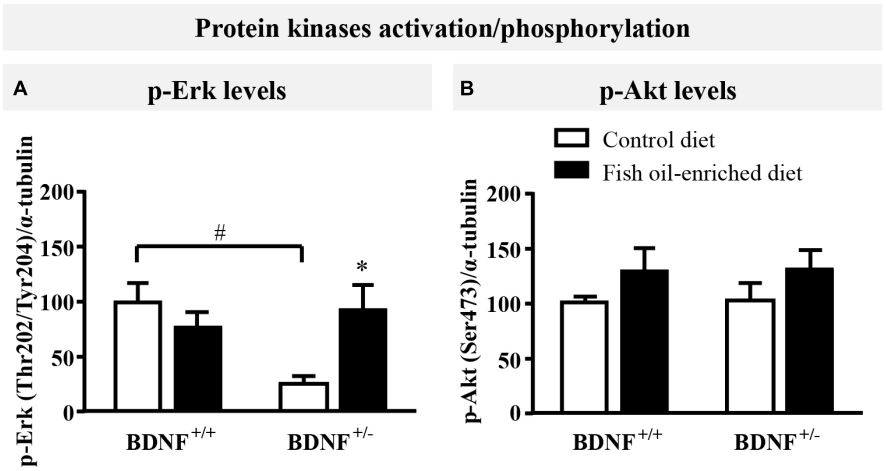


FIGURE 2 | Fish oil-enriched diet increases p-Erk (Thr202/Tyr204) in the hippocampus of BDNF$^{+/-}$ mice. **(A)** Densitometric quantification of immunoblot analysis from p-Erk (Thr202/Tyr204) (ANOVA: [$F_{(3,16)} = 4.2$; $p = 0.02$]) and **(B)** p-Akt (Ser473) (ANOVA: [$F_{(3,16)} = 0.9$; $p = 0.4$]) in the hippocampus of wild-type and BDNF$^{+/-}$ mice fed a control (white bars) or fish oil-enriched diet (black bars) for 12 weeks. Data are expressed as means ± SEM of the ratio p-Erk/α-tubulin or p-Akt/α-tubulin (% of wild-type mice fed a control diet). *$p < 0.05$: diet effect, #$p < 0.05$: genotype effect ($n = 5$ mice/group).

BrdU- and DCX-labeled (BrdU+ and DCX+) cells in the dentate gyrus of mice from both genotypes. Densities of 12-week-old BrdU+ cells were not significantly different among experimental groups (**Figures 4A,B**), indicating that partial BDNF depletion has no long-term impact on hippocampal new cell survival. As regards the density of DCX+ cells, under control diet, neuronal differentiation was not different between wild-type and BDNF$^{+/-}$ mice. Interestingly, although fish-oil diet failed to alter this parameter, a significant increase in the density of DCX+ cells was observed in BDNF$^{+/-}$ mice ($p = 0.05$; **Figures 4C,D**). These results suggest that fish-oil enriched diet increases the pool of immature neurons in the dentate gyrus of BDNF$^{+/-}$ mice.

Fish Oil-Enriched Diet Exerts Antidepressant and Anxiolytic-Like Effects on BDNF$^{+/-}$ Mice

Finally, we tested the effects of fish oil-enriched diet on mice from both genotypes submitted to behavioral paradigms designed to evaluate different symptoms of depressive state such as anxiety in the OF and EPM and despair in the FST.

In the OF test, numbers of entries in the center were not statistically different between wild-type and BDNF$^{+/-}$ mice fed a control diet ($p = 0.06$). In mice fed the fish oil-enriched diet, the number of entries ($p < 0.01$) was significantly increased in BDNF$^{+/-}$ mice ($p < 0.01$), but not in wild-type littermates ($p = 0.7$; **Figure 5A**). Interestingly, similar results were obtained on the time spent in the center (**Supplementary Figure S1A**). To eliminate a putative bias, we verified that the locomotor activity was not different between groups (wild-type control diet: 3195 ± 461 cm during 30 min; BDNF$^{+/-}$ control diet: 2694 ± 209 cm; wild-type fish oil-enriched diet: 2794 ± 305 cm and BDNF$^{+/-}$ fish oil-enriched diet: 3201 ± 154 cm [$F_{(3,20)} = 0.7$; $p = 0.5491$]).

Likewise, in the elevated plus maze, under control diet, no differences were detected between BDNF$^{+/-}$ and their wild-type littermates in the number of entries in the open arms ($p = 0.4$). However, in mice fed the fish oil-enriched diet, this parameter was significantly increased in BDNF$^{+/-}$ mice ($p < 0.01$) but not in wild-type littermates ($p = 0.4$; **Figure 5B**). Again, these results are consistent with those obtained on the time spent in the open arms (**Supplementary Figure S1B**).

Finally, in the FST, under control diet, wild-type and BDNF$^{+/-}$ mice displayed the same duration of immobility ($p = 0.9$). Fish oil significantly decreased the time of immobility in BDNF$^{+/-}$ mice ($p < 0.01$), while it had no effect in wild-type mice fed a control diet ($p = 0.8$; **Figure 5C**).

Overall, these set of behavioral data indicate that fish oil-enriched diet elicited anxiolytic- and antidepressant-like activities specifically in BDNF$^{+/-}$ mice.

DISCUSSION

The present study evaluated the effects of prolonged exposure to fish oil-enriched diet in wild-type and BDNF$^{+/-}$ mice at the molecular, cellular and behavioral levels. BDNF$^{+/-}$ mice offer a good model to study non-conventional therapeutic strategies for anxiety and depression since these mice are less prone to respond to currently available antidepressant drugs including SSRIs (Daws et al., 2007; Guiard et al., 2008; Ibarguen-Vargas et al., 2009). One of the most remarkable results obtained herein is that under control diet, the partial BDNF depletion produced significant changes in the hippocampus, including a decreased activation of the MAP kinase Erk along with an elevated serotonergic tone. However, these effects were not sufficient to impact cell survival and neuronal differentiation in the hippocampus and to

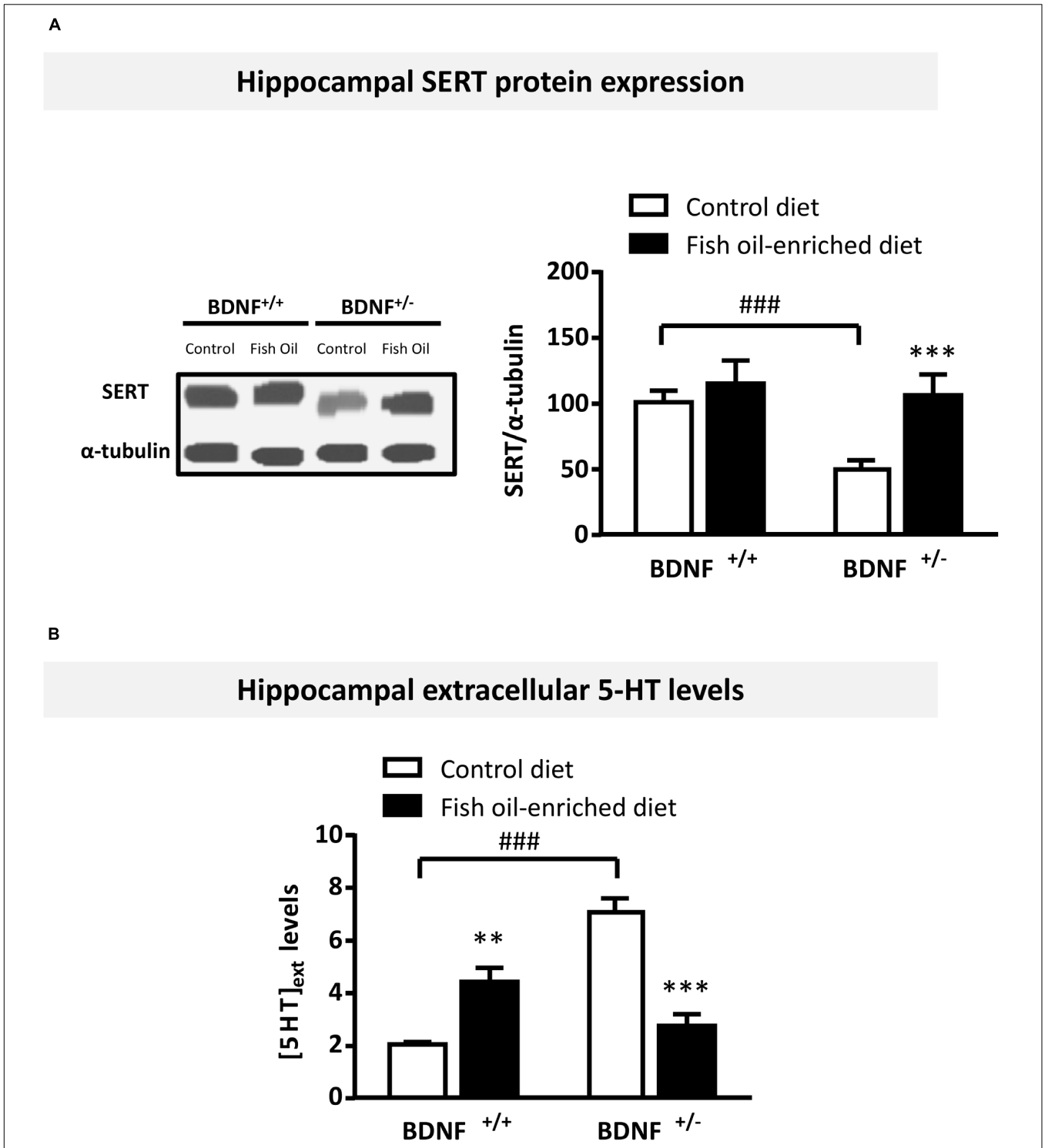


FIGURE 3 | Fish oil-enriched diet decreases serotonergic tone in the hippocampus of BDNF$^{+/-}$ mice. **(A)** Protein expression of the Serotonin Transporter (SERT). (Left panel) Representative blots from SERT. (Right panel) Densitometric quantification of immunoblot analysis from SERT (ANOVA: [$F_{(3,29)}$ = 5.2; p = 0.002]) in the hippocampus of wild-type and BDNF$^{+/-}$ mice fed a control (white bars) or fish oil-enriched diet (black bars) for 12 weeks. Data are means ± SEM of the ratio SERT/α-tubulin (% of wild-type mice fed a control diet). *$p < 0.05$: diet effect; #$p < 0.05$: genotype effect significantly different from wild-type mice fed a control diet (n = 8–9 mice/group). **(B)** Basal extracellular 5-HT levels ([5-HT]$_{ext}$) in the hippocampus. Data are means ± SEM of basal [5-HT]$_{ext}$ (fmol/19 μL) measured for a 60 min-period in the hippocampus of mice from both genotypes fed a control (white) or fish oil-enriched diet (black) for 12 weeks (ANOVA: [$F_{(3,25)}$ = 67.23]; $p < 0.001$). **$p < 0.01$ and ***$p < 0.001$: diet effect; ###$p < 0.001$: significantly different from wild-type mice fed a control diet (n = 6–9 mice/group).

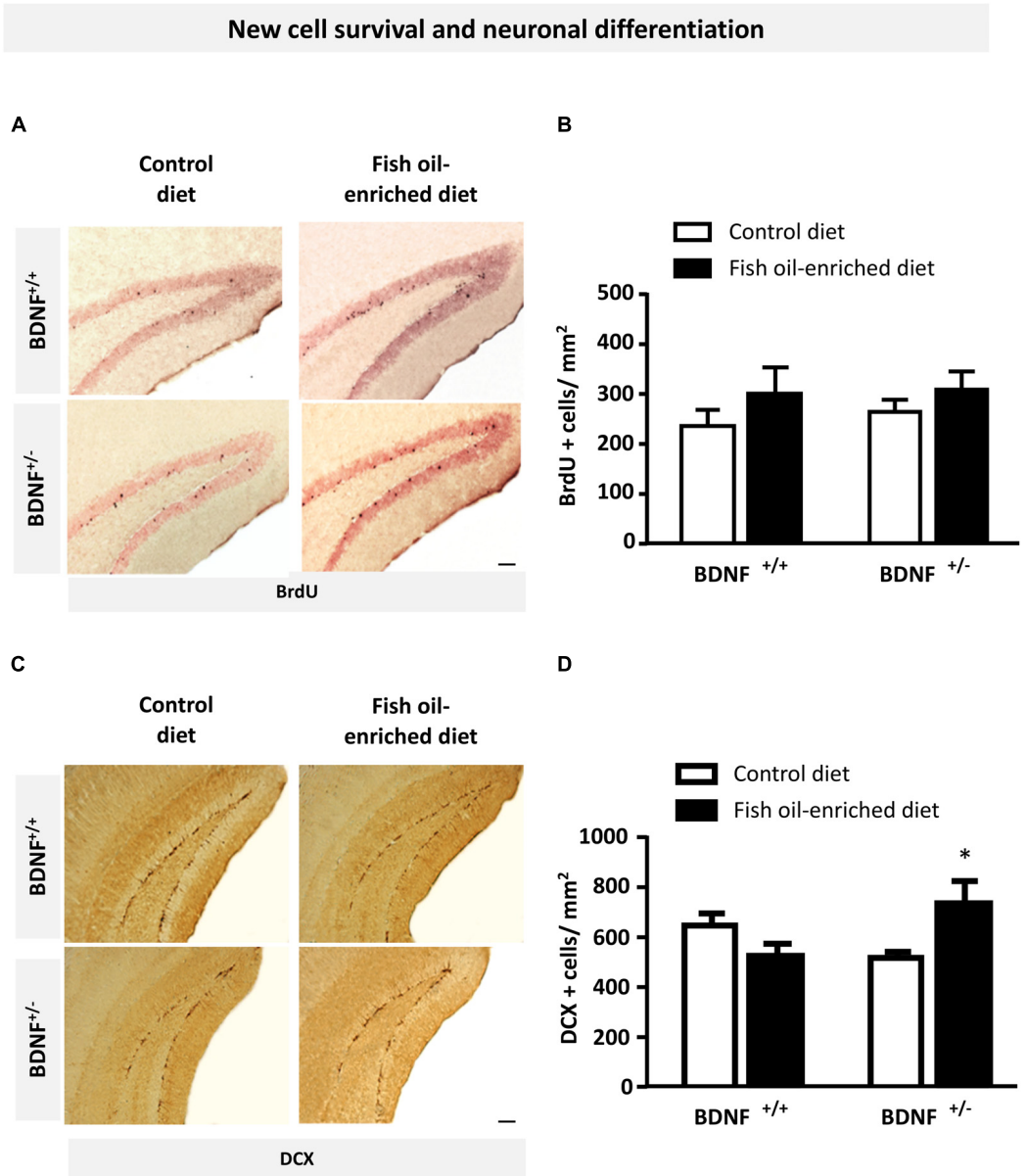


FIGURE 4 | Fish oil-enriched diet does not affect cell survival but increases the density of immature neurons in the hippocampus of BDNF$^{+/-}$ mice. **(A)** Representative images of the dentate gyrus after 5-bromo-2-deoxyuridine (BrdU) immunostaining in each experimental group. Scale bar: 50 μm. **(B)** Density of BrdU-labeled (BrdU+) cells 4 weeks after BrdU injection indicative of new cell survival. Data are means ± SEM of BrdU+ cell counts per mm^2 (ANOVA: [$F_{(3,19)}$ = 0.8; p = 0.4]; n = 5–6 mice/group) in the dentate gyrus of wild-type and BDNF$^{+/-}$ mice fed a control (white) or fish oil-enriched diet (black) for 12 weeks. **(C)** Representative images of the dentate gyrus showing doublecortin-labeled (DCX+) cells in each experimental group. Scale bar: 50 μm. **(D)** Density of DCX+ cells in the dentate gyrus, indicative of the presence of immature neurons. Data are means ± SEM of DCX+ cell counts per mm^2 (ANOVA: [$F_{(3,26)}$ = 3.6; p = 0.025]) in the dentate gyrus of wild-type and BDNF$^{+/-}$ mice fed a control (white) or fish oil-enriched diet (black) for 12 weeks. *p < 0.05: diet effect (n = 6–9 mice/group).

impact anxio-depressive-like behaviors. In a marked contrast, long-term exposure to fish oil-enriched diet in BDNF$^{+/-}$ but not in BDNF$^{+/+}$ mice increased the activation of Erk and decreased serotonergic tone. These molecular changes were accompanied by an enhancement of neuronal differentiation along with reproducible anxiolytic responses and robust antidepressant-like effects. Together these results led us to envision that fish oil could exert its beneficial effects on mood specifically in patients displaying decreased BDNF signaling in the hippocampus.

The effects of fish oil-enriched diet, an omega-3 PUFAs source, on anxiety have been poorly documented. The limited data available in rodents show that supplementation of diet with omega-3 PUFAs favors anxiolytic-like activities (Pérez et al., 2013; Pudell et al., 2014), whereas their deprivation produces opposite effects (Lafourcade et al., 2011; Larrieu et al., 2012).

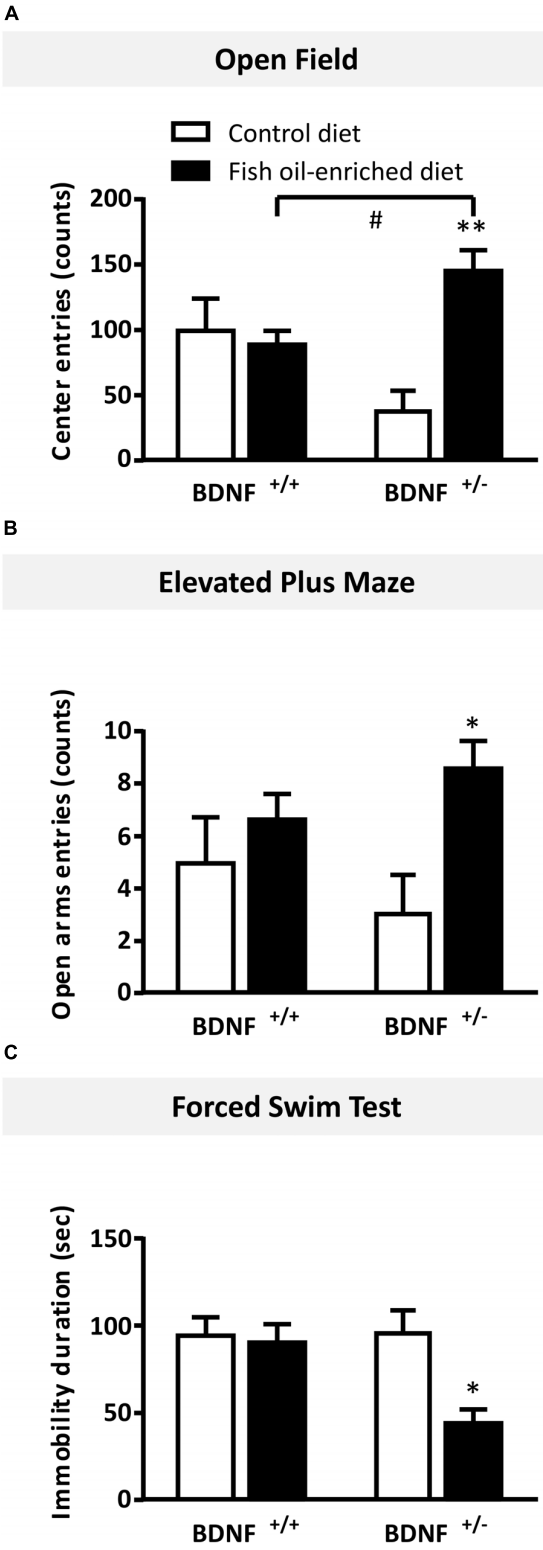


FIGURE 5 | Fish oil-enriched diet induces anxiolytic- and antidepressant-like activities in BDNF$^{+/-}$ mice. **(A)** Anxiety evaluated in the open field. Data are means ± SEM of the number of entries in the center of the arena (ANOVA: [$F_{(3,20)} = 7.1$; $p = 0.002$]). **(B)** Anxiety evaluated in the elevated plus maze.

(Continued)

FIGURE 5 | Continued

Data are means ± SEM of the number of entries in the open arms (ANOVA: [$F_{(3,20} = 2.8$; $p = 0.11$]). **(C)** Antidepressant-like activity evaluated in the forced swim test (FST). Data represent means ± SEM of the immobility time of wild-type and BDNF$^{+/-}$ mice fed a control (white) or fish oil-enriched diet (black) for 12 weeks (ANOVA: [$F_{(3,20)} = 7.1$; $p = 0.002$]). *$p < 0.05$, **$p < 0.01$: diet effect; #$p < 0.05$: genotype effect ($n = 6$ mice/group).

The latter findings are not consistent with the present results since we failed to unveil beneficial effects of fish oil in wild-type BDNF$^{+/+}$ mice submitted to the OF and EPM. One possible explanation for this relies on the fact that the control diet used in the present study was not deficient in omega-3. Indeed, the therapeutic benefits of fish oil supplementation have been found only in omega-3 deficient individuals, while those without baseline deficits were less prone to benefit from supplementation (Horrobin and Bennett, 1999). Alternatively, one would expect that fish oil-enriched diet specifically dampens anxiety under pathological conditions. In agreement with this hypothesis, evidence demonstrated that the anxiolytic-like effects of omega-3 PUFAs are detectable after acute or chronic stress (Harauma and Moriguchi, 2011; Mathieu et al., 2011; Ferreira et al., 2013). Of particularly interest in the context of the present study unveiling anxiolytic-like effects of fish oil in BDNF$^{+/-}$ mice, it has been reported that such diet displays similar behavioral properties in bulbectomized rats, an animal model of depression also characterized by reduced hippocampal BDNF levels (Pudell et al., 2014). In keeping with the latter findings, omega-3 PUFAs supplementation was shown to improve social interaction in a strain of mice displaying a reduction of BDNF levels in various brain regions (Pietropaolo et al., 2014). Collectively, these results suggest that the down-regulation of BDNF may be a prerequisite for the manifestation of fish oil's anxiolytic-like activity. These findings are important since previous studies reported that BDNF$^{+/-}$ mice are not responsive to chronic imipramine treatment (Ibarguen-Vargas et al., 2009) or to acute paroxetine administration (Guiard et al., 2008). Hence, fish oil-enriched diet might be used either alone or as an add-on strategy to antidepressant drugs in treatment-resistant patients (Laino et al., 2010, 2014; Hou and Lai, 2016; Mayor, 2016).

To unravel the putative links between BDNF deficiency and behavioral effects of fish oil-enriched diet, we examined the functional activity of two proteins kinases in the hippocampus (i.e., Erk and Akt). Doing so, we observed that BDNF$^{+/-}$ mice displayed a significant reduction in the level of Erk phosphorylation/activation whereas fish oil diet increased this deficit. Such reversal effect might have contributed, at least in part, to the anxiolytic properties of fish oil diet in these mutants. In support of this hypothesis, we recently demonstrated that an increase in hippocampal p-Erk correlates with a decrease in the latency to feed in the novelty suppressed feeding test (Quesseveur et al., 2015). Conversely, evidence showed that rats microinjected with a specific inhibitor of Erk in the hippocampus for seven consecutive days display anxiety-like behaviors in the open field and the elevated plus maze (Qi et al., 2009). Having shown that fish oil diet increases p-Erk and exert anxiolytic effects specifically in BDNF$^{+/-}$ mice, we

then explored to what extent the serotonergic system could be involved an additional and possible component in the behavioral characteristic of fish oil-enriched diet. Here, we report an increase in basal $[5\text{-HT}]_{ext}$ in the ventral hippocampus of $BDNF^{+/-}$ mice, which is normalized in response to fish oil-enriched diet. Interestingly, evidence indicated that an abnormally elevated 5-HT tone favors anxiety through the activation of specific post-synaptic 5-HT receptors including the $5HT_{1A}$, $5\text{-HT}_{2A/C}$ or 5-HT_3 subtypes (Hamon, 1994). Although the impact of fish oil-enriched diet on hippocampal $[5\text{-HT}]_{ext}$ levels remains elusive, a recent study pointed out that omega-3 PUFAs supplementation in diet increases tissue 5-HT contents in hippocampus and cortex associated to reduced 5-HIAA levels in 3 months-old rats (Vines et al., 2012). The latter findings are relevant in light of our microdialysis data since an accumulation of tissue (i.e., intracellular) 5-HT in fish oil fed animals could reflect lower $[5\text{-HT}]_{ext}$, notably if the release process is dampened (Kodas et al., 2004). It is noteworthy that the putative inhibitory influence of fish oil-enriched diet on $[5\text{-HT}]_{ext}$ levels in the hippocampus of $BDNF^{+/-}$ mice may also result from mechanisms involving the tryptophan hydroxylase 2 (TpH2), the rate-limiting enzyme of 5-HT synthesis, and/or the monoamine oxydase (MAO), an enzyme important in the catabolism of 5-HT. However, the observations that omega-3 PUFAs enhance the expression of tryptophan hydroxylase-2 (TPH-2) (McNamara et al., 2009), while attenuating that of MAO-A/B in the brain (Delion et al., 1997; Chalon et al., 1998; Naveen et al., 2013) are not compatible with our neurochemical data. Because the serotonin transporter SERT is an alternative target through which 5-HT tone may be regulated, we studied this molecular element in the hippocampus. As previously described, we found that $BDNF^{+/-}$ mice exhibited lower levels of SERT protein expression in the hippocampus (Guiard et al., 2008) thereby resulting in an elevated 5-HT tone. Fish oil-enriched diet restored normal hippocampal SERT expression, a process that contributed to normalize 5-HT tone in the hippocampus in $BDNF^{+/-}$ mice and probably in other brain regions. Hence, we demonstrated that fish oil-enriched diet corrected SERT down-regulation directed at minimizing the basal hyperserotonergic phenotype reported in $BDNF^{+/-}$ mice. It is noteworthy that increased anxiety-related behaviors were observed in adult $SERT^{-/-}$ mice (Holmes et al., 2003; Kalueff et al., 2010; Sakakibara et al., 2014) which display spontaneous higher $[5\text{-HT}]_{ext}$. A corollary of this observation is that a functional SERT is necessary to promote long-term fish oil-induced anxiolysis as reported herein. In humans, a short promoter variant in the SERT gene is linked to lower SERT expression, leading to a reduced 5-HT reuptake (Bengel et al., 1998; Murphy and Lesch, 2008). This short variant has also been associated with anxiety-related personality traits (Lesch et al., 1996; Hariri and Holmes, 2006; Canli and Lesch, 2007) and it would be relevant to determine whether long-term exposure to fish oil is effective in this specific population of patients. Again, fish oil-enriched diet had no effect on serotonergic activity in wild-type animals. This is likely due to the fact that these mice display normal levels of anxiety and 5-HT transmission at baseline.

As regards the antidepressant-like effects of fish oil in $BDNF^{+/-}$ mice, it is difficult to envision that decreased immobility observed in the FST relates to the decreased serotonergic tone since an activation of 5-HT neurotransmission is required to hinder behavioral despair in this paradigm (Page et al., 1999). Alternative mechanisms are likely responsible for the antidepressant response induced by long-term exposure to fish oil-enriched diet. It is noteworthy that changes in hippocampal TrkB/BDNF transmission strongly influence MAP kinases signaling pathways, which in turn regulates depressive-related symptoms (Schmidt and Duman, 2010; Lepack et al., 2016). For example, interventions producing antidepressant-like effects

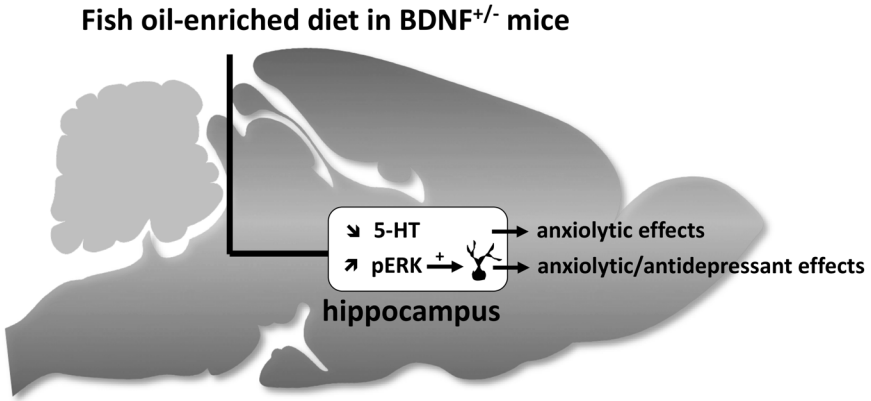


FIGURE 6 | Schematic representation of hippocampal neurochemical, molecular and cellular targets of fish oil-enriched diet and its behavioral effects in $BDNF^{+/-}$ mice. In $BDNF^{+/-}$ mice, a decrease in the extracellular 5-HT concentrations and an increase in the level of Erk phosphorylation are observed in response to long-term exposure to a fish oil-enriched diet. These effects might have contributed to positively reverberate on anxiety and despair. However, because the enhancement of BDNF synthesis/release and related-signaling (Erk activation) in the hippocampus relies on the activation of local 5-HT tone, the results presented herein cannot draw a cause and effect relationship between BDNF signaling and 5-HT neurotransmission. We propose that the beneficial behavioral effects of fish-oil in $BDNF^{+/-}$ mice involved two distinct mechanisms leading on one hand, to decrease extracellular 5-HT concentrations (favorable for anxiolysis) and on the other hand, to stimulate BDNF signaling and neuronal maturation (favorable for anxiolysis and antidepressant response).

such as electroconvulsive shocks or chronic administration of antidepressant drugs are generally associated with an up-regulation of BDNF and downstream signaling pathways in various brain regions including the hippocampus (Nibuya et al., 1995; Balu et al., 2008; Quesseveur et al., 2013). Based on this evidence, we can infer that the ability of fish oil to increase p-Erk levels in BDNF$^{+/-}$ mice leading to a complete recovery of initial hippocampal levels played an important role in the induction of antidepressant-like effects. This is consistent with the observation that addition of DHA to rat primary culture of cortical astrocytes induced BDNF protein expression, an effect blocked by a MAPK inhibitor (Rao et al., 2007). Moreover, it is noteworthy that blunted Erk activation has been observed both in depressed patients and in relevant animal models of depression (Dwivedi et al., 2001; Feng et al., 2003; Gourley et al., 2008; Yuan et al., 2010) whereas inhibition of kinases such as MEK or Erk produced despair-like behaviors and prevented the antidepressant-like effects of SSRIs in rodents (Shirayama et al., 2002; Duman et al., 2007). The observation that fish oil-enriched diet had no effect on Erk activation in wild-type animals is consistent with the lack of effects of this diet on behavioral parameters. The putative enhancement of BDNF signaling, as suggested by Erk phosphorylation/activation in response to fish-oil enriched diet in BDNF$^{+/-}$ mice, also draw our attention because MAP kinases play an important role in the regulation of adult hippocampal neurogenesis including the stimulation of proliferation/differentiation of neural progenitor cells (Lee et al., 2002; Sairanen et al., 2005; Li et al., 2008; Taliaz et al., 2010), the maturation of newborn neurons and their survival (Lee et al., 2002; Sairanen et al., 2005; Rossi et al., 2006; Wang et al., 2015). However, comparing densities of surviving BrdU+ cells, no differences were detected between wild-type and BDNF$^{+/-}$ mice fed a control or a fish oil-enriched diet. However, from these results we cannot provide definitive conclusion on cell survival as long as the number of new generated cells is not assessed in all experimental groups. Given that some studies have described a neurogenic effect of omega-3 PUFAs through its ability to stimulate neuronal maturation (Grundy et al., 2014; McCall et al., 2015), we also quantified immature neurons, using DCX immunolabeling. Interestingly, we observed increased numbers of DCX+ cells in the dentate gyrus of BDNF$^{+/-}$ mice fed a fish oil-enriched diet, suggesting that adult neurogenesis is impacted. Whether increased numbers of DCX+ cells reflect a preferential engagement of newborn cells toward a neuronal fate or a delay in terminal neuronal differentiation remains to be explored.

In an attempt to identify the putative beneficial effects of fish oil, we have to take into consideration the possibility that it might act by modulating inflammatory processes. Indeed, systemic administration of (LPS), widely used to create neuroinflammation, is known to precipitate depression-related behaviors in rodents (Dantzer et al., 2008) whereas evidence indicates that the antidepressant-like effects of fish-oil result, at least in part, from its ability to attenuate this state (Moranis et al., 2012; Delpech et al., 2015; Fourrier et al., 2017). A recent study reported that the TrkB agonist 7,8-dihydroxyflavone (7,8-DHF) reversed LPS-induced depression-like phenotype and morphological changes (i.e., spine density) in the mouse hippocampus (Zhang et al., 2014). These findings suggest that the enhancement of BDNF signaling could be a prerequisite to decrease neuroinflammation (Xu et al., 2017) and to promote the beneficial effects of fish oil in BDNF$^{+/-}$ mice. Nevertheless, different reports indicated that BDNF$^{+/-}$ mice are protected from inflammation not only in the whole brain (Javeri et al., 2010) but also in peripheral tissues such as the heart and the gut (Yang et al., 2010; Halade et al., 2013). Although these findings argue against the fact that BDNF$^{+/-}$ mice could display hallmarks of neuroinflammation, further investigations are warranted to determine to what extent inflammatory processes such as increases in the expression of pro-inflammatory cytokines, activation of ubiquitous indoleamine 2,3-dioxygenase (IDO) or recruitment of microglial cells are altered in these mutants and whether fish-oil enriched diet positively reverberates on these specific markers in the hippocampus.

CONCLUSION

Our data demonstrate that BDNF$^{+/-}$ mice were more sensitive to the effects of fish oil-enriched diet than wild-type mice. As depicted in **Figure 6**, the present study strongly suggests that fish oil positively reverberates on emotionality through its ability to decrease hippocampal extracellular 5-HT levels and to increase the activation of Erk that might contribute by itself to stimulate neuronal plasticity. It should be borne in mind that fish-oil is a mix of omega-3 fatty acids including, for example, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or α-linolenic acid (ALA). Given that these components display distinct effects on behavioral paradigms assessing antidepressant-like activities (Jin and Park, 2015; Choi and Park, 2017), it would be interesting to precise which of them interfere specifically on neurobehavior. This will help optimize an "add-on" strategy based on the combination of fish oil and SSRI in animal models resistant to conventional monoaminergic antidepressant drugs. In particular, it will be interesting to determine the effects of fish oil-enriched diet in mice exposed to chronic stress (e.g., restraint stress, unpredictable chronic mild stress or even social defeat).

ETHICS STATEMENT

The *in vivo* part of this study was conducted in 2013 and the procedures were conducted in conformity with the institutional guidelines in compliance with national and policy (Council directive #87–848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permission #92.196). The *in vitro* part of this study, not subjected to an ethical committee, was conducted between 2015 and 2017.

AUTHOR CONTRIBUTIONS

JZ conducted all the experiments in BDNF wild-type and mutant mice fed a fish oil diet and wrote the manuscript. QR and CG

provided their assistance for the behavioral and Western-blot analyses. DR-R and AG prepared the fish oil diet and analyses. ER and BG were the principal investigators of this study and contributed to the analyses of the results.

ACKNOWLEDGMENTS

The authors are thankful to M. Pereira (Sao Paulo) and P. Robert (Châtenay-Malabry) for animal care. JZ was supported by "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES, Brazil) and "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, Brazil). The authors greatly acknowledge "the Mouse Behavioural Phenotyping Core" (Center of Integrative Biology, Toulouse, France) and more particularly Sebastien Lopez for his expertise and assistance in setting up behavioral apparatus and procedures. The authors also thank Prs A. M. Gardier and D. David for their helpful comments in the preparation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2018.00974/full#supplementary-material

FIGURE S1 | Fish oil-enriched diet induces anxiolytic-like activities in BDNF$^{+/-}$ mice. **(A)** Anxiety evaluated in the open field. Data are means ± SEM of the time spent in the center of the arena (ANOVA: [$F_{(3,20)} = 7.1$; $p = 0.002$]). **(B)** Anxiety evaluated in the elevated plus maze. Data are means ± SEM of the time spent in the open arms (ANOVA: [$F_{(3,20)} = 2.8$; $p = 0.11$]). *$p < 0.05$: significantly different from the corresponding group fed a control diet ($n = 6$ mice/group).

TABLE S1 | Fatty acids content in control and fish oil-enriched diet.

REFERENCES

Able, J. A., Liu, Y., Jandacek, R., Rider, T., Tso, P., and McNamara, R. K. (2014). Omega-3 fatty acid deficient male rats exhibit abnormal behavioral activation in the forced swim test following chronic fluoxetine treatment: association with altered 5-HT1A and alpha2A adrenergic receptor expression. *J. Psychiatr. Res.* 50, 42–50. doi: 10.1016/j.jpsychires.2013.11.008

Balu, D. T., Hoshaw, B. A., Malberg, J. E., Rosenzweig-Lipson, S., Schechter, L. E., and Lucki, I. (2008). Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. *Brain Res.* 1211, 37–43. doi: 10.1016/j.brainres.2008.03.023

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Heils, A., et al. (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol. Pharmacol.* 53, 649–655. doi: 10.1124/mol.53.4.649

Blondeau, N., Nguemeni, C., Debruyne, D. N., Piens, M., Wu, X., Pan, H., et al. (2009). Subchronic alpha-linolenic acid treatment enhances brain plasticity and exerts an antidepressant effect: a versatile potential therapy for stroke. *Neuropsychopharmacology* 34, 2548–2559. doi: 10.1038/npp.2009.84

Calder, P. C. (1998). Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. *Braz. J. Med. Biol. Res.* 31, 467–490. doi: 10.1590/S0100-879X1998000400002

Canli, T., and Lesch, K. P. (2007). Long story short: the serotonin transporter in emotion regulation and social cognition. *Nat. Neurosci.* 10, 1103–1109. doi: 10.1038/nn1964

Chalon, S., Delion-Vancassel, S., Belzung, C., Guilloteau, D., Leguisquet, A. M., Besnard, J. C., et al. (1998). Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. *J. Nutr.* 128, 2512–2519. doi: 10.1093/jn/128.12.2512

Chiu, C. C., Huang, S. Y., Su, K. P., Lu, M. L., Huang, M. C., Chen, C. C., et al. (2003). Polyunsaturated fatty acid deficit in patients with bipolar mania. *Eur. Neuropsychopharmacol.* 13, 99–103. doi: 10.1016/S0924-977X(02)00130-X

Choi, J. E., and Park, Y. (2017). EPA and DHA, but not ALA, have antidepressant effects with 17β-estradiol injection via regulation of a neurobiological system in ovariectomized rats. *J. Nutr. Biochem.* 49, 101–109. doi: 10.1016/j.jnutbio.2017.07.012

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Daws, L. C., Munn, J. L., Valdez, M. F., Frosto-Burke, T., and Hensler, J. G. (2007). Serotonin transporter function, but not the expression, is dependent on brain-derived neurotrophic factor (BDNF): in vivo studies in BDNF-deficient mice. *J. Neurochem.* 101, 641–651. doi: 10.1111/j.1471-4159.2006.04392.x

De Vriese, S. R., Christophe, A. B., and Maes, M. (2003). Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sci.* 73, 3181–3187. doi: 10.1016/j.lfs.2003.02.001

Delion, S., Chalon, S., Guilloteau, D., Lejeune, B., Besnard, J. C., and Durand, G. (1997). Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. *J. Lipid Res.* 38, 680–689.

Delpech, J. C., Thomazeau, A., Madore, C., Bosch-Bouju, C., Larrieu, T., Lacabanne, C., et al. (2015). Dietary n-3 PUFAs deficiency increases vulnerability to inflammation-induced spatial memory impairments. *Neuropsychopharmacology* 40, 2274–2287. doi: 10.1038/npp.2015.127

Deltheil, T., Tanaka, K., Repérant, C., Hen, R., David, D. J., and Gardier, A. M. (2009). Synergistic neurochemical and behavioural effects of acute intra-hippocampal injection of brain-derived neurotrophic factor and antidepressants in adult mice. *Int. J. Neuropsychopharmacol.* 12, 905–915. doi: 10.1017/S1461145709000017

Duman, C. H., Schlesinger, L., Kodama, M., Russell, D. S., and Duman, R. S. (2007). A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol. Psychiatry* 61, 661–670. doi: 10.1016/j.biopsych.2006.05.047

Dwivedi, Y., Rizavi, H. S., Roberts, R. C., Conley, R. C., Tamminga, C. A., and Pandey, G. N. (2001). Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. *J. Neurochem.* 77, 916–928. doi: 10.1046/j.1471-4159.2001.00300.x

Ernfors, P., Lee, K. F., and Jaenisch, R. (1994). Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* 368, 147–150. doi: 10.1038/368147a0

Feng, P., Guan, Z., Yang, X., and Fang, J. (2003). Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. *Brain Res.* 991, 195–205. doi: 10.1016/j.brainres.2003.08.018

Ferreira, C. F., Bernardi, J. R., Krolow, R., Arcego, D. M., Fries, G. R., de Aguiar, B. W., et al. (2013). Vulnerability to dietary n-3 polyunsaturated fatty acid deficiency after exposure to early stress in rats. *Pharmacol. Biochem. Behav.* 107, 11–19. doi: 10.1016/j.pbb.2013.03.006

Fourrier, C., Remus-Borel, J., Greenhalgh, A. D., Guichardant, M., Bernoud-Hubac, N., Lagarde, M., et al. (2017). Docosahexaenoic acid-containing choline phospholipid modulates LPS-induced neuroinflammation in vivo and in microglia in vitro. *J. Neuroinflamm.* 14:170. doi: 10.1186/s12974-017-0939-x

Freeman, M. P., Hibbeln, J. R., Wisner, K. L., Brumbach, B. H., Watchman, M., and Gelenberg, A. J. (2006). Randomized dose-ranging pilot trial of omega-3 fatty acids for postpartum depression. *Acta Psychiatr. Scand.* 113, 31–35. doi: 10.1111/j.1600-0447.2005.00660.x

Gardier, A. M., Malagié, I., Trillat, A. C., Jacquot, C., and Artigas, F. (1996). Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic

antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam. Clin. Pharmacol.* 10, 16–27. doi: 10.1111/j.1472-8206.1996.tb00145.x

Gertsik, L., Poland, R. E., Bresee, C., and Rapaport, M. H. (2012). Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder. *J. Clin. Psychopharmacol.* 32, 61–64. doi: 10.1097/JCP.0b013e31823f3b5f

Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., et al. (2008). Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. *Biol. Psychiatry* 63, 353–359. doi: 10.1016/j.biopsych.2007.07.016

Grundy, T., Toben, C., Jaehne, E. J., Corrigan, F., and Baune, B. T. (2014). Long-term omega-3 supplementation modulates behavior, hippocampal fatty acid concentration, neuronal progenitor proliferation and central TNF-α expression in 7 month old unchallenged mice. *Front. Cell. Neurosci.* 8:399. doi: 10.3389/fncel.2014.00399

Guiard, B. P., David, D. J., Deltheil, T., Chenu, F., Le Maître, E., Renoir, T., et al. (2008). Brain-derived neurotrophic factor-deficient mice exhibit a hippocampal hyperserotonergic phenotype. *Int. J. Neuropsychopharmacol.* 11, 79–92. doi: 10.1017/S1461145707007857

Halade, G. V., Ma, Y., Ramirez, T. A., Zhang, J., Dai, Q., Hensler, J. G., et al. (2013). Reduced BDNF attenuates inflammation and angiogenesis to improve survival and cardiac function following myocardial infarction in mice. *Am. J. Physiol. Heart Circ. Physiol.* 305, H1830-18-42. doi: 10.1152/ajpheart.00224.2013

Hamon, M. (1994). Neuropharmacology of anxiety: perspectives and prospects. *Trends Pharmacol. Sci.* 15, 36–39. doi: 10.1016/0165-6147(94)90104-X

Hamon, M., and Blier, P. (2013). Monoamine neurocircuitry in depression and strategies for new treatments. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 45, 54–63. doi: 10.1016/j.pnpbp.2013.04.009

Harauma, A., and Moriguchi, T. (2011). Dietary n-3 fatty acid deficiency in mice enhances anxiety induced by chronic mild stress. *Lipids* 46, 409–416. doi: 10.1007/s11745-010-3523-z

Hariri, A. R., and Holmes, A. (2006). Genetics of emotional regulation: the role of the serotonin transporter in neural function. *Trends Cogn. Sci.* 10, 182–191. doi: 10.1016/j.tics.2006.02.011

Hibbeln, J. R., Ferguson, T. A., and Blasbalg, T. L. (2006). Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. *Int. Rev. Psychiatry* 18, 107–118. doi: 10.1080/09540260600582967

Holmes, A., Yang, R. J., Lesch, K. P., Crawley, J. N., and Murphy, D. L. (2003). Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology* 28, 2077–2088. doi: 10.1038/sj.npp.1300266

Horrobin, D. F., and Bennett, C. N. (1999). Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing and osteoporosis, *Possible candidate genes, Prostaglandins Leukot. Essent. Fatty Acids* 60, 217–234. doi: 10.1054/plef.1999.0037

Hou, Y. C., and Lai, C. H. (2016). Enhancing therapeutic effects after augmentation of omega-3 fatty acid with long-term antidepressant therapy in a chronic case of panic disorder. *Psychiatry Clin. Neurosci.* 70, 72–83. doi: 10.1111/pcn.12373

Ibarguen-Vargas, Y., Surget, A., Vourc'h, P., Leman, S., Andres, C. R., Gardier, A. M., et al. (2009). Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. *Behav. Brain Res.* 202, 245–251. doi: 10.1016/j.bbr.2009.03.040

Javeri, S., Rodi, M., Tary-Lehmann, M., Lehmann, P. V., Addicks, K., and Kuerten, S. (2010). Involvement of brain-derived neurotrophic factor (BDNF) in MP4-induced autoimmune encephalomyelitis. *Clin. Immunol.* 137, 181–189. doi: 10.1016/j.clim.2010.08.001

Jazayeri, S., Tehrani-Doost, M., Keshavarz, S. A., Hosseini, M., Djazayery, A., Amini, H., et al. (2008). Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. *Aust. N. Z. J. Psychiatry* 42, 192–198. doi: 10.1080/00048670701827275

Jin, Y., and Park, Y. (2015). N-3 polyunsaturated fatty acids and 17β-estradiol injection induce antidepressant-like effects through regulation of serotonergic neurotransmission in ovariectomized rats. *J. Nutr. Biochem.* 26, 970–977. doi: 10.1016/j.jnutbio.2015.04.005

Kalueff, A. V., Olivier, J. D., Nonkes, L. J., and Homberg, J. R. (2010). Conserved role for the serotonin transporter gene in rat and mouse neurobehavioral endophenotypes. *Neurosci. Biobehav. Rev.* 34, 373–386. doi: 10.1016/j.neubiorev.2009.08.003

Kodas, E., Galineau, L., Bodard, S., Vancassel, S., Guilloteau, D., Besnard, J. C., et al. (2004). Serotoninergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. *J. Neurochem.* 89, 695–702. doi: 10.1111/j.1471-4159.2004.02401.x

Kolbeck, R., Bartke, I., Eberle, W., and Barde, Y. A. (1999). Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. *J. Neurochem.* 75, 1930–1938. doi: 10.1046/j.1471-4159.1999.0721930.x

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., and Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. U.S.A.* 92, 8856–8860. doi: 10.1073/pnas.92.19.8856

Lafourcade, M., Larrieu, T., Mato, S., Duffaud, A., Sepers, M., Matias, I., et al. (2011). Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. *Nat. Neurosci.* 14, 345–350. doi: 10.1038/nn.2736

Laino, C. H., Fonseca, C., Sterin-Speziale, N., Slobodianik, N., and Reinés, A. (2010). Potentiation of omega-3 fatty acid antidepressant-like effects with low non-antidepressant doses of fluoxetine and mirtazapine. *Eur. J. Pharmacol.* 648, 117–126. doi: 10.1016/j.ejphar.2010.08.047

Laino, C. H., Garcia, P., Podestá, M. F., Höcht, C., Slobodianik, N., and Reinés, A. (2014). Fluoxetine potentiation of omega-3 fatty acid antidepressant effect: evaluating pharmacokinetic and brain fatty acid-related aspects in rodents. *J. Pharm. Sci.* 103, 3316–3325. doi: 10.1002/jps.24123

Lakhwani, L., Tongia, S. K., Pal, V. S., Agrawal, R. P., Nyati, P., and Phadnis, P. (2007). Omega-3 fatty acids have antidepressant activity in forced swimming test in Wistar rats. *Acta Pol. Pharm.* 64, 271–276.

Larrieu, T., Madore, C., Joffre, C., and Layé, S. (2012). Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice. *J. Physiol. Biochem.* 68, 671–681. doi: 10.1007/s13105-012-0179-6

Lecrubier, Y. (2001). Prescribing patterns for depression and anxiety worldwide. *J. Clin. Psychiatry* 62 (Suppl. 13), 31–36; discussion 37–38.

Lee, J., Duan, W., and Mattson, M. P. (2002). Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.* 82, 1367–1375. doi: 10.1046/j.1471-4159.2002.01085.x

Lepack, A. E., Bang, E., Lee, B., Dwyer, J. M., and Duman, R. S. (2016). Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures. *Neuropharmacology* 111, 242–252. doi: 10.1016/j.neuropharm.2016.09.011

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Li, Y., Luikart, B. W., Birnbaum, S., Chen, J., Kwon, C. H., Kernie, S. G., et al. (2008). TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron* 59, 399–412; Erratum in: Neuron 2008 60, 730. doi: 10.1016/j.neuron.2008.06.023

Lin, P. Y., Huang, S. Y., and Su, K. P. (2010). A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol. Psychiatry* 15, 140–147. doi: 10.1016/j.biopsych.2010.03.018

Lin, P. Y., and Su, K. P. (2007). A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *J. Clin. Psychiatry* 68, 1056–1061. doi: 10.4088/JCP.v68n0712

Luellen, B. A., Bianco, L. E., Schneider, L. M., and Andrews, A. M. (2007). Reduced brain-derived neurotrophic factor is associated with a loss of serotonergic innervation in the hippocampus of aging mice. *Gene Brain Behav.* 6, 482–490. doi: 10.1111/j.1601-183X.2006.00279.x

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., et al. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15239–15244. doi: 10.1073/pnas.96.26.15239

MacQueen, G. M., Ramakrishnan, K., Croll, S. D., Siuciak, J. A., Yu, G., Young, L. T., et al. (2001). Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav. Neurosci.* 115, 1145–1153. doi: 10.1037/0735-7044.115.5.1145

Mamounas, L. A., Altar, C. A., Blue, M. E., Kaplan, D. R., Tessarollo, L., and Lyons, W. E. (2000). BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. *J. Neurosci.* 20, 771–782. doi: 10.1523/JNEUROSCI.20-02-00771.2000

Marangell, L. B., Martinez, J. M., Zboyan, H. A., Kertz, B., Kim, H. F., and Puryear, L. J. (2003). A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. *Am. J. Psychiatry* 160, 996–998. doi: 10.1176/appi.ajp.160.5.996

Mathieu, G., Oualian, C., Denis, I., Lavialle, M., Gisquet-Verrier, P., and Vancassel, S. (2011). Dietary n-3 polyunsaturated fatty acid deprivation together with early maternal separation increases anxiety and vulnerability to stress in adult rats. *Prostaglandins Leukot Essent Fatty Acids* 85, 129–136. doi: 10.1016/j.plefa.2011.07.001

Mayor, S. (2016). Supplements such as fish oils improve antidepressant effectiveness, review finds. *BMJ* 27:i2418. doi: 10.1136/bmj.i2418

McCall, N., Mahadevia, D., Corriveau, J. A., and Glenn, M. J. (2015). Adult emotionality and neural plasticity as a function of adolescent nutrient supplementation in male rats. *Pharmacol. Biochem. Behav.* 132, 125–135. doi: 10.1016/j.pbb.2015.03.004

McGrath, J., Drummond, G., Kilkenny, C., and Wainwright, C. (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br. J. Pharmacol.* 160, 1573–1576. doi: 10.1111/j.1476-5381.2010.00873.x

McNamara, R. K., Able, J., Liu, Y., Jandacek, R., Rider, T., Tso, P., et al. (2009). Omega-3 fatty acid deficiency during perinatal development increases serotonin turnover in the prefrontal cortex and decreases midbrain tryptophan hydroxylase-2 expression in adult female rats: dissociation from estrogenic effects. *J. Psychiatr. Res.* 43, 656–663. doi: 10.1016/j.jpsychires.2008.09.011

McNamara, R. K., Hahn, C. G., Jandacek, R., Rider, T., Tso, P., Stanford, K. E., et al. (2007). Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biol. Psychiatry* 62, 17–24. doi: 10.1016/j.biopsych.2006.08.026

Mocking, R. J., Harmsen, I., Assies, J., Koeter, M. W., Ruhé, H. G., and Schene, A. H. (2016). Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Transl. Psychiatry* 6:e756. doi: 10.1038/tp.2016.29

Monteggia, L. M., Barrot, M., Powell, C. M., Berton, O., Galapis, V., Gemelli, T., et al. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10827–10832. doi: 10.1073/pnas.0402141101

Monteggia, L. M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., et al. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol. Psychiatry* 61, 187–197. doi: 10.1016/j.biopsych.2006.03.021

Montgomery, P., and Richardson, A. J. (2008). Omega-3 fatty acids for bipolar disorder. *Cochrane Database Syst Rev.* 2:CD005169. doi: 10.1002/14651858.CD005169.pub2

Moranis, A., Delpech, J. C., De Smedt-Peyrusse, V., Aubert, A., Guesnet, P., Lavialle, M., et al. (2012). Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice. *Brain Behav. Immun.* 26, 721–731. doi: 10.1016/j.bbi.2011.11.001

Murphy, D. L., and Lesch, K. P. (2008). Targeting the murine serotonin transporter: insights into human neurobiology. *Nat. Rev. Neurosci.* 9, 85–96. doi: 10.1038/nrn2284

Naveen, S., Siddalingaswamy, M., Singsit, D., and Khanum, F. (2013). Antidepressive effect of polyphenols and omega-3 fatty acid from pomegranate peel and flax seed in mice exposed to chronic mild stress. *Psychiatry Clin. Neurosci.* 67, 501–508. doi: 10.1111/pcn.12100

Nibuya, M., Morinobu, S., and Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* 15, 7539–7547. doi: 10.1523/JNEUROSCI.15-11-07539.1995

Owen, C., Rees, A. M., and Parker, G. (2008). The role of fatty acids in the development and treatment of mood disorders. *Curr. Opin. Psychiatry* 21, 19–24. doi: 10.1097/YCO.0b013e3282f29841

Page, M. E., Detke, M. J., Dalvi, A., Kirby, L. G., and Lucki, I. (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berl.)* 147, 162–167. doi: 10.1007/s002130051156

Park, Y., Moon, H. J., and Kim, S. H. (2012). N-3 polyunsaturated fatty acid consumption produces neurobiological effects associated with prevention of depression in rats after the forced swimming test. *J. Nutr. Biochem.* 23, 924–928. doi: 10.1016/j.jnutbio.2011.04.018

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., and Pieke-Dahl, S. (2006). The use of behavioral test batteries II: effect of test interval. *Physiol. Behav.* 87, 95–102. doi: 10.1016/j.physbeh.2005.09.002

Peet, M., and Horrobin, D. F. (2002). A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch. Gen. Psychiatry* 59, 913–919. doi: 10.1001/archpsyc.59.10.913

Peet, M., Murphy, B., Shay, J., and Horrobin, D. (1998). Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol. Psychiatry* 43, 315–319. doi: 10.1016/S0006-3223(97)00206-0

Pérez, M. Á., Terreros, G., and Dagnino-Subiabre, A. (2013). Long-term ω-3 fatty acid supplementation induces anti-stress effects and improves learning in rats. *Behav. Brain Funct.* 9:25. doi: 10.1186/1744-9081-9-25

Pietropaolo, S., Goubran, M. G., Joffre, C., Aubert, A., Lemaire-Mayo, V., Crusio, W. E., et al. (2014). Dietary supplementation of omega-3 fatty acids rescues fragile X phenotypes in Fmr1-Ko mice. *Psychoneuroendocrinology* 49, 119–129. doi: 10.1016/j.psyneuen.2014.07.002

Pudell, C., Vicente, B. A., Delattre, A. M., Carabelli, B., Mori, M. A., Suchecki, D., et al. (2014). Fish oil improves anxiety-like, depressive-like and cognitive behaviors in olfactory bulbectomised rats. *Eur. J. Neurosci.* 39, 266–274. doi: 10.1111/ejn.12406

Qi, X., Lin, W., Wang, D., Pan, Y., Wang, W., and Sun, M. (2009). A role for the extracellular signal-regulated kinase signal pathway in depressive-like behavior. *Behav. Brain Res.* 199, 203–209. doi: 10.1016/j.bbr.2008.11.051

Quesseveur, G., David, D. J., Gaillard, M. C., Pla, P., Wu, M. V., Nguyen, H. T., et al. (2013). BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities. *Transl. Psychiatry* 3:e253. doi: 10.1038/tp.2013.30

Quesseveur, G., Portal, B., Basile, J. A., Ezan, P., Mathou, A., Halley, H., et al. (2015). Attenuated levels of hippocampal connexin 43 and its phosphorylation correlate with antidepressant- and anxiolytic-like activities in mice. *Front. Cell Neurosci.* 9:490. doi: 10.3389/fncel.2015.00490

Rao, J. S., Ertley, R. N., Lee, H. J., DeMar, J. C. Jr., Arnold, J. T., Rapoport, S. I., et al. (2007). n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. *Mol. Psychiatry* 12, 36–46. doi: 10.1038/sj.mp.4001888

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *J. Nutr.* 27(5 Suppl.), 838S–841S. doi: 10.1093/jn/127.5.838S

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., et al. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur. J. Neurosci.* 24, 1850–1856. doi: 10.1111/j.1460-9568.2006.05059.x

Rousseau, D., Helies-Toussaint, C., Moreau, D., Raederstorff, D., and Grynberg, A. (2003). Dietary n-3 PUFAs affect the blood pressure rise and cardiac impairments in a hyperinsulinemia rat model in vivo. *Am. J. Physiol. Heart Circ. Physiol.* 285, H1294–H1302. doi: 10.1152/ajpheart.00651.2002

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., et al. (2003). Activation of TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J. Neurosci.* 23, 349–357. doi: 10.1523/JNEUROSCI.23-01-00349.2003

Sairanen, M., Lucas, G., Ernfors, P., Castren, M., and Castren, E. (2005). Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J. Neurosci.* 25, 1089–1094. doi: 10.1523/JNEUROSCI.3741-04.2005

Sakakibara, Y., Kasahara, Y., Hall, F. S., Lesch, K. P., Murphy, D. L., Uhl, G. R., et al. (2014). Developmental alterations in anxiety and cognitive behavior in serotonin transporter mutant mice. *Psychopharmacology (Berl.)* 231, 4119–4133. doi: 10.1007/s00213-014-3554-x

Schmidt, H. D., and Duman, R. S. (2007). The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatements and animal models of depressive-like behavior. *Behav. Pharmacol.* 18, 391–418. doi: 10.1097/FBP.0b013e3282ee2aa8

Schmidt, H. D., and Duman, R. S. (2010). Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology* 35, 2378–2391. doi: 10.1038/npp.2010.114

Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S., and Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.* 22, 3251–3261. doi: 10.1523/JNEUROSCI.22-08-03251.2002

Silvers, K. M., Woolley, C. C., Hamilton, F. C., Watts, P. M., and Watson, R. A. (2005). Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. *Prostaglandins Leukot Essent Fatty Acids* 72, 211–218. doi: 10.1016/j.plefa.2004.11.004

Siuciak, J. A., Boylan, C., Fritsche, M., Altar, C. A., and Lindsay, R. M. (1996). BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. *Brain Res.* 710, 11–20. doi: 10.1016/0006-8993(95)01289-3

Su, K. P., Huang, S. Y., Chiu, T. H., Huang, K. C., Huang, C. L., Chang, H. C., et al. (2008). Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychiatry* 69, 644–651. doi: 10.4088/JCP.v69n0418

Taliaz, D., Stall, N., Dar, D. E., and Zangen, A. (2010). Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Mol. Psychiatry* 15, 80–92. doi: 10.1038/mp.2009.67

Tang, M., Jiang, P., Li, H., Cai, H., Liu, Y., Gong, H., et al. (2015). Antidepressant-like effect of n-3 PUFAs in CUMS rats: role of tPA/PAI-1 system. *Physiol. Behav.* 139, 210–215. doi: 10.1016/j.physbeh.2014.11.054

Venna, V. R., Deplanque, D., Allet, C., Belarbi, K., Hamdane, M., and Bordet, R. (2009). PUFA induce antidepressant-like effects in parallel to structural and molecular changes in the hippocampus. *Psychoneuroendocrinology* 34, 199–211. doi: 10.1016/j.psyneuen.2008.08.025

Vines, A., Delattre, A. M., Lima, M. M., Rodrigues, L. S., Suchecki, D., Machado, R. B., et al. (2012). The role of 5-HT_{1A} receptors in fish oil-mediated increased BDNF expression in the rat hippocampus and cortex: a possible antidepressant mechanism. *Neuropharmacology* 62, 184–191. doi: 10.1016/j.neuropharm.2011.06.017

Wang, L., Chang, X., She, L., Xu, D., Huang, W., and Poo, M. M. (2015). Autocrine action of BDNF on dendrite development of adult-born hippocampal neurons. *J. Neurosci.* 3, 8384–8393. doi: 10.1523/JNEUROSCI.4682-14.2015

Warensjo, E., Rosell, M., Hellenius, M. L., Vessby, B., De Faire, U., and Riserus, U. (2009). Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: links to obesity and insulin resistance. *Lipids Health Dis.* 8:37. doi: 10.1186/1476-511X-8-37

Wu, A., Ying, Z., and Gomez-Pinilla, F. (2004). Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J. Neurotrauma* 21, 1457–1467. doi: 10.1089/neu.2004.21.1457

Wu, Y. Q., Dang, R. L., Tang, M. M., Cai, H. L., Li, H. D., Liao, D. H., et al. (2016). Long chain omega-3 polyunsaturated fatty acid supplementation alleviates doxorubicin-induced depressive-like behaviors and neurotoxicity in rats: involvement of oxidative stress and neuroinflammation. *Nutrients* 8:243. doi: 10.3390/nu8040243

Xu, D., Lian, D., Wu, J., Liu, Y., Zhu, M., Sun, J., et al. (2017). Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental *Streptococcus* pneumoniae meningitis. *J. Neuroinflamm.* 14:156. doi: 10.1186/s12974-017-0930-6

Yang, J., Yu, Y., Yu, H., Zuo, X., Liu, C., Gao, L., et al. (2010). The role of brain-derived neurotrophic factor in experimental inflammation of mouse gut. *Eur. J. Pain.* 14, 574–579. doi: 10.1016/j.ejpain.2009.10.007

Yuan, P., Zhou, R., Wang, Y., Li, X., Li, J., Chen, G., et al. (2010). Altered levels of extracellular signal-regulated kinase signaling proteins in post-mortem frontal cortex of individuals with mood disorders and schizophrenia. *J. Affect. Disord.* 124, 164–169. doi: 10.1016/j.jad.2009.10.017

Zhang, J. C., Wu, J., Fujita, Y., Yao, W., Ren, Q., Yang, C., et al. (2014). Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. *Int. J. Neuropsychopharmacol.* 18:pyu077.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Zemdegs, Rainer, Grossmann, Rousseau-Ralliard, Grynberg, Ribeiro and Guiard. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

BRIEF RESEARCH REPORT
published: 06 February 2019
doi: 10.3389/fnins.2019.00070

Integrin αVβ3 Function Influences Citalopram Immobility Behavior in the Tail Suspension Test

*Hope Pan, Michael R. Dohn, Rody Kingston and Ana M. D. Carneiro**

Department of Pharmacology, Nashville, TN, United States

Human studies first identified genetic and expression interactions between integrin β3 and serotonin (5-HT) transporter (SERT) genes. This association has been further strengthened by our discovery that integrin β3-containing receptors (αvβ3) physically interact with, and thereby define, a subpopulation of SERTs that may represent the main target of selective serotonin reuptake inhibitors (SSRIs). In this study, we examine how integrin αvβ3 function influences the behavioral response to the highly SSRI citalopram in the tail suspension test. Mice bearing a conditional deletion of the integrin β3 gene in neurons, or those expressing a constitutively active αvβ3 receptor, have decreased sensitivity to citalopram, when compared to wild-type littermates. To identify potential signaling pathways downstream of integrin αvβ3 that could be altered in these mouse lines, and consequently influence citalopram response *in vivo*, we performed antibody array analyses of midbrain synaptosomes isolated from mice bearing genetically altered integrin β3. We then pharmacologically targeted focal adhesion (FAK) and extracellular-signal-regulated (ERK) kinases and determined that FAK and ERK activity are critical for the actions of citalopram. Taken together, our studies have revealed a complex relationship between integrin αvβ3 function, SERT-dependent 5-HT uptake, and the effective dose of citalopram in the TST, thus implicating a role for integrin signaling pathways in the behavioral response to SSRIs.

Keywords: antidepressant, TST, signaling network, integrin, citalopram, mouse model

OPEN ACCESS

Edited by:
Nasser Haddjeri, Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Bruno Pierre Guiard, Université de Toulouse, France
Adeline Etiévant, Centre Hospitalier Universitaire de Besançon, France

****Correspondence:***
Ana M. D. Carneiro
ana.carneiro@vanderbilt.edu

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *03 October 2018*
Accepted: *23 January 2019*
Published: *06 February 2019*

Citation:
Pan H, Dohn MR, Kingston R and Carneiro AMD (2019) Integrin αVβ3 Function Influences Citalopram Immobility Behavior in the Tail Suspension Test. Front. Neurosci. 13:70. doi: 10.3389/fnins.2019.00070

INTRODUCTION

Multiple genetic and environmental factors influence antidepressant response (Keers et al., 2011; Klengel and Binder, 2013). The tail suspension test (TST) is a simple and quick paradigm with strong predictive validity for the positive therapeutic outcomes for most antidepressants, including tricyclic and selective serotonin reuptake inhibitors (SSRIs) (Cryan et al., 2005; Castagne et al., 2011). With the exception of modifications in the serotonin system, there is no obvious genetic link between alterations in immobility time in the TST by acute antidepressant administration and reduction of symptoms upon chronic SSRI treatment.

We first identified the integrin β3 subunit as a modulator of peripheral and central serotonin homeostasis via its interactions with the high-affinity serotonin transporter (SERT) (Carneiro et al., 2008; Whyte et al., 2014; Mazalouskas et al., 2015; Dohn et al., 2017). Haploinsufficiency in the murine integrin β3 gene (*Itgb3*) leads to a reduction in plasma-membrane levels of SERTs, which are the main target of SSRIs (Mazalouskas et al., 2015). These effects cause an increased potency

of citalopram and paroxetine in the TST (Mazalouskas et al., 2015). Recapitulation of a coding polymorphism in the human *ITGB3* gene (Oliver et al., 2014) by the knock-in of $Pro^{32}Pro^{33}$ in *Itgb3* also reduces SERT serotonin reuptake, via integrin αvβ3's actions on intracellular signaling pathways (Dohn et al., 2017). Studies in human and mouse models also have linked integrin β3 with antidepressant response (Fabbri et al., 2013; Probst-Schendzielorz et al., 2015; Rzezniczek et al., 2016; Oved et al., 2017).

In this study, we explore the role of integrin αvβ3 in modulating citalopram response in the TST. We capitalized on common signaling features observed in genetically altered *Itgb3* mice to identify novel pathways that can be targeted for antidepressant response in the future. These are the first studies examining the role of integrin αvβ3 in antidepressant response, beyond those focusing on the serotonin system.

MATERIALS AND METHODS

Animals

Mouse studies were performed following Vanderbilt Institutional Animal Care and Use Committee guidelines under protocols M/12/167 and M/15/014. Conditional deletion of *Itgb3* was obtained by crossing floxed *Itgb3* mice (Morgan et al., 2010) with *Nestin*-Cre mice [B6.Cg-Tg (Nes-cre)1Kln/J; Jackson Lab, #003771 (Tronche et al., 1999)], which were backcrossed five generations into C57BL/6 background. Knock-in mice used in this study were generated from crosses of heterozygous C57BL/6 mice expressing one $Pro^{32}Pro^{33}$ knock-in *Itgb3* allele (Oliver et al., 2014). All other experiments were performed on C57BL/6 mice bred in house. Mice were group-housed with their littermates, maintained on a 12-h light-dark cycle, and provided with food and water *ad libitum*. We utilized mice of both sexes (8–20 weeks of age). All experimenters were blinded to the genotypes.

Tail Suspension Test

An automated TST device (Med Associates, St. Albans, VT, United States) was used to measure the duration of behavioral immobility. Each mouse had its tail passed through a clear 3 cm plastic tube before being suspended by the tail with tape to a vertical aluminum bar connected to a strain gauge. The following settings were used in all experiments: threshold 1: 7; gain: 8; time constant: 0.25; and resolution: 200 ms. Citalopram (R/S citalopram hydrobromide; Sigma, St. Louis, MO, United States) was prepared fresh daily by dissolving the powder in 0.9% sterile saline. Drug was administered by intraperitoneal injection in a volume of 0.01 ml/g body weight and the dose was 0, 5, 20, or 30 mg/kg, calculated as the weight of the base. Mice were injected with drug or saline 30 min before a 6 min TST. Each mouse was tested two times in the TST, with 1 week between testings, which did not significant alter immobility time (**Figure 1A**). A counterbalanced design was used, where half of the animals of each genotype received citalopram in 1 week and the other half in the following week. Data was analyzed by two-way repeated measures ANOVA over the 6 min period for drug vs. genotype comparisons.

A second set of experiments tested immobility responses to citalopram in the presence of kinase inhibitors (ToCris, Minneapolis, MN, United States). Three cohorts were used: two for the FAK inhibitor PF-573228 (prepared in DMSO, diluted in saline with a final concentration of 12.5% DMSO and 2.5 mM of inhibitor) and one for the MEK inhibitor SL-327 (prepared in DMSO, diluted with saline with a final DMSO concentration of 12.5% and 1.5 mM SL-327). In these cohorts, mice received saline or citalopram via intraperitoneal injection. After 10 min, kinase inhibitor or 12.5% DMSO in saline (vehicle) were administered intranasally (2.5 μl per nostril) and were then tested in the TST after 20 min. Drugs were administered intranasally as it allows the delivery of compounds that do not cross the blood–brain barrier directly into the brain (Hanson and Frey, 2008; Hanson et al., 2013). Mice were anesthetized by inhaled isoflurane at 5% and a single volume (2.5 μl/nostril) of drug or vehicle were delivered slowly dropwise to the nares using a pipetman while the mouse was in a supine position. Each mouse was randomly assigned to a combination of saline/vehicle, saline/inhibitor, citalopram/vehicle or citalopram/inhibitor for week 1 and another combination for testing on a second week. In these experiments, data was analyzed by a two-way ANOVA and group comparisons were performed using Bonferroni corrections. Detailed statistical results showing $F_{(DFn,DFd)}$ and P values for each experiment are described in the figure legends.

Marble Burying

A novel cage was prepared with a layer of Harlan T.7089 Diamond Soft bedding (Harlan Laboratories, Indianapolis, IN, United States) covering the floor. This layer was 3 cm thick to allow burying of glass marbles of 1.5 cm diameter. Each mouse was removed from the TST apparatus and allowed to acclimate in the novel cage for 30 min. Following the acclimation period, the mouse was briefly removed from the novel cage, and 20 blue glass marbles were placed in a four-by-five grid on top of the bedding, with each marble spaced 2 cm apart. The mouse was then returned to the novel cage and given 30 additional minutes to explore and interact with the marbles without interference. After this period, the number of marbles buried was quantified. Data was analyzed using two-way ANOVA and post-tests were corrected for multiple testing using Bonferroni. Detailed statistics are presented in the figure legends.

Isolation of Presynaptic Boutons and Antibody Array

Each array experiment consisted of two mice, one genetically modified (*Nestin*-Cre cKO or $Pro^{32}Pro^{33}$ *Itgb3* KI) and one wild-type littermate, euthanized for dissections of midbrains. A total of three biological replicates were performed for cKO mice, and two for KI mice. Each array was completed in 1 day, including synaptosomal preparation, protein labeling, and incubation with microarray slide, and imaged the following day. We utilized a commercially available microarray kit (Panorama® Antibody Microarray (Cat. Number CSAA1), Sigma-Aldrich; St. Louis,

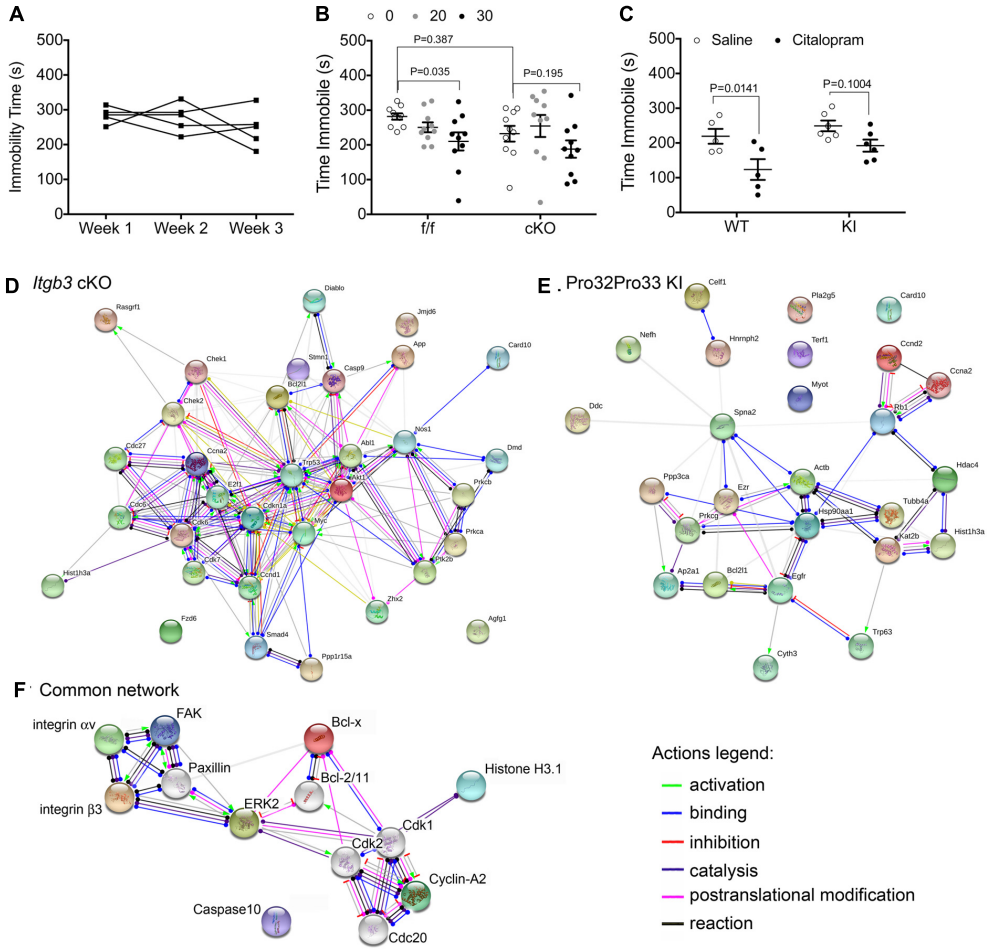


FIGURE 1 | Genetic alterations in integrin αvβ3 function disrupt citalopram responses in the TST. **(A)** Effects of repeated TST testing on immobility time in C57BL/6 mice. Mice were tested after injection with saline for 6 min in the TST. Experiment was repeated once weekly, for three consecutive weeks. Repeated measures one-way ANOVA: Time $F_{(1.647,6.590)} = 1.064$, $P = 0.3825$; Individual (between rows) $F_{(4,8)} = 0.7768$, $P = 0.5703$. **(B)** Citalopram dose–response curve in floxed *Itgb3* lacking or expressing Cre under the control of the *Nestin* promoter (cKO). Two-way repeated measures (RM) ANOVA citalopram effect: $F_{(2,36)} = 6.172$, $P = 0.005$; genotype effect: $F_{(1,18)} = 0.8719$, $P = 0.3628$; interaction effect: $F_{(2,36)} = 1.057$, $P = 0.379$; subject (matching): $F_{(18,36)} = 2.597$, $P = 0.0072$. Bonferroni-corrected post-tests: f/f: saline vs. 30 mg/kg: $P = 0.035$, $N = 10$; cKO: saline vs. 30 mg/kg: $P = 0.195$, $N = 10$. Saline f/f vs. cKO: $P = 0.387$. **(C)** Immobility time in mice expressing $Ser^{32}Gln^{33}$ (WT) or $Pro^{32}Pro^{33}$ (KI) integrin β3 after dosing intraperitoneally (IP) with 30 mg/kg citalopram or saline control. Two-way repeated measures (RM) ANOVA citalopram effect: $F_{(1,9)} = 16.70$, $P = 0.0027$; genotype effect: $F_{(1,9)} = 4.557$, $P = 0.0615$; interaction effect: $F_{(1,9)} = 1.081$, $P = 0.3257$; subject (matching): $F_{(9,9)} = 1.536$, $P = 0.2664$. Bonferroni-corrected post-tests: WT: saline vs. 30 mg/kg: $P = 0.0141$, $N = 5$; KI: saline vs. 30 mg/kg: $P = 0.1004$, $N = 6$. **(D–F)** Schematic diagrams of protein networks identified in kinome studies. Synaptosomes were isolated from *Nestin*Cre and floxed littermates **(D)** or WT and KI littermates **(E)**, and protein extracts were analyzed using antibody microarrays. Target proteins were converted to mouse gene codes for input into the online STRING software, where network analysis was performed. Shown here are action outputs where each line linking gene nodes denotes a molecular action, as depicted in the legends. **(D)** Network linking gene products with altered expression or phosphorylation levels between *Nestin*Cre and floxed littermates. **(E)** Network of gene products that are altered in KI samples when compared to wild-type littermates. **(F)** Network of proteins that are commonly modified by genetic alterations in *Itgb3*. In this diagram, we replaced *Mus musculus* gene names by protein names for clarity. Colored nodes, including both subunits of the integrin αvβ3 receptor, FAK, and ERK2, were added during input. Nodes shown in white were added by STRING.

MO, United States) and followed the instructions for protein extraction, labeling, hybridization, and analysis as provided by the manufacturer. Synaptosomes were prepared as described previously (Phillips et al., 2001). Detailed description of the antibody array procedures can be found in the **Supplementary Material**. In the first biological replicate genetically modified samples were labeled with Cy3, whereas the wild-type control was labeled with Cy5. In the following replicate, the labels were reversed to compensate potential bias of binding of Cy3 and Cy5 to the protein samples. After incubation with proteins samples, the slides were washed and scanned using an Odyssey® Imaging system (LI-COR Biotechnology, Lincoln, NE, United States). Images of scanned antibody microarrays were gridded and linked to a protein print list. A blinded reviewer identified missing spots and background signal. Two levels of normalization were used: log ratios of Cy5 to Cy3 were determined between array replicates to determine whether there was a bias for the fluorophore (e.g., compare cKO Cy3 and cKO Cy5 from two experiments). Then,

within each biological replicate, data was normalized to GAPDH fluorescence intensity. The Cy5 results were divided by the Cy3 results for each individual protein; proteins of interest were identified by a Cy5/Cy3 ratio higher than 2 or lower than 0.5 in all biological replicates. The list of proteins was converted into a list of *Mus musculus* genes for network analysis in STRING[1] using the actions output.

RESULTS

Genetic Alteration in *Itgb3* Prevents Citalopram From Reducing Immobility Time in the TST

To examine the role of integrin αvβ3 on the citalopram response in the TST, we generated mice lacking integrin β3 expression in neuronal and glial precursors in the brain (cKO). A dose–response curve for citalopram revealed that, while 30 mg/kg citalopram elicited decreases in immobility in floxed littermates, no reductions in immobility time were observed in cKO mice (**Figure 1B**). We then tested the effects of 30 mg/kg citalopram on mice expressing either $Ser^{32}Gln^{33}$ (WT) or $Pro^{32}Pro^{33}$ (KI) integrin β3, as the latter present alterations in the serotonin system (Dohn et al., 2017). No significant reductions in immobility were observed in KI mice, whereas citalopram reduced immobility times in WT controls (**Figure 1C**).

Kinome Analysis of Synaptosomes Isolated From cKO and KI *Itgb3* Mice

As both genetic deletion of *Itgb3* in the brain and constitutive activation of integrin αvβ3 led to diminished sensitivity to citalopram in the TST, we hypothesized that this acute response to citalopram depends on common signaling pathways modified in both mouse lines. To identify potential signaling pathways that are commonly altered by integrin αvβ3 loss- or gain-of-function, we performed kinome analysis using antibody arrays. We utilized a commercially available antibody microarray that allows for simultaneous quantification of phosphorylated and non-phosphorylated proteins in control and target groups (Kopf et al., 2005). Pathway analysis comparing cKO and floxed *Itgb3* controls (**Figure 1D**) revealed enrichment in proteins involved in the regulation of cell cycle (GO: 0051726. False discovery rate $P = 6.59^{-13}$: *Abl1*, *Akt1*, *App*, *Bcl2l1*, *Ccnd1*, *Ccna2*, *Cdkn1a*, *Cdc6*, *Cdc27*, *Cdk6*, *Cdk7*, *Check1*, *Check2*, *E2f1*, *Myc1*, *Prkca*, *Trp53*), some of which are also involved in intracellular signal transduction (GO: 0035556. False discovery rate: 2.09^{-10}: *Abl1*, *Akt1*, *Card10*, *Ccna2*, *Casp9*, *Chek2*, *Diablo*, *Dmd*, *E2f1*, *Myc*, *Nos1*, *Prkca*, *Ptk2b*, *Rasgrf1*, and *Smad4*). The analysis of proteins altered by constitutive activation of αvβ3 revealed 25 gene products altered in KI samples, 11 of which participate in macromolecular subunit organization (**Figure 1E**. GO: 0043933. False discovery rate: 0.000453: *Actb*, *Ezr*, *Hdac4*, *Hist1h3a*, *Hsp90aa1*, *Kat2b*, *Tubb4a*, *Trp63*, and *Rb* form a protein complex, whereas *Terf1*, *Nefh*, and *Celf1* do not participate in a macromolecular complex).

[1]https://string-db.org

The list of common proteins altered by both gain- and loss-of-function in integrin αvβ3 consists of: Bcl-x (encoded by *Bcl2l1*), caspase recruitment domain family member 10 (*Card10*), cyclin A2 (*Ccna2*) and histone H3.1 (*Hist1h3a*), which do not, as a group, consist of a single signaling pathway. To identify potential kinases that are proximal to integrin αvβ3 that could alter the proteins identified by the antibody array, we added *Itgav* and *Itgb3* and *Ptk2* (which encodes for FAK, the downstream focal adhesion kinase) and allowed STRING to add up to 10 nodes, generating the network shown in **Figure 1F**. In addition to FAK, paxillin and ERK2 (mitogen activated protein kinase 1, encoded by *Mapk1*) were necessary nodes linking the integrin αvβ3 receptor and the cell cycle proteins identified in the antibody arrays. Thus, we targeted those pathways in the behavioral experiments that followed.

Inhibition of FAK and ERK Prevents Citalopram From Reducing Immobility Time in the TST

Immediately downstream of integrin αvβ3 activation lies FAK recruitment to focal adhesions, phosphorylation, and activation, which are all necessary steps for ERK activation. Therefore, we tested whether inhibition of FAK by intranasal administration of PF-573228 (2.5 μl per nostril at 2.5 mM) could potentiate a suboptimal dose of citalopram in C57BL/6 mice. When administered intraperitoneally, neither 15 mg/kg citalopram (Crowley et al., 2005, 2006) or PF-573228 administration had effects on immobility time (**Figure 2A**). To examine whether these negative results were due to an inability of the drugs to reach the central nervous system, we exposed mice to the marble burying test. In this test, intranasal PF-573228 significantly decreased the number of marbles buried when compared to vehicle alone without enhancing the effect of citalopram (**Figure 2B**). These data suggest that FAK inhibition may have anxiolytic effects, but not antidepressant effects when tested in a behavior despair paradigm.

We then examined the potential for inhibition of FAK to influence the effective dose of citalopram (30 mg/kg). In this paradigm, we observed that intranasal PF-573228 administration prevented citalopram from reducing time immobile in the TST, recapitulating the effects observed in genetically modified *Itgb3* mice (**Figure 2C**).

We then examined whether downregulation of ERK1/2 alter citalopram responses by the inhibiting upstream kinase MEK1 with SL-327 (2.5 μl/nostril at 1.5 mM, or vehicle). We observed that mice dosed with citalopram had a significant reduction in immobility time, whereas those dosed with both SL-327 and citalopram had no alterations in immobility time, when compared to vehicle controls (**Figure 2D**). Taken together, these data indicate that inhibition of either FAK or ERK signaling pathways prevent the positive actions of citalopram in the TST.

DISCUSSION

Here we provide evidence that appropriate integrin αvβ3 function is necessary for citalopram response

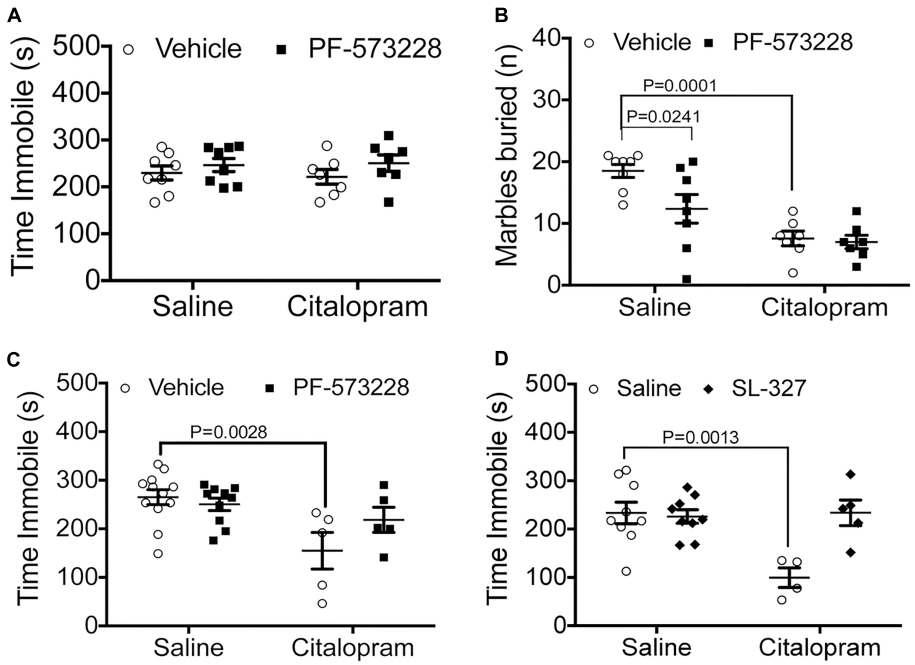


FIGURE 2 | Pharmacological manipulation of FAK and ERK alter the efficacy of cirtalopram in the TST. **(A,B)** Mice were dosed IN with the FAK inhibitor PF-573228 (2.5 mM) or saline 10 min after receiving an IP injection with a sub-optimal dose of citalopram (15 mg/kg). **(A)** FAK inhibition on immobility time in the TST. Two-way ANOVA: citalopram effect: $F_{(1,28)} = 0.6715$, $P = 0.4195$; PF-573228 effect: $F_{(1,28)} = 0.4201$, $P = 0.5222$; interaction effect: $F_{(1,28)} = 0.1139$, $P = 0.7383$. **(B)** FAK inhibition on marble burying behavior. Two-way ANOVA interaction effect: $F_{(1,26)} = 3.161$, $P = 0.0871$; FAK inhibitor effect: $F_{(1,26)} = 4.595$, $P = 0.0416$; Citalopram effect: $F_{(1,26)} = 27.24$, $P < 0.0001$. Bonferroni-corrected post-tests: $saline_{IP}$ + $vehicle_{IN}$ vs. $citalopram_{IP}$ + $vehicle_{IN}$: $P = 0.0001$, $Saline_{IP}$ + $vehicle_{IN}$ vs. $saline_{IP}$ + $PF\text{-}573228_{IN}$: $P = 0.0241$, $Saline_{IP}$ + $vehicle_{IN}$ vs. citalopram + $PF\text{-}573228_{IN}$: $P = 0 < 0.0001$. Number of mice for **(A,B)**: $Saline_{IP}$ + $vehicle_{IN}$ $N = 8$; $citalopram_{IP}$ + $vehicle_{IN}$ $N = 7$; $Saline_{IP}$ + PF-573228 $N = 8$; $Citalopram_{IP}$ + $PF\text{-}573228_{IN}$ $N = 7$. **(C)** Citalopram response (30 mg/kg) in the TST was measured after intranasal (IN) administration of the FAK inhibitor PF-573228 (2.5 mM). Two-way ANOVA interaction effect: $F_{(1,28)} = 3.357$, $P = 0.0776$; FAK inhibitor effect: $F_{(1,28)} = 1.300$, $P = 0.2639$; citalopram effect: $F_{(1,28)} = 11.07$, $P = 0.0025$. Bonferroni post-tests: $Saline_{IP}$ + $vehicle_{IN}$ vs. $citalopram_{IP}$ + $vehicle_{IN}$: $P = 0.0028$. Number of animals: $saline_{IP}$ + $vehicle_{IN}$ $N = 12$; $citalopram_{IP}$ + $vehicle_{IN}$ $N = 5$; $saline_{IP}$ + PF-573228 $N = 10$; $Citalopram_{IP}$ + $PF\text{-}573228_{IN}$ $N = 5$. **(D)** Citalopram response in the TST was measured after intranasal (IN) administration of the MEK inhibitor SL-327 (1.5 mM). Two-way ANOVA interaction effect: $F_{(1,23)} = 10.06$, $P = 0.0043$; MEK inhibitor effect: $F_{(1,23)} = 8.059$, $P = 0.0093$; citalopram effect: $F_{(1,23)} = 7.989$, $P = 0.0096$. Bonferroni-corrected post-tests: $Saline_{IP}$ + $vehicle_{IN}$ vs. $citalopram_{IP}$ + $vehicle_{IN}$: $P = 0.0013$. Number of animals: $saline_{IP}$ + $vehicle_{IN}$ $N = 9$; $citalopram_{IP}$ + $vehicle_{IN}$ $N = 4$; $saline_{IP}$ + $SL\text{-}327_{IN}$ $N = 9$; $citalopram_{IP}$ + $SL\text{-}327_{IN}$ $N = 5$.

in the TST. We show that genetic alteration in the murine integrin β3 gene (*Itgb3*) and inhibition of signaling pathways downstream of integrin αvβ3 prevent citalopram from reducing immobility time in this *in vivo* model.

The genetic models utilized in this study differentially alter integrin function in the brain: the *Nestin*Cre conditional knockout line (cKO) eliminates integrin αvβ3 activity, whereas the $Pro^{32}Pro^{33}$ knock-in line (KI) has constitutively activated FAK-dependent signaling (Dohn et al., 2017). Elevated FAK phosphorylation in serotonergic synapses in the $Pro^{32}Pro^{33}$ line likely results in reduced focal adhesion turnover, which could lead to reduced neuronal motility or synapse formation/pruning that occur during development (Beggs et al., 2003; Xie et al., 2003; Rico et al., 2004; Xie and Tsai, 2004; Chacon et al., 2012; An et al., 2018). As both these lines have in common the loss of citalopram response in the TST, either dynamic activation of integrin αvβ3 signaling or integrin αvβ3-dependent circuit formation is necessary for the increased fighting response triggered by citalopram. We tested the former hypothesis by first identifying pathways that are altered in both mouse lines, followed by acute inhibition of kinases that are converging nodes in the cKO/KI signaling pathways. We exposed mice to citalopram, followed by FAK or MEK inhibitors, and observed no antidepressant response in the TST. Although the role of ERK in stress response has been examined, where ERK phosphorylation is enhanced upon exposure to the TST (Iniguez et al., 2010; Galeotti and Ghelardini, 2012; Leem et al., 2014) and ERK phosphorylation is modified with antidepressant use (Carlini et al., 2012; Licznerski and Duman, 2013), few studies have examined the involvement of ERK phosphorylation in the actions of behavior paradigms in response to antidepressants (Zeni et al., 2012). Importantly, these inhibitors had no effects on their own, indicating that FAK and ERK modulate citalopram response, but do not exert antidepressant effects by themselves. Finally, these results suggest that TST immobility is altered by integrin αvβ3 pathways in a SERT-independent fashion, and may reveal more effective targets.

The significance of these studies is thus far limited to citalopram responses in this acute measurement of behavior despair. Still, understanding the relationship between the acute

effects of pharmacological treatment in the TST and the chronic effects of these drugs in the clinical setting is important to identify novel molecular targets that may be more efficacious in treatment of mood disorders. Many of the studies targeted at revealing the genetic basis for antidepressant response have yielded few results. Single-cell analysis of peripheral cells have pointed to alterations in multiple signaling pathways (Lago et al., 2018), and pharmacogenomics of antidepressant response fail to generate consistent results (Fabbri et al., 2018; Madsen et al., 2018; Rosenblat et al., 2018). Here, we propose that taking into consideration the role of serotonin in mood disorders, and utilizing a murine model with strong predictive value, can reveal molecular targets that may have higher efficacy in the clinic.

AUTHOR CONTRIBUTIONS

HP and RK performed experiments and analyzed data. AC and MD designed and performed experiments, analyzed data, and wrote the manuscript.

FUNDING

HP was supported by the Vanderbilt University Interdisciplinary Program in Neuroscience for Undergraduates. RK was supported by a Leadership Alliance Summer Fellowship. This study was supported by the grant MH090256 (AC, MD).

ACKNOWLEDGMENTS

We would like to acknowledge John Allison for assistance with training our students in behavioral tests and general administration and organization of the Vanderbilt Murine Neurobehavioral Core.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00070/full#supplementary-material

REFERENCES

An, L., Li, W., Hu, X., Zhang, W., and Zhao, S. (2018). Abundant focal adhesion kinase causes aberrant neuronal migration via its phosphorylation at Tyr925. *J. Mol. Neurosci.* 64, 102–110. doi: 10.1007/s12031-017-1010-1

Beggs, H. E., Schahin-Reed, D., Zang, K., Goebbels, S., Nave, K. A., Gorski, J., et al. (2003). FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron* 40, 501–514. doi: 10.1016/S0896-6273(03)00666-4

Carlini, V. P., Poretti, M. B., Rask-Andersen, M., Chavan, R. A., Ponzio, M. F., Sawant, R. S., et al. (2012). Differential effects of fluoxetine and venlafaxine on memory recognition: possible mechanisms of action. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 38, 159–167. doi: 10.1016/j.pnpbp.2012.03.004

Carneiro, A. M., Cook, E. H., Murphy, D. L., and Blakely, R. D. (2008). Interactions between integrin alphaIIbbeta3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J. Clin. Invest.* 118, 1544–1552. doi: 10.1172/JCI33374

Castagne, V., Moser, P., Roux, S., and Porsolt, R. D. (2011). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr. Protoc. Neurosci.* 55, 8.10A.1–A.14. doi: 10.1002/0471142301.ns0810as55

Chacon, M. R., Navarro, A. I., Cuesto, G., del Pino, I., Scott, R., Morales, M., et al. (2012). Focal adhesion kinase regulates actin nucleation and neuronal filopodia formation during axonal growth. *Development* 139, 3200–3210. doi: 10.1242/dev.080564

Crowley, J. J., Blendy, J. A., and Lucki, I. (2005). Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology* 183, 257–264. doi: 10.1007/s00213-005-0166-5

Crowley, J. J., Brodkin, E. S., Blendy, J. A., Berrettini, W. H., and Lucki, I. (2006). Pharmacogenomic evaluation of the antidepressant citalopram in the mouse tail suspension test. *Neuropsychopharmacology* 31, 2433–2442. doi: 10.1038/sj.npp.1301065

Cryan, J. F., Mombereau, C., and Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci. Biobehav. Rev.* 29, 571–625. doi: 10.1016/j.neubiorev.2005.03.009

Dohn, M. R., Kooker, C. G., Bastarache, L., Jessen, T., Rinaldi, C., Varney, S., et al. (2017). The gain-of-function integrin beta3 Pro33 variant alters the serotonin system in the mouse brain. *J. Neurosci.* 37, 11271–11284. doi: 10.1523/JNEUROSCI.1482-17.2017

Fabbri, C., Di Girolamo, G., and Serretti, A. (2013). Pharmacogenetics of antidepressant drugs: an update after almost 20 years of research. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 162, 487–520. doi: 10.1002/ajmg.b.32184

Fabbri, C., Tansey, K. E., Perlis, R. H., Hauser, J., Henigsberg, N., Maier, W., et al. (2018). New insights into the pharmacogenomics of antidepressant response from the GENDEP and STAR*D studies: rare variant analysis and high-density imputation. *Pharmacogenomics J.* 18, 413–421. doi: 10.1038/tpj.2017.44

Galeotti, N., and Ghelardini, C. (2012). Regionally selective activation and differential regulation of ERK, JNK and p38 MAP kinase signalling pathway by protein kinase C in mood modulation. *Int. J. Neuropsychopharmacol.* 15, 781–793. doi: 10.1017/S1461145711000897

Hanson, L. R., Fine, J. M., Svitak, A. L., and Faltesek, K. A. (2013). Intranasal administration of CNS therapeutics to awake mice. *J. Vis. Exp.* e4440. doi: 10.3791/4440

Hanson, L. R., and Frey, W. H. II (2008). Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. *BMC Neurosci.* 9(Suppl. 3):S5. doi: 10.1186/1471-2202-9-S3-S5

Iniguez, S. D., Vialou, V., Warren, B. L., Cao, J. L., Alcantara, L. F., Davis, L. C., et al. (2010). Extracellular signal-regulated kinase-2 within the ventral tegmental area regulates responses to stress. *J. Neurosci.* 30, 7652–7663. doi: 10.1523/JNEUROSCI.0951-10.2010

Keers, R., Uher, R., Huezo-Diaz, P., Smith, R., Jaffee, S., Rietschel, M., et al. (2011). Interaction between serotonin transporter gene variants and life events predicts response to antidepressants in the GENDEP project. *Pharmacogenomics J.* 11, 138–145. doi: 10.1038/tpj.2010.14

Klengel, T., and Binder, E. B. (2013). Gene × environment interactions in the prediction of response to antidepressant treatment. *Int. J. Neuropsychopharmacol* 16, 701–711. doi: 10.1017/S1461145712001459

Kopf, E., Shnitzer, D., and Zharhary, D. (2005). Panorama Ab microarray cell signaling kit: a unique tool for protein expression analysis. *Proteomics* 5, 2412–2416. doi: 10.1002/pmic.200401305

Lago, S. G., Tomasik, J., van Rees, G. F., Ramsey, J. M., Haenisch, F., Cooper, J. D., et al. (2018). Exploring the neuropsychiatric spectrum using high-content functional analysis of single-cell signaling networks. *Mol. Psychiatry* doi: 10.1038/s41380-018-0123-4 [Epub ahead of print].

Leem, Y. H., Yoon, S. S., Kim, Y. H., and Jo, S. A. (2014). Disrupted MEK/ERK signaling in the medial orbital cortex and dorsal endopiriform nuclei of the

prefrontal cortex in a chronic restraint stress mouse model of depression. *Neurosci. Lett.* 580, 163–168. doi: 10.1016/j.neulet.2014.08.001

Licznerski, P., and Duman, R. S. (2013). Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression. *Neuroscience* 251, 33–50. doi: 10.1016/j.neuroscience.2012.09.057

Madsen, M. B., Kogelman, L. J. A., Kadarmideen, H. N., and Rasmussen, H. B. (2018). Systems genetics analysis of pharmacogenomics variation during antidepressant treatment. *Pharmacogenomics J.* 18, 144–152. doi: 10.1038/tpj.2016.68

Mazalouskas, M., Jessen, T., Varney, S., Sutcliffe, J. S., Veenstra-VanderWeele, J., Cook, E. H., et al. (2015). Integrin beta3 haploinsufficiency modulates serotonin transport and antidepressant-sensitive behavior in mice. *Neuropsychopharmacology* 40, 2015–2024. doi: 10.1038/npp.2015.51

Morgan, E. A., Schneider, J. G., Baroni, T. E., Uluckan, O., Heller, E., Hurchla, M. A., et al. (2010). Dissection of platelet and myeloid cell defects by conditional targeting of the beta3-integrin subunit. *FASEB J.* 24, 1117–1127. doi: 10.1096/fj.09-138420

Oliver, K. H., Jessen, T., Crawford, E. L., Chung, C. Y., Sutcliffe, J. S., and Carneiro, A. M. (2014). Pro32Pro33 mutations in the integrin beta3 PSI domain result in alphaIIbbeta3 priming and enhanced adhesion: reversal of the hypercoagulability phenotype by the Src inhibitor SKI-606. *Mol. Pharmacol.* 85, 921–931. doi: 10.1124/mol.114.091736

Oved, K., Farberov, L., Gilam, A., Israel, I., Haguel, D., Gurwitz, D., et al. (2017). MicroRNA-mediated regulation of *ITGB3* and *CHL1* is implicated in SSRI action. *Front. Mol. Neurosci.* 10:355. doi: 10.3389/fnmol.2017.00355

Phillips, G. R., Huang, J. K., Wang, Y., Tanaka, H., Shapiro, L., Zhang, W., et al. (2001). The presynaptic particle web: ultrastructure, composition, dissolution, and reconstitution. *Neuron* 32, 63–77. doi: 10.1016/S0896-6273(01)00450-0

Probst-Schendzielorz, K., Scholl, C., Efimkina, O., Ersfeld, E., Viviani, R., Serretti, A., et al. (2015). *CHL1*, *ITGB3* and *SLC6A4* gene expression and antidepressant drug response: results from the Munich Antidepressant Response Signature (MARS) study. *Pharmacogenomics* 16, 689–701. doi: 10.2217/pgs.15.31

Rico, B., Beggs, H. E., Schahin-Reed, D., Kimes, N., Schmidt, A., and Reichardt, L. F. (2004). Control of axonal branching and synapse formation by focal adhesion kinase. *Nat. Neurosci.* 7, 1059–1069. doi: 10.1038/nn1317

Rosenblat, J. D., Lee, Y., and McIntyre, R. S. (2018). The effect of pharmacogenomic testing on response and remission rates in the acute treatment of major depressive disorder: a meta-analysis. *J. Affect Dis.* 241, 484–491. doi: 10.1016/j.jad.2018.08.056

Rzezniczek, S., Obuchowicz, M., Datka, W., Siwek, M., Dudek, D., Kmiotek, K., et al. (2016). Decreased sensitivity to paroxetine-induced inhibition of peripheral blood mononuclear cell growth in depressed and antidepressant treatment-resistant patients. *Transl. Psychiatry* 6:e827. doi: 10.1038/tp.2016.90

Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P. C., et al. (1999). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat. Genet.* 23, 99–103. doi: 10.1038/12703

Whyte, A., Jessen, T., Varney, S., and Carneiro, A. M. (2014). Serotonin transporter and integrin beta 3 genes interact to modulate serotonin uptake in mouse brain. *Neurochem. Int.* 73, 122–126. doi: 10.1016/j.neuint.2013.09.014

Xie, Z., Sanada, K., Samuels, B. A., Shih, H., and Tsai, L. H. (2003). Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. *Cell* 114, 469–482. doi: 10.1016/S0092-8674(03)00605-6

Xie, Z., and Tsai, L. H. (2004). Cdk5 phosphorylation of FAK regulates centrosome-associated miocrotubules and neuronal migration. *Cell Cycle* 3, 108–110. doi: 10.4161/cc.3.2.646

Zeni, A. L., Zomkowski, A. D., Maraschin, M., Rodrigues, A. L., and Tasca, C. I. (2012). Involvement of PKA, CaMKII, PKC, MAPK/ERK and PI3K in the acute antidepressant-like effect of ferulic acid in the tail suspension test. *Pharmacol. Biochem. Behav.* 103, 181–186. doi: 10.1016/j.pbb.2012.08.020

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Pan, Dohn, Kingston and Carneiro. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

REVIEW
published: 12 February 2019
doi: 10.3389/fnins.2019.00091

Multifaceted Regulations of the Serotonin Transporter: Impact on Antidepressant Response

Anne Baudry[1,2], Mathea Pietri[1,2], Jean-Marie Launay[3,4], Odile Kellermann[1,2] and Benoit Schneider[1,2]*

[1] INSERM UMR-S 1124, Paris, France, [2] Université Paris Descartes, Sorbonne Paris Cité, UMR-S 1124, Paris, France, [3] Hôpital Lariboisière, AP-HP, INSERM UMR-S 942, Paris, France, [4] Pharma Research Department, Hoffmann-La Roche Ltd., Basel, Switzerland

Serotonin transporter, SERT (*SLC64A* for solute carrier family 6, member A4), is a twelve transmembrane domain (TMDs) protein that assumes the uptake of serotonin (5-HT) through dissipation of the Na^+ gradient established by the electrogenic pump Na/K ATPase. Abnormalities in 5-HT level and signaling have been associated with various disorders of the central nervous system (CNS) such as depression, obsessive-compulsive disorder, anxiety disorders, and autism spectrum disorder. Since the 50s, SERT has raised a lot of interest as being the target of a class of antidepressants, the Serotonin Selective Reuptake Inhibitors (SSRIs), used in clinics to combat depressive states. Because of the refractoriness of two-third of patients to SSRI treatment, a better understanding of the mechanisms regulating SERT functions is of priority. Here, we review how genetic and epigenetic regulations, post-translational modifications of SERT, and specific interactions between SERT and a set of diverse partners influence SERT expression, trafficking to and away from the plasma membrane and activity, in connection with the neuronal adaptive cell response to SSRI antidepressants.

Keywords: SERT, SSRIs, microRNAs, trafficking, phosphorylation, Na/K ATPase

OPEN ACCESS

Edited by:
Thorsten Lau,
Central Institute of Mental Health, Germany

Reviewed by:
Citlali Trueta,
Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM), Mexico
Augusto Pasini,
University of Rome Tor Vergata, Italy

****Correspondence:***
Odile Kellermann
odile.kellermann@parisdescartes.fr

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *01 October 2018*
Accepted: *25 January 2019*
Published: *12 February 2019*

Citation:
Baudry A, Pietri M, Launay J-M, Kellermann O and Schneider B (2019) Multifaceted Regulations of the Serotonin Transporter: Impact on Antidepressant Response. Front. Neurosci. 13:91. doi: 10.3389/fnins.2019.00091

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) signaling in the central nervous system (CNS) modulates several physiological functions, including sleep, mood, anxiety, appetite, cognition as well as memory, and perception (for review see Olivier, 2015 and references therein), and in the periphery gut and platelet functions (Mercado and Kilic, 2010; Mawe and Hoffman, 2013). The precise extent and temporal dynamics of 5-HT signaling depend on control mechanisms that notably rely on the clearance of the released neurotransmitter by the high affinity serotonin transporter (SERT or 5-HTT), and to a lesser extent, the low affinity, high capacity organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT) at the cell surface, and the vesicular monoamine transporter (VMAT) in storage granules (Amphoux et al., 2006; Koepsell et al., 2007; Duan and Wang, 2010; Matthaeus et al., 2015).

Serotonin transporter (*SLC64A* for solute carrier family 6, member A4) belongs to the *SLC6* gene super family of Na^+/Cl^--dependent transporters. The SERT encoding gene was first cloned from rat brain and basophilic leukemia cells in 1991 (Blakely et al., 1991; Hoffman et al., 1991). Two years after, the human SERT gene was cloned: it is present on chromosome 17q11.2 and contains 14/15

exons spanning around 40 kb (Ramamoorthy et al., 1993). In 1992, SERT protein was purified to homogeneity from human platelets (Launay et al., 1992). SERT is a 12 transmembrane domain (TMDs) protein containing two sites of N-linked glycosylation (Launay et al., 1992; **Figure 1**). This transporter is mainly located in cholesterol-rich membrane microdomains, also called lipid-rafts that act as platforms for the regulated assembly and functioning of signaling receptors and transporters (Allen et al., 2007). The N- and C-terminal regions of SERT dip into the cytosol and interact with several proteins that define, at least in part, the localization, stability and activity of SERT. Cytoplasmic domains located between TMDs also contain sites of post-translational modifications, showing that 5-HT transport is a highly regulated process.

Serotonin transporter assumes the active co-uptake of 5-HT and Cl^- ion using as the energy force the Na^+ gradient created by the plasma membrane Na/K ATPase (Rudnick, 1977). It is generally admitted that SERT-mediated uptake of one 5-HT (a monovalent cation at physiological pH) with one Na^+ and one Cl^- is electroneutral as the transport of the transmitter and ions is coupled to the efflux of one K^+ ion (Rudnick and Nelson, 1978). However, SERT-mediated 5-HT uptake was shown to generate currents and to be electrogenic. This means the fixed stoichiometry of 5-HT and ions is probably not the only valid model for 5-HT transport and SERT may display ion channel-like property (for review, see De Felice, 2016 and references therein). In any case, in serotonergic neurons, serotonin uptaken by SERT adds to that synthesized *de novo* and increases the intracellular neurotransmitter pool. Three-Dimensional-Quantitative Structure-Activity Relationships studies allowed to show that SERT selectively uptakes specific 5-HT conformers with anti, −gauche and +gauche side-chain conformation, and to identify chemical determinants of the 5-HT molecule critical for 5-HT interaction with SERT (Pratuangdejkul et al., 2005, 2008).

Dysregulation of 5-HT signaling has been linked to several CNS-associated disorders such as depression, obsessive-compulsive disorder, anxiety disorders, and autism spectrum disorder (Murphy et al., 2004; Murphy and Lesch, 2008). Intensive researches have been carried out from the 50s to develop therapeutic compounds that antagonize SERT activity in order to maintain a tonic concentration of 5-HT at the synapse and/or in the surrounding milieu of serotonergic neurons. A particularity of the serotonergic system is to release 5-HT from extrasynaptic sites, the soma (Kaushalya et al., 2008; Trueta et al., 2012) and neuritic varicosities (Tork, 1990). 5-HT then acts as a volume transmitter involved in paracrine neuromodulation effects (Fuxe et al., 2007). In this context, drugs targeting SERT are serotonin reuptake inhibitors such as tricyclic inhibitors (e.g., imipramine), selective serotonin reuptake inhibitors (SSRIs, e.g., paroxetine and fluoxetine [Prozac®]), or compounds that lead to transport reversal (e.g., drugs of abuse such as amphetamines derivatives like MDMA, "ectasy"). However, the 3-week delay for beneficial effects of SSRIs treatment in patients with a depressive disorder [(Machado-Vieira et al., 2010) and references therein], and the fact that two thirds of the patients do not respond to antidepressants (Fava, 2003; Fekadu et al., 2009), reflect complex regulatory mechanisms of SERT that deserve considerations to better understand the functioning of SERT molecule and refine the options that would modulate 5-HT clearance by SERT in patients suffering from neuropsychiatric disorders. We will here review our current knowledge of the mechanisms that contribute to regulation of SERT activity and how specific regulation pathways render SERT responsive to antidepressant action.

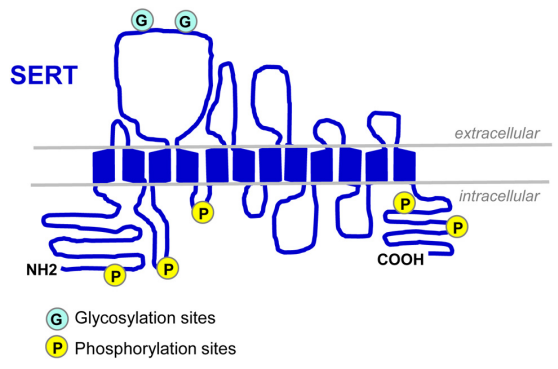


FIGURE 1 | Schematic representation of SERT. SERT protein displays two N-linked glycosylation sites in the extracellular loop 2 (Launay et al., 1992; Tate and Blakely, 1994) and several sites of phosphorylation located in the N- and C-termini and in intracellular loops 1 and 2 (Vaughan, 2004; Sorensen et al., 2014).

GENETIC AND TRANSCRIPTIONAL REGULATIONS OF SERT GENE EXPRESSION

Several lines of evidence support that SERT expression and activity are influenced by genetic variations of SERT encoding gene. Variations in the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR) located ∼1.2 kb upstream of the transcription start site have been shown to influence SERT expression and function. Lesch et al. (1996) found that the short promoter variant (S) containing 14 tandem repeats is associated with lower transcriptional activity, leading to reduced expression of SERT compared to the long (L) allelic variant composed of 16 repeats. Based on epidemiological studies, a link between such functional polymorphism in the SERT promoter and the susceptibility to life stress related depression has been proposed (Caspi et al., 2003). Ten years later, the identification of the single-nucleotide polymorphisms (SNPs) rs25531 (Hu et al., 2006) and rs25532 (Wendland et al., 2008) close to the 5-HTTLPR added allele variants that in combination with the L and S alleles contribute to the modulation of SERT expression and activity. Other mutations have also been described in SERT exon sequences (SERT I425V, G56A. . .) (Glatt et al., 2001; Prasad et al., 2005). Some of them lead to an increase of 5-HT uptake (Kilic et al., 2003) and/or alter SERT regulation by PKG and p38-MAPK signaling pathways (Prasad et al., 2005).

A second polymorphism region was evidenced in intron 2 (STin2) of the human SERT gene (Ogilvie et al., 1996). This region consists of a variable number of tandem repeats

(VNTR) with 9 (STin2.9), 10 (STin2.10), or 12 (STin2.12) copies of a 16/17 bp element. Using mouse embryos or embryonic stem cells, the STin2 polymorphism was shown to function as transcriptional regulator (Fiskerstrand et al., 1999; MacKenzie and Quinn, 1999). Further *in vitro* analyses demonstrated that STin2 and 5-HTTLPR can contribute in concert to the gene expression (Ali et al., 2010) suggesting that combinations of VNTRs could modulate *in vivo* the level of expression of SERT, and thus the amount of SERT present at the plasma membrane and thereby modifying the efficacy of SERT-mediated 5-HT uptake.

In the 3′-untranslated (UTR) region of the SERT gene, two polyadenylation sites located 567 and 690 bp downstream of the stop codon have been reported, as well as a common SNP (rs3813034) present in the distal polyA signal (Battersby et al., 1999; Gyawali et al., 2010). This polymorphism of the 3′-UTR may influence the translation, localization, and stability of SERT mRNA. However, it is presently unknown how polymorphisms in the 3′-UTR of SERT mRNA (i.e., mutations, SNP, polyA tail length,...) impact on the capacity of (i) the translation machinery, (ii) RNA binding factors that pilot localization of mRNA in polarized cells, (iii) RNA binding proteins that stabilize the transcripts, and/or (iv) microRNAs that regulate mRNA expression, to interact with the SERT mRNA.

Since the complete characterization of SERT gene promoter in 1998 (Heils et al., 1998), a combination of positive and negative signals and factors were shown to influence SERT transcription in developing and adult brain serotonergic neurons of raphe nuclei. This notably includes the Pet-1 transcription factor that plays a critical role in the speciation, development and regulation of the serotonergic system (Hendricks et al., 1999; Goridis and Rohrer, 2002). A Pet-1 binding site is located upstream of the SERT encoding gene and Pet-1 binding was shown to increase SERT gene transcription *in vitro* (Hendricks et al., 1999). Lmx1b is another transcription factor that acts with Pet-1 to regulate the expression of SERT, as well as of other proteins related to the serotonergic system, including tryptophan hydroxylase (TPH) and VMAT. Selective inactivation of Lmx1b in serotonergic neurons of the raphe nuclei of adult mice leads to dramatic down-regulation of SERT, TPH, and VMAT expression, despite normal Pet1 level (Song et al., 2011).

Because of the presence of a cyclic AMP (cAMP) response element-like motif (CRE) in SERT promoter, cAMP signaling can also stimulate SERT gene transcription (Heils et al., 1998). In line with this, SERT transcription in the mouse midbrain is influenced by the dark/light alternation, with higher levels of SERT mRNA during the dark phase than during the light phase. In mouse, the expression of SERT mRNA follows a 24 h oscillation rhythm and depends on the clockwork system as well as the ATF4 transcription factor that sustains circadian oscillations of CRE-mediated gene expression and binds the CRE site of the SERT promoter (Ushijima et al., 2012). At the mRNA and protein level, the ATF4 transcription factor itself was shown to vary in the mid-brain of mice according to a 24-h rhythm with higher levels in the dark phase (Ushijima et al., 2012). Such time-dependent variations of ATF4 amount depend on the clockwork system as they are no longer observed in *Clock* mutant mice (Ushijima et al., 2012). With the help of the 1C11 inducible neuronal stem cell line that differentiates into serotonergic neuronal cells ($1C11^{5-HT}$) (Buc et al., 1990; Mouillet-Richard et al., 2000), our laboratory provided evidence that the onset of SERT protein and activity obeys to a mechanism linked to the neuronal differentiation of 1C11 stem cells (Launay et al., 2006). SERT encoding mRNAs are present at the stem cell stage, but are dormant. Exposure of 1C11 precursors to dibutyril cyclic AMP (dbcAMP) to recruit the serotonergic neuronal program does not affect SERT mRNA level, but promotes a rise in the length of SERT mRNA polyA tail (+200 base pairs) that precedes SERT translation and trafficking to the plasma membrane (Launay et al., 2006). Yammamoto et al. (2013) also reported dbcAMP-dependent induction of SERT expression along neural differentiation of SERT-transfected RN46A cells derived from embryonic rat raphe nuclei that depended on protein kinase A (PKA) activity and was further associated with reduced degradation rate of the SERT protein. cAMP signaling thus contributes to the control of SERT expression at multiple levels: gene transcription by acting on the SERT promoter through CRE regions, polyadenylation of the SERT mRNA along neuronal differentiation that unlocks the translation of SERT, and increased stability of the SERT protein.

Glucocorticoid signaling was also shown to positively influence SERT gene transcription in human lymphoblastoid cells (Glatz et al., 2003) as well as in serotonergic neurons (Lau et al., 2013). In the latter case, glucocorticoid-induced SERT expression likely underlies the contribution of the hypothalamo-pituitary-adrenal axis (also called stress axis) to modulation of serotonergic functions in raphe nuclei (Lau et al., 2013) and the possible protective function of glucocorticoids in the regulation of emotional behavior as shown by reduced exploratory behavior in zebrafish mutants defective for the expression of glucocorticoid receptor (Ziv et al., 2013). Of note, dysregulation of the stress axis indeed represents a hallmark of major depression in human patients (Pittenger and Duman, 2008; Keller et al., 2017).

A growing body of evidence indicates a role of the immune system and inflammation in the pathophysiology of neurological disorders, including depression (Furtado and Katzman, 2015; Robson et al., 2017; Leonard, 2018), anxiety (Salim et al., 2012; Reader et al., 2015) or autism spectrum disorders (Gesundheit et al., 2013). Among cytokines and interleukins produced along the inflammation process, interleukin 1 (IL1) upregulates SERT translation in JAR human placental choriocarcinoma cells (Ramamoorthy et al., 1995), possibly through signaling pathways involving MAP kinases and NF-kappaB transcription factor (Kekuda et al., 2000). On the contrary, interleukin 6 (IL6) was shown to negatively act on SERT gene transcription. SERT expression in the hippocampus is reduced upon mouse treatment with IL6, which can be counteracted upon inhibition of the STAT3 transcription factor (Kong et al., 2015). One would expect an anti-depressive action of IL6 treatment that contrasts with sustained elevation of IL6 levels in patients with major depressive disorders and depressive-like phenotype of mice injected with recombinant IL-6 (Sukoff Rizzo et al., 2012).

Further investigations are needed to delineate how IL6-mediated SERT reduction leads to depressive states.

REGULATION OF SERT TRAFFICKING

A second layer of SERT regulations concerns the trafficking and bioavailability of the serotonin transporter at specific plasma membrane subdomains to achieve localized clearance of 5-HT.

First evidence comes from the observation that depletion of cholesterol in membranes of human embryonic kidney 293 cells stably expressing rat SERT decreases SERT activity (Scanlon et al., 2001). Magnani et al. (2004) then reported a partitioning of SERT molecules to a subpopulation of lipid rafts of the plasma membrane in the rat brain. Cholesterol present in the membrane bilayer affects the conformation of SERT and its transport kinetics parameters through binding to the SERT conserved cholesterol site 1 located in a hydrophobic groove between TMD1a, TMD5, and TMD7 (Laursen et al., 2018). Beyond cholesterol, SERT activity, and SERT trafficking to the plasma membrane could be influenced by phosphatidylinositol-4,5-biphosphate (PIP2), whose binding to SERT in the endoplasmic reticulum (ER) was reported to drive the oligomerization of SERT, to target SERT homo-oligomers to the cell surface and to positively impact on SERT activity (Anderluh et al., 2017). How PIP2 level impact on SERT present at the plasma membrane is presently unknown, since PIP2 levels drop down at the plasma membrane due to its conversion into inositol triphosphate (IP3) and diacylglycerol (DAG) by phospholipase C.

From a structural point of view, the carboxyl terminus of SERT is a critical domain of the transporter necessary for its delivery to the plasma membrane. SERT molecules truncated for 17–30 amino-acid residues in the C-terminal region in fact lack mature glycosylation and fail to reach the cell surface during the synthesis/secretory process (Larsen et al., 2006; Nobukuni et al., 2009). Specific subdomains of the C-terminus would influence human SERT folding and the formation of a docking site for a coat protein (COPII) component necessary to export SERT from the ER to the plasma membrane (El-Kasaby et al., 2010), and/or limit the influence of Heat Shock Proteins on SERT retention in the ER (El-Kasaby et al., 2014). The study by Ahmed et al. (2008) reinforces the role of the C-terminus of SERT for translocation of the transporter from intracellular compartments to the plasma membrane. Starting from the observation that the density of SERT molecules is reduced at the cell surface of platelets when plasmatic concentrations of 5-HT are elevated, the authors provided evidence that this high level of 5-HT causes 5-HT transamidation (serotonylation) of the small GTPase Rab4 within platelets, leading to the stabilization of Rab4 in its active GTP-bound form, binding of Rab4 to the cytoplasmic C-terminus part of SERT and retention of the transporter in intracellular compartments (Ahmed et al., 2008). Such impact of an excess of 5-HT on SERT trafficking in platelets can be viewed as a way to maintain an external tonic 5-HT concentration for regulating/amplifying blood functions, such as platelet aggregation (Walther et al., 2003). On the other hand, the direct interaction of the C-terminus of SERT with active integrin αIIbβ3 enhances SERT activity that correlates with increased SERT expression at the surface of transfected HEK293 cells (Carneiro et al., 2008). Refining the functional interaction between integrin β3 and SERT, Mazalouskas et al. (2015) provided evidence for tight modulation of the activity of a subpopulation of SERT molecules by the αvβ3 integrin receptor subtype in the midbrain at serotonergic synapses. Partial neuronal depletion of integrin β3 subunit in mice reduces SERT-mediated 5-HT uptake in midbrain synaptosomes by scaling down the population size of active SERT molecules (Mazalouskas et al., 2015). In this context, polymorphism in human integrin β3 has been suggested to impact on the responsiveness of some patients to SSRIs. Interestingly, decrease of SERT cell surface localization and 5-HT uptake was reported upon interaction of Nitric Oxide synthase, through its PDZ domain, with SERT C-terminus (Chanrion et al., 2007). Thus, SERT trafficking to or away from the plasma membrane depends on the protagonists interacting with the carboxy-terminal domain of SERT.

Association of diverse proteins to SERT regions distinct from the C-terminus has also been shown to influence the trafficking of SERT. This includes the secretory carrier membrane protein 2 (SCAMP2) that interacts with the N-terminus of SERT, leading to subcellular redistribution of SERT, with a reduction of its density at the cell surface (Muller et al., 2006). Some other proteins, whose SERT binding site has not yet been identified, also impact on SERT localization. α-synuclein, a protein mainly known for its implication in Parkinson's disease, binds SERT through direct protein-protein interactions via the non-Aβ-amyloid component domain of the α-synuclein protein and promotes SERT internalization, accounting for reduced 5-HT uptake (Wersinger et al., 2006; Wersinger and Sidhu, 2009). The membrane glycoprotein M6B, a proteolipid notably expressed in neurons and oligodendrocytes in the brain, interacts with SERT, down-regulates its trafficking to and/or stability at the plasma membrane, and thereby decreases SERT-mediated serotonin uptake in transfected cells (Fjorback et al., 2009). Formation of a hetero-complex between SERT and ASCT2 (for alanine-serine-cysteine-threonine2), a solute carrier 1 family member co-expressed with SERT in serotonergic neurons and involved in the plasma membrane transport of neutral amino acids, also reduces cell surface localization of SERT and SERT activity (Seyer et al., 2016). Although the mechanism remains elusive, interaction of SERT with the vesicle-associated membrane protein 2 (VAMP-2), a SNARE protein involved in the vesicle fusion with the plasma membrane, positively influences SERT translocation to the plasma membrane and thereby SERT function (Muller et al., 2014).

A complex signaling network involving kinases and phosphatases has also been shown to largely influence the presence of SERT at the cell surface. Exploiting the non-neuronal HEK-293 cell system, Ramamoorthy et al. (1998) reported the internalization of the transfected human SERT through a protein kinase C (PKC)-dependent pathway, leading to reduced 5-HT uptake. PKC-dependent internalization of SERT would depend on SERT interaction with the LIM domain adaptor protein Hic-5 (Carneiro and Blakely, 2006). The same group further showed in rat basophilic leukemia 2H3 cells that protein kinase

G (PKG)-connected pathways enhance SERT surface trafficking (Zhu et al., 2004). Another protagonist that positively influences SERT presence at the plasma membrane is the Akt/PKB protein kinase, as the inhibition of Akt1 and Akt2 reduces SERT export to the plasma membrane, possibly through post-translational modification of SERT by phosphorylation (Rajamanickam et al., 2015). The Ca^{2+}-activated protein phosphatase calcineurin was shown to influence both *in vitro* and *in vivo* SERT trafficking to the plasma membrane (Seimandi et al., 2013): both catalytic and regulatory subunits of calcineurin bind SERT C-terminus and the physical association of calcineurin with SERT depends on calcineurin phosphatase activity. Calcineurin interaction with SERT was shown to prevent reduction of SERT uptake activity induced by PKC-mediated SERT phosphorylation. Unexpectedly, constitutive calcineurin activation in mice generates antidepressant-like effects, that is, reduced immobility in the forced swim test (Seimandi et al., 2013). How calcineurin contributes to antidepressant effects remains largely elusive and needs extensive investigations (Seimandi et al., 2013).

Supporting the idea that modification of SERT trafficking could underlie the beneficial action of antidepressants, Schloss's laboratory showed that exposure of 1C11-derived serotonergic neurons to citalopram, and to a lesser extent, fluoxetine, paroxetine, and sertraline, reduces the level of SERT molecules present at the neuronal cell surface (notably in neurites) through SERT internalization and redistribution to the cell body (Lau et al., 2008; Kittler et al., 2010; Matthaus et al., 2016). The consequence is a reduction of 5-HT uptake and increase of the external 5-HT concentration. How SSRI antidepressants promote endocytosis of SERT molecules and their redistribution to the soma is unknown and needs further investigations.

REGULATION OF SERT UPTAKE ACTIVITY

Beyond the impact of phosphorylations on SERT trafficking/residence at the plasma membrane, phosphorylation steps also impact on SERT uptake activity. In SERT-transfected CHO cells, activation of p38-MAP kinase downstream from PKG in adenosine receptor-coupled signaling pathways with a critical implication of the protein phosphatase 2A was shown to up-regulate SERT catalytic activity (Zhu et al., 2005). Although it remains controversial (Andreetta et al., 2013; Schwamborn et al., 2016), implication of p38-MAPK in the upregulation of SERT activity induced by IL1β and TNFα was observed in the mouse midbrain and striatal synaptosomes (Zhu et al., 2006, 2010). Ramamoorthy et al. (2007) further provided evidence that PKG activation does not affect SERT surface abundance, excluding that the increase of SERT activity originates from enhanced trafficking of SERT to the plasma membrane. In that study, the authors firstly identified Thr276 of the SERT molecule, as a site whose phosphorylation by PKG augments the Vmax of SERT-mediated 5-HT uptake (Zhang et al., 2016). In addition to Thr276, five other residues located in the N- and C-termini and in intracellular loop 1 and 2 of the SERT molecule were identified by liquid-chromatography-tandem mass spectrometry as phosphorylation sites. These are Ser149/Ser277/Thr603 for PKC, Ser13 for CaMKII, and Thr616 for p38MAPK (Sorensen et al., 2014). Tyrosine phosphorylations of SERT by signaling pathways coupled to Src kinase activity, including Syk, were also shown to positively influence SERT-mediated 5-HT accumulation in dense granules of platelets (Zarpellon et al., 2008; Pavanetto et al., 2011), but Tyr residue(s) involved remain(s) to be identified.

In human placenta choriocarcinoma cell line (Sakai et al., 1997) as well as in rat platelets (Jayanthi et al., 2005), activation of PKC by phorbol esters triggers rapid inhibition of SERT uptake activity associated with decreased Vmax and sometimes increased Km. Such reduction of SERT intrinsic activity depends on PKC-mediated phosphorylations of SERT present at the plasma membrane on Ser residues. This step precedes the PKC-mediated internalization of SERT, due to additional phosphorylations on Thr residues, thus sustaining strong reduction of SERT activity because of enhanced endocytosis of SERT (Jayanthi et al., 2005; Carneiro and Blakely, 2006). The direct phosphorylation of SERT by PKC has been questioned in a study by Sakai et al. (2000), which showed that the negative action of PKC on SERT activity rather depends, at least in part, on the disruption of F-actin cytoskeleton and morphological cell changes.

Interestingly, in HEK-293 cells transfected with SERT, 5-HT counteracts PKC-dependent SERT phosphorylation and rescues SERT uptake activity (Ramamoorthy and Blakely, 1999). With the help of prefrontocortical synaptosomes, Awtry and colleagues further report that PKA, downstream nicotinic acetylcholine receptors-coupled signaling pathways, increases SERT activity. Nicotine-induced increase of 5-HT levels in the brain prefrontal cortex may account for the effects of nicotine on behaviors such as cognition, reward and memory (Awtry et al., 2006). Through pharmacological approaches using agonists and antagonists of serotonergic 5-HT_{2B} receptor (5-HT_{2B}R) subtype, our laboratory showed that 5-HT_{2B}R signaling underlies the action of 5-HT on the control of SERT uptake activity and its energy source, the Na/K ATPase electrogenic pump (**Figure 2**; Launay et al., 2006). In serotonergic $1C11^{5-HT}$ neuronal cells as well as in primary neuronal culture derived from embryonic raphe nuclei, 5-HT_{2B}R governs the phosphorylation state of both SERT and Na/K ATPase. At low 5-HT concentration [1–2 nM, as in *in vivo* physiological conditions (Brand and Anderson, 2011)], the intrinsic 5-HT_{2B}R coupling to NOS and the subsequent activation of PKG ensure SERT phosphorylation to basal level that correlates with maximal 5-HT uptake. In these conditions, SERT molecules are fully competent for binding cocaine and paroxetine. In excess of 5-HT, 5-HT_{2B}R coupling to the IP_3/PKC pathway promotes additional phosphorylation of SERT and in parallel enhances the phosphorylation level of the Na/K ATPase. PKC-mediated phosphorylation of Ser residues in the N-terminus of the alpha subunit of Na/K ATPase was shown to reduce pump activity through mechanisms that remain elusive, but that could relate to the acquisition of an altered structure caused by the phosphorylations and/or the endocytosis of the Na/K ATPase (for review, see Poulsen et al., 2010 and references therein). In any case, SERT hyperphosphorylation combined to the

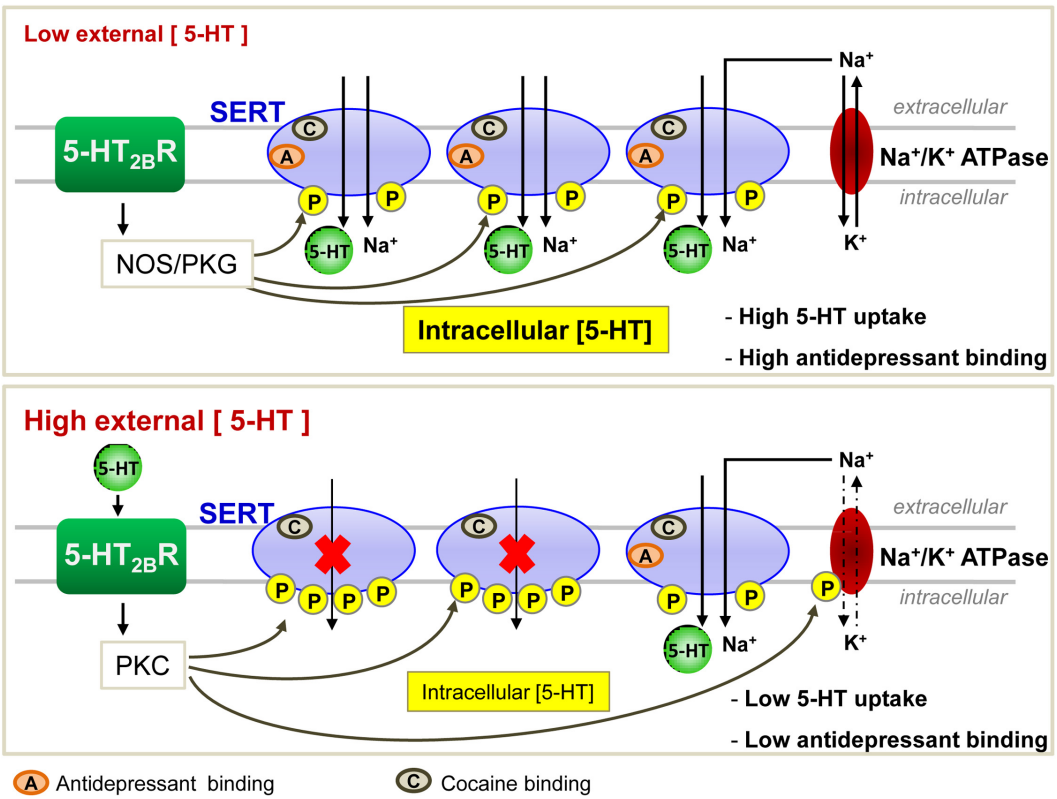


FIGURE 2 | Regulation of SERT function by the serotonergic receptor 5-HT_{2B} in $1C11^{5-HT}$ neuronal cells. At low 5-HT concentration, 5-HT_{2B}Rs through its NO signaling coupling ensure a basal phosphorylation level of SERT, endowing it with an optimal 5-HT uptake activity. All SERT molecules are able to bind antidepressants. At high level of 5-HT, 5-HT_{2B}R-dependent protein kinase C (PKC) couplings provoke hyperphosphorylation of SERT that impairs its ability to uptake 5-HT. Under these conditions, only 1/3 of SERT molecules bind antidepressants.

phosphorylation rise of Na/K ATPase reduces 5-HT transport efficacy (**Figure 2**). Importantly, the population of SERT molecules hyperphosphorylated upon 5-HT_{2B}R stimulation loose their ability to bind paroxetine. As hyperphosphorylated SERT keeps its capacity to bind cocaine at the plasma membrane of neuronal cells, reduced SERT uptake activity and reduced SERT binding of SSRIs are not attributable to SERT internalization induced by excess of 5-HT. The impact of 5-HT and 5-HT_{2B}R signaling on SERT uptake activity can be viewed as a feedback loop that *in fine* permits keeping extracellular serotonin at tonic concentrations necessary for regulating sleep, mood, appetite, and some cognitive functions. In addition to influencing SERT function, 5-HT_{2B}R-dependent control of Na/K ATPase activity would have a broader action by impacting other transporters whose activity depends on this electrogenic pump. Of note, the fact that stimulation of 5-HT_{2B}R promotes SERT hyperphosphorylation and thereby reduces SERT sensitivity to antidepressants may explain, at least partly, the resistance of some patients to antidepressant treatment and/or the time-delay of the antidepressant response. Whatever their phosphorylation state, the half-life of SERT molecules present at the plasma membrane of serotonergic neurons is unknown. Considering that hyperphosphorylated SERT, which is non-competent for antidepressant recognition and thereby confers resistance to antidepressant action, may stay at the surface of serotonergic neurons even if external 5-HT concentration collapses (depressive situations), binding of SSRIs to SERT will depend on the neo-synthesis and trafficking of non-, or poorly phosphorylated SERT to the plasma membrane, a process whose kinetics could take several days. Further investigations are therefore needed to appreciate the stability of the different phosphorylated forms of SERT at the plasma membrane and their turnovers in fully integrated serotonergic systems.

EPIGENETIC REGULATION OF SERT EXPRESSION AND ACTIVITY

Over the past decade, evidence has accumulated that SERT expression and thereby its functions are also governed by epigenetic regulation, including DNA methylation, histone modifications and microRNAs. In this respect, it has been shown that in lymphoblast cell lines, methylation of CpG island nearby the untranslated exon1A of SERT encoding gene is associated with reduced levels of SERT mRNA, an effect that depends on 5HTTLPR genotype (Philibert et al., 2007). Accordingly, Kinnally et al. (2010) reported that in rhesus macaques, the short allele

rh5HTTLRP presented higher level of CpG methylation than the longer one, which was associated with reduced SERT expression in peripheral blood mononuclear cells. Further, using a luciferase reporter construct, Wang et al. showed *in vitro* reduced SERT transcription when its promoter is methylated in JAR cells (Wang et al., 2012). However, a role of DNA methylation in SERT regulation is still at the heart of debates since Wankerl et al. (2014) observed in their cohort of 133 healthy young participants that variations of SERT mRNA level appear to be independent of DNA methylation profiles within the SERT CpG island.

In few reports, modifications in the acetylation status of histones were found to impact on SERT expression. Histone deacetylases (HDAC) inhibitors [butyrate, trichostatin A (TSA)] or knockdown of HDAC2 (but not HDAC1) by RNA interference in the human intestinal epithelial cell line Caco-2 reduced SERT mRNA and protein levels (Gill et al., 2013). By contrast, in several tumor cell lines (HD11, HepG2, THP-1...), addition of TSA or siRNA-mediating silencing of HDAC1 enhanced the expression of SERT mRNA, leading to an increase of 5-HT uptake (Phi van et al., 2015).

Another layer of epigenetic control of SERT level relies on microRNAs that are small non-coding RNA able to interact with the 3′ untranslated region (3′UTR) of the mRNA of target genes and block their translation. In 2010, our laboratory identified the microRNA miR-16 as a regulator of SERT expression (Baudry et al., 2010; **Figure 3**). The level of miR-16 is low in serotonergic neurons ($1C11^{5-HT}$ cells or raphe nuclei), where SERT is expressed. In contrast, miR-16 is abundant in noradrenergic neurons ($1C11^{NE}$ cells or locus coeruleus) and prevents the translation of SERT mRNA. We established that variation of miR-16 level sustains the antidepressant action of fluoxetine (Baudry et al., 2010; Launay et al., 2011). In mice, fluoxetine increases miR-16 in serotonergic neurons of raphe nuclei, which in turn decreases SERT protein level as observed in Prozac®-treated patients (Benmansour et al., 2002), thus increasing external 5-HT concentration. From a mechanistic point of view, the rise of miR-16 induced by fluoxetine in serotonergic neurons results from an increased maturation of pri/pre-miR-16 through a GSK3β-dependent process. Moreover, the fluoxetine action on raphe creates a dialog between raphe and locus coeruleus, and between raphe and hippocampus:

(i) Fluoxetine provokes the release of the neurotrophic factor S100β by serotonergic neurons that acts on noradrenergic neurons of the locus coeruleus. S100β signaling reduces miR-16 level in noradrenergic neurons, which unlocks the expression of SERT, but also of other serotonergic functions, including the expression of TPH. Noradrenergic neurons acquired a mixed noradrenergic/serotonergic phenotype and thus become a new source of serotonin in the brain (Baudry et al., 2010; **Figure 4**).

(ii) Fluoxetine also provokes the secretion of BNDF, Wnt2, and 15dPGJ2 by serotonergic neurons that act in synergy on another raphe-connected structure, the hippocampus. The cocktail of the three molecules decreases miR-16 levels in the hippocampus, which again unlocks SERT expression, but also enhances the level of a second miR-16 target, the anti-apoptotic factor bcl2, and sustains neurogenesis (Launay et al., 2011). Of note, the levels of BDNF, Wnt2, and 15dPGJ2 are augmented *in vivo* not only in the CSFs of mice but also in patients treated with Prozac® (Launay et al., 2011).

Finally, using mouse models of depression, we observed that the upregulation of miR-16 in raphe or the downregulation of miR-16 in the locus coeruleus (Baudry et al., 2010) or in the hippocampus (Launay et al., 2011) provoke behavioral responses that compare to those induced by fluoxetine. Accordingly, Yang et al. (2017) reported that miR-16 administration in raphe nuclei or intragastric injections of fluoxetine in chronic unpredicted mild stress model rats for 3 weeks lead to similar improvement of depressive behavioral changes.

Can the fluoxetine-induced dialogs between raphe and locus coeruleus, or raphe and hippocampus account for the time-delay of action of antidepressants? Depression seems strongly related to a loss in the number of neurons and synapses in several brain structures, including the hippocampus and prefrontal cortex (Drevets et al., 1997; Hercher et al., 2009). Post-mortem studies indeed revealed hippocampal atrophy in cases of major depressive disorder, which can be reversed by antidepressant treatment (Duman, 2004; MacQueen and Frodl, 2011) through stimulation of hippocampal neurogenesis and synaptogenesis (Duman, 2004; Boldrini et al., 2009). Of note, these processes are slow. In response to antidepressants, the neuronal plasticity of noradrenergic neurons, but more likely the hippocampal neurogenesis orchestrated by the raphe nuclei, might account for the delay in the action of antidepressants.

Nevertheless, the picture is likely more complicated. SERT mRNA occurs in two alternative polyadenylated forms: a short form and a ∼125 bp longer form, which is associated with reduced anxiety-related behavior (Gyawali et al., 2010; Hartley et al., 2012). miR-16 blocks translation of both polyadenylated forms of the SERT mRNA (Yoon et al., 2013). However, Yoon et al. showed that the miR-16 regulation of SERT expression was modulated by a RNA binding protein hnRNPK after S100β addition in rat C6 astroglioma cells and RN46A brain raphe cells. S100β-induced Src kinases phosphorylate hnRNPK, which in turn displaces miR-16 from long polyadenylated SERT mRNA and allows its translation (Yoon et al., 2013). Thus, hnRNPK adds another layer for regulation of SERT expression specific to long polyadenylation forms of SERT mRNAs.

In addition to miR-16, other microRNAs were found to interact with the 3′UTR of the SERT mRNA and inhibit its translation (**Figure 3**). Among those, miR-15a, which belongs to the same cluster as miR-16, was shown to regulate SERT expression in the rat RN46A serotonergic cell line as well as in human JAR cells (Moya et al., 2013). In 2014, Issler and colleagues evidenced that viral-mediated overexpression of another miR, miR-135a, in mouse raphe induces a decrease of SERT protein level and reduces adverse effects of chronic social defeat. Conversely, miR-135a knockdown leads to a raise of SERT expression, an increase of anxiety-like behavior and a reduced response to SSRIs. Upon SSRI antidepressant administration to mice, upregulation of miR-135a level was

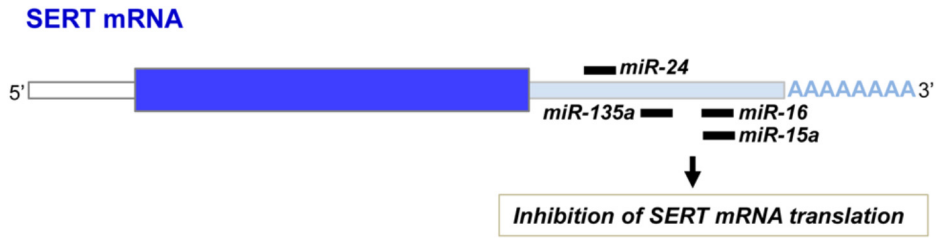


FIGURE 3 | miR-16, miR-15a, miR-135a, and miR-24 binding sites on the 3′ untranslated region of the SERT mRNA.

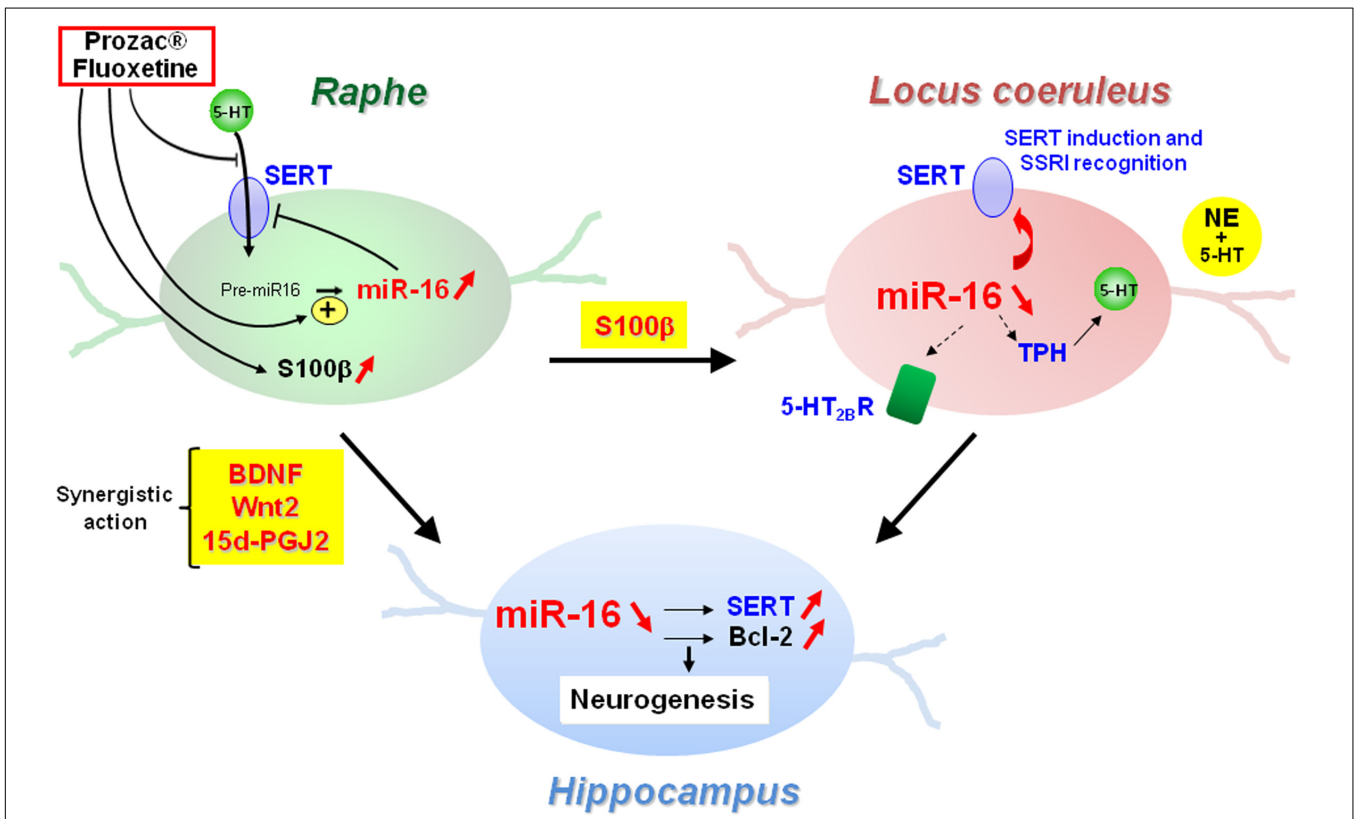


FIGURE 4 | Central role of miR-16 in the adaptive response of neurons from raphe, locus coeruleus, and hippocampus to SSRI antidepressant treatment.

observed in raphe nuclei (Issler et al., 2014). As for miR-16, increase of miR-135a induced by antidepressants in serotonergic neurons will repress SERT expression and thereby SERT-mediated serotonin uptake, thus contributing to the beneficial effect of antidepressants. miR-24 was identified as a regulator of SERT expression in intestinal mucosa epithelial cells (Liao et al., 2016). Patients suffering of irritable bowel syndrome (IBS) as well as a IBS mouse model display high levels of miR-24 and correlatively decreased SERT expression. Treatment of IBS mice with a miR-24 inhibitor increases SERT protein level and alleviates intestinal pain and inflammation (Liao et al., 2016). More recently, miR-195 and miR-322, in addition to miR-15 and miR-16, were found to inhibit SERT expression after transfection in smooth muscle cells (Gu et al., 2017).

Given the above findings, which receptors and downstream signaling pathways regulate the level of SERT-targeting microRNAs upon antidepressant action remain to be identified. Finally, it will be also critical to evaluate how antidepressants, by modulating microRNA expressions, modify the neuronal phenotype and, as for miR-16, promote changes of neurotransmission in raphe-connected brain regions.

CONCLUSION

Since the enunciation in the 50s of the "monoamine theory of affective disorders" that stipulates decreased levels of bioamines in depressed patients, and the consecutive pharmaceutical development of SSRI antidepressants in the 60s, how SSRIs exert

their antidepressant action remained largely obscure for almost 50 years. By showing that fluoxetine (Prozac®) binding to SERT not only blocks 5-HT uptake in serotonergic neurons, but also creates a new source of serotonin in raphe nuclei-connected locus coeruleus through modulations of miR-16 level, a huge breakthrough has been made in our understanding of the mode of action of some SSRI antidepressants that notably relies on the neuronal plasticity of noradrenergic and hippocampal neurons (Baudry et al., 2010; Launay et al., 2011). However, it is clear that a consequent fraction of depressed patients is hyporesponsive to SSRI antidepressants. The absence of antidepressant response likely relates to a molecular state of SERT incompetent for SSRI recognition. We illustrate in this review that multiple factors, including SERT polymorphism, post-translational modifications of SERT by phosphorylations, SERT partners, or SERT trafficking, influence the presence of SERT at the plasma membrane and competency to bind SSRI antidepressants. In this context, to predict the success of a SSRI treatment in depressed patients, the clinical rational would be first to determine SERT status in those patients. Even if non-invasive imaging techniques permit to delineate the density of SERT molecules in the human brain (Meyer, 2007), post-translational modifications of SERT cannot be addressed. Interestingly, SERT molecules expressed by blood platelets display pharmacological properties highly comparable to those of SERT in the CNS, in terms of 5-HT uptake and sensitivity to antidepressants (Tuomisto and Tukiainen, 1976; Tuomisto et al., 1979; Da Prada et al., 1988; Launay et al., 2006). As the SERT state in blood platelets would likely mirror the SERT state in serotonergic neurons of raphe nuclei, characterizing SERT post-translational modifications in blood platelets would be helpful to predict the response or refractoriness of depressed patients to one type of SSRIs prior to proposing a personalized medicine tailored to each individual depressed patient.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by the French Agence Nationale de la Recherche (grant miR-Dep, reference ANR-13-SAMA-0001) and INSERM.

REFERENCES

Ahmed, B. A., Jeffus, B. C., Bukhari, S. I., Harney, J. T., Unal, R., Lupashin, V. V., et al. (2008). Serotonin transamidates Rab4 and facilitates its binding to the C terminus of serotonin transporter. *J. Biol. Chem.* 283, 9388–9398. doi: 10.1074/jbc.M706367200

Ali, F. R., Vasiliou, S. A., Haddley, K., Paredes, U. M., Roberts, J. C., Miyajima, F., et al. (2010). Combinatorial interaction between two human serotonin transporter gene variable number tandem repeats and their regulation by CTCF. *J. Neurochem.* 112, 296–306. doi: 10.1111/j.1471-4159.2009.06453.x

Allen, J. A., Halverson-Tamboli, R. A., and Rasenick, M. M. (2007). Lipid raft microdomains and neurotransmitter signalling. *Nat. Rev. Neurosci.* 8, 128–140. doi: 10.1038/nrn2059

Amphoux, A., Vialou, V., Drescher, E., Bruss, M., Mannoury La Cour, C., Rochat, C., et al. (2006). Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. *Neuropharmacology* 50, 941–952. doi: 10.1016/j.neuropharm.2006.01.005

Anderluh, A., Hofmaier, T., Klotzsch, E., Kudlacek, O., Stockner, T., Sitte, H. H., et al. (2017). Direct PIP2 binding mediates stable oligomer formation of the serotonin transporter. *Nat. Commun.* 8:14089. doi: 10.1038/ncomms14089

Andreetta, F., Barnes, N. M., Wren, P. B., and Carboni, L. (2013). p38 MAP kinase activation does not stimulate serotonin transport in rat brain: implications for sickness behaviour mechanisms. *Life Sci.* 93, 30–37. doi: 10.1016/j.lfs.2013.05.014

Awtry, T. L., Frank, J. G., and Werling, L. L. (2006). In vitro regulation of serotonin transporter activity by protein kinase A and nicotinic acetylcholine receptors in the prefrontal cortex of rats. *Synapse* 59, 342–349. doi: 10.1002/syn.20251

Battersby, S., Ogilvie, A. D., Blackwood, D. H., Shen, S., Muqit, M. M., Muir, W. J., et al. (1999). Presence of multiple functional polyadenylation signals and a single nucleotide polymorphism in the 3′ untranslated region of the human serotonin transporter gene. *J. Neurochem.* 72, 1384–1388. doi: 10.1046/j.1471-4159.1999.721384.x

Baudry, A., Mouillet-Richard, S., Schneider, B., Launay, J. M., and Kellermann, O. (2010). miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science* 329, 1537–1541. doi: 10.1126/science.1193692

Benmansour, S., Owens, W. A., Cecchi, M., Morilak, D. A., and Frazer, A. (2002). Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. *J. Neurosci.* 22, 6766–6772. doi: 10.1523/JNEUROSCI.22-15-06766.2002

Blakely, R. D., Berson, H. E., Fremeau, R. T. Jr., Caron, M. G., Peek, M. M., Prince, H. K., et al. (1991). Cloning and expression of a functional serotonin transporter from rat brain. *Nature* 354, 66–70. doi: 10.1038/354066a0

Boldrini, M., Underwood, M. D., Hen, R., Rosoklija, G. B., Dwork, A. J., John Mann, J., et al. (2009). Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology* 34, 2376–2389. doi: 10.1038/npp.2009.75

Brand, T., and Anderson, G. M. (2011). The measurement of platelet-poor plasma serotonin: a systematic review of prior reports and recommendations for improved analysis. *Clin. Chem.* 57, 1376–1386. doi: 10.1373/clinchem.2011.163824

Buc, M. H., Launay, J. M., Lamblin, D., and Kellermann, O. (1990). Serotonin uptake, storage and synthesis in an immortalized commited cell line derived from mouse teratocarcinoma. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1922–1926. doi: 10.1073/pnas.87.5.1922

Carneiro, A. M., and Blakely, R. D. (2006). Serotonin-, protein kinase C-, and Hic-5-associated redistribution of the platelet serotonin transporter. *J. Biol. Chem.* 281, 24769–24780. doi: 10.1074/jbc.M603877200

Carneiro, A. M., Cook, E. H., Murphy, D. L., and Blakely, R. D. (2008). Interactions between integrin alphaIIbbeta3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J. Clin. Invest.* 118, 1544–1552. doi: 10.1172/JCI33374

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301, 386–389. doi: 10.1126/science.1083968

Chanrion, B., Mannoury la Cour, C., Bertaso, F., Lerner-Natoli, M., Freissmuth, M., Millan, M. J., et al. (2007). Physical interaction between the serotonin transporter and neuronal nitric oxide synthase underlies reciprocal modulation of their activity. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8119–8124. doi: 10.1073/pnas.0610964104

Da Prada, M., Cesura, A. M., Launay, J. M., and Richards, J. G. (1988). Platelets as a model for neurones? *Experientia* 44, 115–126.

De Felice, L. J. (2016). A current view of serotonin transporters. *F1000Res.* 5:pii:F1000FacultyRev–1884. doi: 10.12688/f1000research.8384.1

Drevets, W. C., Price, J. L., Simpson, J. R. Jr., Todd, R. D., Reich, T., Vannier, M., et al. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* 386, 824–827. doi: 10.1038/386824a0

Duan, H., and Wang, J. (2010). Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. *J. Pharmacol. Exp. Ther.* 335, 743–753. doi: 10.1124/jpet.110.170142

Duman, R. S. (2004). Depression: a case of neuronal life and death? *Biol. Psychiatry* 56, 140–145. doi: 10.1016/j.biopsych.2004.02.033

El-Kasaby, A., Just, H., Malle, E., Stolt-Bergner, P. C., Sitte, H. H., Freissmuth, M., et al. (2010). Mutations in the carboxyl-terminal SEC24 binding motif of the serotonin transporter impair folding of the transporter. *J. Biol. Chem.* 285, 39201–39210. doi: 10.1074/jbc.M110.118000

El-Kasaby, A., Koban, F., Sitte, H. H., Freissmuth, M., and Sucic, S. (2014). A cytosolic relay of heat shock proteins HSP70-1A and HSP90beta monitors the folding trajectory of the serotonin transporter. *J. Biol. Chem.* 289, 28987–29000. doi: 10.1074/jbc.M114.595090

Fava, M. (2003). Diagnosis and definition of treatment-resistant depression. *Biol. Psychiatry* 53, 649–659. doi: 10.1016/S0006-3223(03)00231-2

Fekadu, A., Wooderson, S. C., Markopoulo, K., Donaldson, C., Papadopoulos, A., and Cleare, A. J. (2009). What happens to patients with treatment-resistant depression? A systematic review of medium to long term outcome studies. *J. Affect. Disord.* 116, 4–11. doi: 10.1016/j.jad.2008.10.014

Fiskerstrand, C. E., Lovejoy, E. A., and Quinn, J. P. (1999). An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. *FEBS Lett.* 458, 171–174. doi: 10.1016/S0014-5793(99)01150-3

Fjorback, A. W., Muller, H. K., and Wiborg, O. (2009). Membrane glycoprotein M6B interacts with the human serotonin transporter. *J. Mol. Neurosci.* 37, 191–200. doi: 10.1007/s12031-008-9092-4

Furtado, M., and Katzman, M. A. (2015). Examining the role of neuroinflammation in major depression. *Psychiatry Res.* 229, 27–36. doi: 10.1016/j.psychres.2015.06.009

Fuxe, K., Dahlstrom, A., Hoistad, M., Marcellino, D., Jansson, A., Rivera, A., et al. (2007). From the Golgi-Cajal mapping to the transmitter-based characterization of the neuronal networks leading to two modes of brain communication: wiring and volume transmission. *Brain Res. Rev.* 55, 17–54. doi: 10.1016/j.brainresrev.2007.02.009

Gesundheit, B., Rosenzweig, J. P., Naor, D., Lerer, B., Zachor, D. A., Prochazka, V., et al. (2013). Immunological and autoimmune considerations of autism spectrum disorders. *J. Autoimmun.* 44, 1–7. doi: 10.1016/j.jaut.2013.05.005

Gill, R. K., Kumar, A., Malhotra, P., Maher, D., Singh, V., Dudeja, P. K., et al. (2013). Regulation of intestinal serotonin transporter expression via epigenetic mechanisms: role of HDAC2. *Am. J. Physiol. Cell Physiol.* 304, C334–C341. doi: 10.1152/ajpcell.00361.2012

Glatt, C. E., DeYoung, J. A., Delgado, S., Service, S. K., Giacomini, K. M., Edwards, R. H., et al. (2001). Screening a large reference sample to identify very low frequency sequence variants: comparisons between two genes. *Nat. Genet.* 27, 435–438. doi: 10.1038/86948

Glatz, K., Mossner, R., Heils, A., and Lesch, K. P. (2003). Glucocorticoid-regulated human serotonin transporter (5-HTT) expression is modulated by the 5-HTT gene-promotor-linked polymorphic region. *J. Neurochem.* 86, 1072–1078. doi: 10.1046/j.1471-4159.2003.01944.x

Goridis, C., and Rohrer, H. (2002). Specification of catecholaminergic and serotonergic neurons. *Nat. Rev. Neurosci.* 3, 531–541. doi: 10.1038/nrn871

Gu, J., Zhang, H., Ji, B., Jiang, H., Zhao, T., Jiang, R., et al. (2017). Vesicle miR-195 derived from endothelial cells inhibits expression of serotonin transporter in vessel smooth muscle cells. *Sci. Rep.* 7:43546. doi: 10.1038/srep43546

Gyawali, S., Subaran, R., Weissman, M. M., Hershkowitz, D., McKenna, M. C., Talati, A., et al. (2010). Association of a polyadenylation polymorphism in the serotonin transporter and panic disorder. *Biol. Psychiatry* 67, 331–338. doi: 10.1016/j.biopsych.2009.10.015

Hartley, C. A., McKenna, M. C., Salman, R., Holmes, A., Casey, B. J., Phelps, E. A., et al. (2012). Serotonin transporter polyadenylation polymorphism modulates the retention of fear extinction memory. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5493–5498. doi: 10.1073/pnas.1202044109

Heils, A., Wichems, C., Mossner, R., Petri, S., Glatz, K., Bengel, D., et al. (1998). Functional characterization of the murine serotonin transporter gene promoter in serotonergic raphe neurons. *J. Neurochem.* 70, 932–939. doi: 10.1046/j.1471-4159.1998.70030932.x

Hendricks, T., Francis, N., Fyodorov, D., and Deneris, E. S. (1999). The ETS domain factor Pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. *J. Neurosci.* 19, 10348–10356. doi: 10.1523/JNEUROSCI.19-23-10348.1999

Hercher, C., Turecki, G., and Mechawar, N. (2009). Through the looking glass: examining neuroanatomical evidence for cellular alterations in major depression. *J. Psychiatr. Res.* 43, 947–961. doi: 10.1016/j.jpsychires.2009.01.006

Hoffman, B. J., Mezey, E., and Brownstein, M. J. (1991). Cloning of a serotonin transporter affected by antidepressants. *Science* 254, 579–580. doi: 10.1126/science.1948036

Hu, X. Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., Taubman, J., Greenberg, B. D., et al. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am. J. Hum. Genet.* 78, 815–826. doi: 10.1086/503850

Issler, O., Haramati, S., Paul, E. D., Maeno, H., Navon, I., Zwang, R., et al. (2014). MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. *Neuron* 83, 344–360. doi: 10.1016/j.neuron.2014.05.042

Jayanthi, L. D., Samuvel, D. J., Blakely, R. D., and Ramamoorthy, S. (2005). Evidence for biphasic effects of protein kinase C on serotonin transporter function, endocytosis, and phosphorylation. *Mol. Pharmacol.* 67, 2077–2087. doi: 10.1124/mol.104.009555

Kaushalya, S. K., Desai, R., Arumugam, S., Ghosh, H., Balaji, J., and Maiti, S. (2008). Three-photon microscopy shows that somatic release can be a quantitatively significant component of serotonergic neurotransmission in the mammalian brain. *J. Neurosci. Res.* 86, 3469–3480. doi: 10.1002/jnr.21794

Kekuda, R., Leibach, F. H., Furesz, T. C., Smith, C. H., and Ganapathy, V. (2000). Polarized distribution of interleukin-1 receptors and their role in regulation of serotonin transporter in placenta. *J. Pharmacol. Exp. Ther.* 292, 1032–1041.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G. M., et al. (2017). HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. *Mol. Psychiatry* 22, 527–536. doi: 10.1038/mp.2016.120

Kilic, F., Murphy, D. L., and Rudnick, G. (2003). A human serotonin transporter mutation causes constitutive activation of transport activity. *Mol. Pharmacol.* 64, 440–446. doi: 10.1124/mol.64.2.440

Kinnally, E. L., Capitanio, J. P., Leibel, R., Deng, L., LeDuc, C., Haghighi, F., et al. (2010). Epigenetic regulation of serotonin transporter expression and behavior in infant rhesus macaques. *Genes Brain Behav.* 9, 575–582. doi: 10.1111/j.1601-183X.2010.00588.x

Kittler, K., Lau, T., and Schloss, P. (2010). Antagonists and substrates differentially regulate serotonin transporter cell surface expression in serotonergic neurons. *Eur. J. Pharmacol.* 629, 63–67. doi: 10.1016/j.ejphar.2009.12.010

Koepsell, H., Lips, K., and Volk, C. (2007). Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm. Res.* 24, 1227–1251. doi: 10.1007/s11095-007-9254-z

Kong, E., Sucic, S., Monje, F. J., Savalli, G., Diao, W., Khan, D., et al. (2015). STAT3 controls IL6-dependent regulation of serotonin transporter function and depression-like behavior. *Sci. Rep.* 5:9009. doi: 10.1038/srep09009

Larsen, M. B., Fjorback, A. W., and Wiborg, O. (2006). The C-terminus is critical for the functional expression of the human serotonin transporter. *Biochemistry* 45, 1331–1337. doi: 10.1021/bi0508688

Lau, T., Heimann, F., Bartsch, D., Schloss, P., and Weber, T. (2013). Nongenomic, glucocorticoid receptor-mediated regulation of serotonin transporter cell surface expression in embryonic stem cell derived serotonergic neurons. *Neurosci. Lett.* 554, 115–120. doi: 10.1016/j.neulet.2013.08.070

Lau, T., Horschitz, S., Berger, S., Bartsch, D., and Schloss, P. (2008). Antidepressant-induced internalization of the serotonin transporter in serotonergic neurons. *FASEB J.* 22, 1702–1714. doi: 10.1096/fj.07-095471

Launay, J. M., Geoffroy, C., Mutel, V., Buckle, M., Cesura, A., Alouf, J. E., et al. (1992). One-step purification of the serotonin transporter located at the human platelet plasma membrane. *J. Biol. Chem.* 267, 11344–11351.

Launay, J. M., Mouillet-Richard, S., Baudry, A., Pietri, M., and Kellermann, O. (2011). Raphe-mediated signals control the hippocampal response to SRI antidepressants via miR-16. *Transl. Psychiatry* 1:e56. doi: 10.1038/tp.2011.54

Launay, J. M., Schneider, B., Loric, S., Da Prada, M., and Kellermann, O. (2006). Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT2B receptor signaling in serotonergic neuronal cells. *FASEB J.* 20, 1843–1854. doi: 10.1096/fj.06-5724com

Laursen, L., Severinsen, K., Kristensen, K. B., Periole, X., Overby, M., Muller, H. K., et al. (2018). Cholesterol binding to a conserved site modulates the conformation, pharmacology, and transport kinetics of the human serotonin transporter. *J. Biol. Chem.* 293, 3510–3523. doi: 10.1074/jbc.M117.809046

Leonard, B. E. (2018). Inflammation and depression: a causal or coincidental link to the pathophysiology? *Acta Neuropsychiatr.* 30, 1–16. doi: 10.1017/neu.2016.69

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Liao, X. J., Mao, W. M., Wang, Q., Yang, G. G., Wu, W. J., and Shao, S. X. (2016). MicroRNA-24 inhibits serotonin reuptake transporter expression and aggravates irritable bowel syndrome. *Biochem. Biophys. Res. Commun.* 469, 288–293. doi: 10.1016/j.bbrc.2015.11.102

Machado-Vieira, R., Baumann, J., Wheeler-Castillo, C., Latov, D., Henter, I. D., Salvadore, G., et al. (2010). The timing of antidepressant effects: a comparison of diverse pharmacological and somatic treatments. *Pharmaceuticals* 3, 19–41. doi: 10.3390/ph3010019

MacKenzie, A., and Quinn, J. (1999). A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15251–15255. doi: 10.1073/pnas.96.26.15251

MacQueen, G., and Frodl, T. (2011). The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol. Psychiatry* 16, 252–264. doi: 10.1038/mp.2010.80

Magnani, F., Tate, C. G., Wynne, S., Williams, C., and Haase, J. (2004). Partitioning of the serotonin transporter into lipid microdomains modulates transport of serotonin. *J. Biol. Chem.* 279, 38770–38778. doi: 10.1074/jbc.M400831200

Matthaeus, F., Schloss, P., and Lau, T. (2015). Differential uptake mechanisms of fluorescent substrates into stem-cell-derived serotonergic neurons. *ACS Chem. Neurosci.* 6, 1906–1912. doi: 10.1021/acschemneuro.5b00219

Matthaus, F., Haddjeri, N., Sanchez, C., Marti, Y., Bahri, S., Rovera, R., et al. (2016). The allosteric citalopram binding site differentially interferes with neuronal firing rate and SERT trafficking in serotonergic neurons. *Eur. Neuropsychopharmacol.* 26, 1806–1817. doi: 10.1016/j.euroneuro.2016.09.001

Mawe, G. M., and Hoffman, J. M. (2013). Serotonin signalling in the gut–functions, dysfunctions and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol.* 10, 473–486. doi: 10.1038/nrgastro.2013.105

Mazalouskas, M., Jessen, T., Varney, S., Sutcliffe, J. S., Veenstra-VanderWeele, J., Cook, E. H., et al. (2015). Integrin beta3 haploinsufficiency modulates serotonin transport and antidepressant-sensitive behavior in mice. *Neuropsychopharmacology* 40, 2015–2024. doi: 10.1038/npp.2015.51

Mercado, C. P., and Kilic, F. (2010). Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. *Mol. Interv.* 10, 231–241. doi: 10.1124/mi.10.4.6

Meyer, J. H. (2007). Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J. Psychiatry Neurosci.* 32, 86–102.

Mouillet-Richard, S., Mutel, V., Loric, S., Tournois, C., Launay, J. M., and Kellermann, O. (2000). Regulation by neurotransmitter receptors of serotonergic or catecholaminergic neuronal cell differentiation. *J. Biol. Chem.* 275, 9186–9192. doi: 10.1074/jbc.275.13.9186

Moya, P. R., Wendland, J. R., Salemme, J., Fried, R. L., and Murphy, D. L. (2013). miR-15a and miR-16 regulate serotonin transporter expression in human placental and rat brain raphe cells. *Int. J. Neuropsychopharmacol.* 16, 621–629. doi: 10.1017/S1461145712000454

Muller, H. K., Kragballe, M., Fjorback, A. W., and Wiborg, O. (2014). Differential regulation of the serotonin transporter by vesicle-associated membrane protein 2 in cells of neuronal versus non-neuronal origin. *PLoS One* 9:e97540. doi: 10.1371/journal.pone.0097540

Muller, H. K., Wiborg, O., and Haase, J. (2006). Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. *J. Biol. Chem.* 281, 28901–28909. doi: 10.1074/jbc.M602848200

Murphy, D. L., Lerner, A., Rudnick, G., and Lesch, K. P. (2004). Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol. Interv.* 4, 109–123. doi: 10.1124/mi.4.2.8

Murphy, D. L., and Lesch, K. P. (2008). Targeting the murine serotonin transporter: insights into human neurobiology. *Nat. Rev. Neurosci.* 9, 85–96. doi: 10.1038/nrn2284

Nobukuni, M., Mochizuki, H., Okada, S., Kameyama, N., Tanaka, A., Yamamoto, H., et al. (2009). The C-terminal region of serotonin transporter is important for its trafficking and glycosylation. *J. Pharmacol. Sci.* 111, 392–404. doi: 10.1254/jphs.09195FP

Ogilvie, A. D., Battersby, S., Bubb, V. J., Fink, G., Harmar, A. J., Goodwim, G. M., et al. (1996). Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *Lancet* 347, 731–733. doi: 10.1016/S0140-6736(96)90079-3

Olivier, B. (2015). Serotonin: a never-ending story. *Eur. J. Pharmacol.* 753, 2–18. doi: 10.1016/j.ejphar.2014.10.031

Pavanetto, M., Zarpellon, A., Borgo, C., Donella-Deana, A., and Deana, R. (2011). Regulation of serotonin transport in human platelets by tyrosine kinase Syk. *Cell Physiol. Biochem.* 27, 139–148. doi: 10.1159/000325216

Phi van, D. K., Muhlbauer, E., and Phi-van, L. (2015). Histone deacetylase HDAC1 downregulates transcription of the serotonin transporter (5-HTT) gene in tumor cells. *Biochim. Biophys. Acta* 1849, 909–918. doi: 10.1016/j.bbagrm.2015.05.008

Philibert, R., Madan, A., Andersen, A., Cadoret, R., Packer, H., and Sandhu, H. (2007). Serotonin transporter mRNA levels are associated with the methylation of an upstream CpG island. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 144B, 101–105. doi: 10.1002/ajmg.b.30414

Pittenger, C., and Duman, R. S. (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* 33, 88–109. doi: 10.1038/sj.npp.1301574

Poulsen, H., Khandelia, H., Morth, J. P., Bublitz, M., Mouritsen, O. G., Egebjerg, J., et al. (2010). Neurological disease mutations compromise a C-terminal ion pathway in the Na(+)/K(+)-ATPase. *Nature* 467, 99–102. doi: 10.1038/nature09309

Prasad, H. C., Zhu, C. B., McCauley, J. L., Samuvel, D. J., Ramamoorthy, S., Shelton, R. C., et al. (2005). Human serotonin transporter variants display altered sensitivity to protein kinase G and p38 mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11545–11550. doi: 10.1073/pnas.0501432102

Pratuangdejkul, J., Schneider, B., Jaudon, P., Rosilio, V., Baudoin, E., Loric, S., et al. (2005). Definition of an uptake pharmacophore of the serotonin transporter through 3D-QSAR analysis. *Curr. Med. Chem.* 12, 2393–2410. doi: 10.2174/0929867054864813

Pratuangdejkul, J., Schneider, B., Launay, J. M., Kellermann, O., and Manivet, P. (2008). Computational approaches for the study of serotonin and its membrane transporter SERT: implications for drug design in neurological sciences. *Curr. Med. Chem.* 15, 3214–3227. doi: 10.2174/092986708786848523

Rajamanickam, J., Annamalai, B., Rahbek-Clemmensen, T., Sundaramurthy, S., Gether, U., Jayanthi, L. D., et al. (2015). Akt-mediated regulation of antidepressant-sensitive serotonin transporter function, cell-surface expression and phosphorylation. *Biochem. J.* 468, 177–190. doi: 10.1042/BJ20140826

Ramamoorthy, S., Bauman, A. L., Moore, K. R., Han, H., Yang-Feng, T., Chang, A. S., et al. (1993). Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc. Natl. Acad. Sci. U.S.A.* 90, 2542–2546. doi: 10.1073/pnas.90.6.2542

Ramamoorthy, S., and Blakely, R. D. (1999). Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. *Science* 285, 763–766. doi: 10.1126/science.285.5428.763

Ramamoorthy, S., Giovanetti, E., Qian, Y., and Blakely, R. D. (1998). Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. *J. Biol. Chem.* 273, 2458–2466. doi: 10.1074/jbc.273.4.2458

Ramamoorthy, S., Ramamoorthy, J. D., Prasad, P. D., Bhat, G. K., Mahesh, V. B., Leibach, F. H., et al. (1995). Regulation of the human serotonin transporter by

interleukin-1 beta. *Biochem. Biophys. Res. Commun.* 216, 560–567. doi: 10.1006/bbrc.1995.2659

Ramamoorthy, S., Samuvel, D. J., Buck, E. R., Rudnick, G., and Jayanthi, L. D. (2007). Phosphorylation of threonine residue 276 is required for acute regulation of serotonin transporter by cyclic GMP. *J. Biol. Chem.* 282, 11639–11647. doi: 10.1074/jbc.M611353200

Reader, B. F., Jarrett, B. L., McKim, D. B., Wohleb, E. S., Godbout, J. P., and Sheridan, J. F. (2015). Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. *Neuroscience* 289, 429–442. doi: 10.1016/j.neuroscience.2015.01.001

Robson, M. J., Quinlan, M. A., and Blakely, R. D. (2017). Immune system activation and depression: roles of serotonin in the central nervous system and periphery. *ACS Chem. Neurosci.* 8, 932–942. doi: 10.1021/acschemneuro.6b00412

Rudnick, G. (1977). Active transport of 5-hydroxytryptamine by plasma membrane vesicles isolated from human blood platelets. *J. Biol. Chem.* 252, 2170–2174.

Rudnick, G., and Nelson, P. J. (1978). Platelet 5-hydroxytryptamine transport, an electroneutral mechanism coupled to potassium. *Biochemistry* 17, 4739–4742. doi: 10.1021/bi00615a021

Sakai, N., Kodama, N., Ohmori, S., Sasaki, K., and Saito, N. (2000). Involvement of the actin cytoskeleton in the regulation of serotonin transporter (SET) activity: possible mechanism underlying SET regulation by protein kinase C. *Neurochem. Int.* 36, 567–579. doi: 10.1016/S0197-0186(99)00160-6

Sakai, N., Sasaki, K., Nakashita, M., Honda, S., Ikegaki, N., and Saito, N. (1997). Modulation of serotonin transporter activity by a protein kinase C activator and an inhibitor of type 1 and 2A serine/threonine phosphatases. *J. Neurochem.* 68, 2618–2624. doi: 10.1046/j.1471-4159.1997.68062618.x

Salim, S., Chugh, G., and Asghar, M. (2012). Inflammation in anxiety. *Adv. Protein Chem. Struct. Biol.* 88, 1–25. doi: 10.1016/B978-0-12-398314-5.00001-5

Scanlon, S. M., Williams, D. C., and Schloss, P. (2001). Membrane cholesterol modulates serotonin transporter activity. *Biochemistry* 40, 10507–10513. doi: 10.1021/bi010730z

Schwamborn, R., Brown, E., and Haase, J. (2016). Elevation of cortical serotonin transporter activity upon peripheral immune challenge is regulated independently of p38 mitogen-activated protein kinase activation and transporter phosphorylation. *J. Neurochem.* 137, 423–435. doi: 10.1111/jnc.13596

Seimandi, M., Seyer, P., Park, C. S., Vandermoere, F., Chanrion, B., Bockaert, J., et al. (2013). Calcineurin interacts with the serotonin transporter C-terminus to modulate its plasma membrane expression and serotonin uptake. *J. Neurosci.* 33, 16189–16199. doi: 10.1523/JNEUROSCI.0076-13.2013

Seyer, P., Vandermoere, F., Cassier, E., Bockaert, J., and Marin, P. (2016). Physical and functional interactions between the serotonin transporter and the neutral amino acid transporter ASCT2. *Biochem. J.* 473, 1953–1965. doi: 10.1042/BCJ20160315

Song, N. N., Xiu, J. B., Huang, Y., Chen, J. Y., Zhang, L., Gutknecht, L., et al. (2011). Adult raphe-specific deletion of Lmx1b leads to central serotonin deficiency. *PLoS One* 6:e15998. doi: 10.1371/journal.pone.0015998

Sorensen, L., Stromgaard, K., and Kristensen, A. S. (2014). Characterization of intracellular regions in the human serotonin transporter for phosphorylation sites. *ACS Chem. Biol.* 9, 935–944. doi: 10.1021/cb4007198

Sukoff Rizzo, S. J., Neal, S. J., Hughes, Z. A., Beyna, M., Rosenzweig-Lipson, S., Moss, S. J., et al. (2012). Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. *Transl. Psychiatry* 2:e199. doi: 10.1038/tp.2012.120

Tate, C. G., and Blakely, R. D. (1994). The effect of N-linked glycosylation on activity of the Na(+)- and Cl(-)-dependent serotonin transporter expressed using recombinant baculovirus in insect cells. *J. Biol. Chem.* 269, 26303–26310.

Tork, I. (1990). Anatomy of the serotonergic system. *Ann. N. Y. Acad. Sci.* 600, 9–34; discussion 34–35. doi: 10.1111/j.1749-6632.1990.tb16870.x

Trueta, C., Kuffler, D. P., and De-Miguel, F. F. (2012). Cycling of dense core vesicles involved in somatic exocytosis of serotonin by leech neurons. *Front. Physiol.* 3:175. doi: 10.3389/fphys.2012.00175

Tuomisto, J., and Tukiainen, E. (1976). Decreased uptake of 5-hydroxytryptamine in blood platelets from depressed patients. *Nature* 262, 596–598. doi: 10.1038/262596a0

Tuomisto, J., Tukiainen, E., and Ahlfors, U. G. (1979). Decreased uptake of 5-hydroxytryptamine in blood platelets from patients with endogenous depression. *Psychopharmacology* 65, 141–147. doi: 10.1007/BF00433040

Ushijima, K., Koyanagi, S., Sato, Y., Ogata, T., Matsunaga, N., Fujimura, A., et al. (2012). Role of activating transcription factor-4 in 24-hour rhythm of serotonin transporter expression in the mouse midbrain. *Mol. Pharmacol.* 82, 264–270. doi: 10.1124/mol.112.079079

Vaughan, R. A. (2004). Phosphorylation and regulation of psychostimulant-sensitive neurotransmitter transporters. *J. Pharmacol. Exp. Ther.* 310, 1–7. doi: 10.1124/jpet.103.052423

Walther, D. J., Peter, J. U., Winter, S., Holtje, M., Paulmann, N., Grohmann, M., et al. (2003). Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 115, 851–862. doi: 10.1016/S0092-8674(03)01014-6

Wang, D., Szyf, M., Benkelfat, C., Provencal, N., Turecki, G., Caramaschi, D., et al. (2012). Peripheral SLC6A4 DNA methylation is associated with in vivo measures of human brain serotonin synthesis and childhood physical aggression. *PLoS One* 7:e39501. doi: 10.1371/journal.pone.0039501

Wankerl, M., Miller, R., Kirschbaum, C., Hennig, J., Stalder, T., and Alexander, N. (2014). Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. *Transl. Psychiatry* 4:e402. doi: 10.1038/tp.2014.37

Wendland, J. R., Moya, P. R., Kruse, M. R., Ren-Patterson, R. F., Jensen, C. L., Timpano, K. R., et al. (2008). A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive-compulsive disorder. *Hum. Mol. Genet.* 17, 717–723. doi: 10.1093/hmg/ddm343

Wersinger, C., Rusnak, M., and Sidhu, A. (2006). Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. *Eur. J. Neurosci.* 24, 55–64. doi: 10.1111/j.1460-9568.2006.04900.x

Wersinger, C., and Sidhu, A. (2009). Partial regulation of serotonin transporter function by gamma-synuclein. *Neurosci. Lett.* 453, 157–161. doi: 10.1016/j.neulet.2009.02.033

Yammamoto, H., Tanaka, S., Tanaka, A., Hide, I., Seki, T., and Sakai, N. (2013). Long-term exposure of RN46A cells expressing serotonin transporter (SERT) to a cAMP analog up-regulates SERT activity and is accompanied by neural differentiation of the cells. *J. Pharmacol. Sci.* 121, 25–38. doi: 10.1254/jphs.12229FP

Yang, Y., Hu, Z., Du, X., Davies, H., Huo, X., and Fang, M. (2017). miR-16 and fluoxetine both reverse autophagic and apoptotic change in chronic unpredictable mild stress model rats. *Front. Neurosci.* 11:428. doi: 10.3389/fnins.2017.00428

Yoon, Y., McKenna, M. C., Rollins, D. A., Song, M., Nuriel, T., Gross, S. S., et al. (2013). Anxiety-associated alternative polyadenylation of the serotonin transporter mRNA confers translational regulation by hnRNPK. *Proc. Natl. Acad. Sci. U.S.A.* 110, 11624–11629. doi: 10.1073/pnas.1301485110

Zarpellon, A., Donella-Deana, A., Folda, A., Turetta, L., Pavanetto, M., and Deana, R. (2008). Serotonin (5-HT) transport in human platelets is modulated by Src-catalysed Tyr-phosphorylation of the plasma membrane transporter SERT. *Cell Physiol. Biochem.* 21, 87–94. doi: 10.1159/000113750

Zhang, Y. W., Turk, B. E., and Rudnick, G. (2016). Control of serotonin transporter phosphorylation by conformational state. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2776–E2783. doi: 10.1073/pnas.1603282113

Zhu, C. B., Blakely, R. D., and Hewlett, W. A. (2006). The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology* 31, 2121–2131. doi: 10.1038/sj.npp.1301029

Zhu, C. B., Carneiro, A. M., Dostmann, W. R., Hewlett, W. A., and Blakely, R. D. (2005). p38 MAPK activation elevates serotonin transport activity via

a trafficking-independent, protein phosphatase 2A-dependent process. *J. Biol. Chem.* 280, 15649–15658. doi: 10.1074/jbc.M410858200

Zhu, C. B., Hewlett, W. A., Feoktistov, I., Biaggioni, I., and Blakely, R. D. (2004). Adenosine receptor, protein kinase G, and p38 mitogen-activated protein kinase-dependent up-regulation of serotonin transporters involves both transporter trafficking and activation. *Mol. Pharmacol.* 65, 1462–1474. doi: 10.1124/mol.65.6.1462

Zhu, C. B., Lindler, K. M., Owens, A. W., Daws, L. C., Blakely, R. D., and Hewlett, W. A. (2010). Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology* 35, 2510–2520. doi: 10.1038/npp.2010.116

Ziv, L., Muto, A., Schoonheim, P. J., Meijsing, S. H., Strasser, D., Ingraham, H. A., et al. (2013). An affective disorder in zebrafish with mutation of the glucocorticoid receptor. *Mol. Psychiatry* 18, 681–691. doi: 10.1038/mp.2012.64

Conflict of Interest Statement: J-ML has non-financial competing interests with Hoffmann-La Roche Ltd., laboratories. He acts as an expert witness for Hoffmann-La Roche Ltd., laboratories. This does not alter his adherence to all Frontiers in Neuroscience policies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Baudry, Pietri, Launay, Kellermann and Schneider. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

MINI REVIEW
published: 27 February 2019
doi: 10.3389/fnins.2019.00156

Targeting Serotonin Transporters in the Treatment of Juvenile and Adolescent Depression

Melodi A. Bowman[1] *and Lynette C. Daws*[1,2]*

[1] Department of Cellular and Integrative Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, [2] Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

Depression is a serious public health concern. Many patients are not effectively treated, but in children and adolescents this problem is compounded by limited pharmaceutical options. Currently, the Food and Drug Administration approves only two antidepressants for use in these young populations. Both are selective serotonin reuptake inhibitors (SSRIs). Compounding matters further, they are therapeutically less efficacious in children and adolescents than in adults. Here, we review clinical and preclinical literature describing the antidepressant efficacy of SSRIs in juveniles and adolescents. Since the high-affinity serotonin transporter (SERT) is the primary target of SSRIs, we then synthesize these reports with studies of SERT expression/function during juvenile and adolescent periods. Preclinical literature reveals some striking parallels with clinical studies, primary among them is that, like humans, juvenile and adolescent rodents show reduced antidepressant-like responses to SSRIs. These findings underscore the utility of preclinical assays designed to screen drugs for antidepressant efficacy across ages. There is general agreement that SERT expression/function is lower in juveniles and adolescents than in adults. It is well established that chronic SSRI treatment decreases SERT expression/function in adults, but strikingly, SERT expression/function in adolescents is increased following chronic treatment with SSRIs. Finally, we discuss a putative role for organic cation transporters and/or plasma membrane monoamine transporter in serotonergic homeostasis in juveniles and adolescents. Taken together, fundamental differences in SERT, and putatively in other transporters capable of serotonin clearance, may provide a mechanistic basis for the relative inefficiency of SSRIs to treat pediatric depression, relative to adults.

Keywords: serotonin, transporters, depression, antidepressants, development, children, juveniles, adolescents

OPEN ACCESS

Edited by:
Guillaume Lucas,
INSERM U1215 Neurocentre Magendie, France

Reviewed by:
Renato Corradetti,
Università degli Studi di Firenze, Italy
Thorsten Lau,
Central Institute for Mental Health, Germany
Ove Wiborg,
Aalborg University, Denmark

***Correspondence:**
Lynette C. Daws
daws@uthscsa.edu

Received: *03 November 2018*
Accepted: *11 February 2019*
Published: *27 February 2019*

Citation:
Bowman MA and Daws LC (2019) Targeting Serotonin Transporters in the Treatment of Juvenile and Adolescent Depression. Front. Neurosci. 13:156. doi: 10.3389/fnins.2019.00156

CHILD AND ADOLESCENT DEPRESSION: OVERVIEW

Major depressive disorder, one of the most common forms of depression, affects individuals of all ages with approximately 5.7% of children and 11.3% of adolescents experiencing major depressive episodes (Fuhrmann et al., 2014; Mojtabai et al., 2016). Hallmarks of major depressive disorder include feelings of sadness for long periods, anhedonia, significant increases or decreases in weight, sleeping disturbances, psychomotor retardation or agitation, lethargy, feelings of helplessness

and hopelessness, difficulty concentrating and/or decision making, and suicidal thoughts (American Psychiatric Association, 2013). Diagnosis of major depressive disorder is based on an individual having at least five of these symptoms for two or more weeks. However, symptoms of depression do not present themselves the same in all ages. Children and adolescents have a more pernicious onset of symptoms and experience more irritability than adults (Hazell, 2009).

Depression is a serious public health concern. Many patients are not effectively treated, but in children and adolescents this problem is compounded by limited pharmaceutical options (for review, see Bylund and Reed, 2007). Only two selective serotonin reuptake inhibitors (SSRIs), fluoxetine (Prozac), and escitalopram (Lexapro), are currently approved by the United States Food and Drug Administration (FDA) for use in these young populations. However, children and adolescents are less effectively treated by these antidepressants compared with adults, further exacerbating the situation (Tsapakis et al., 2008; Hetrick et al., 2010, 2012). Moreover, treatment response to escitalopram is especially poor in children and adolescents carrying a common variant of the serotonin transporter (SERT) gene, which confers reduced SERT functionality (Kronenberg et al., 2007). Given the high prevalence of adolescent depression, affecting 4–8% of the population with a potential lifetime incidence as high as 25% (Kessler et al., 2001; Cheung et al., 2005; Bujoreanu et al., 2011; Thapar et al., 2012; Maughan et al., 2013) and, of great concern, the high incidence of suicide in this young population ("the third leading cause of death in the 15–19 years age group"; Reed et al., 2008), there is a clear and urgent need to establish the neural mechanisms underlying these differences between children and adolescents on the one hand and adults on the other, with the goal to discover novel targets for the development of improved treatments.

In spite of numerous reports documenting that the antidepressant response of children and adolescents is less than that of adults (Hazell et al., 2002; Bylund and Reed, 2007; Tsapakis et al., 2008; Hetrick et al., 2010, 2012), studies investigating the underlying mechanisms are lacking. That the brain is still undergoing rapid development during childhood and adolescent periods (Lenroot and Giedd, 2006) no doubt contributes, at least in part, to different treatment responses between children and adolescents, and adults. However, the neural reasons for the age-dependency of antidepressant response remain poorly understood. SSRIs, the most commonly prescribed antidepressants, and the only class approved for children and adolescents, act by inhibiting serotonin uptake via SERT, the high-affinity transporter for serotonin. This focused review aims to synthesize current clinical and preclinical literature investigating the utility of the SSRI class of antidepressant in the treatment of childhood and adolescent depression, together with what is known about SERT expression and function during these developmental periods. Importantly, we discuss findings from preclinical studies that are beginning to shed light on putative mechanisms accounting for the relatively poor response of children and adolescents to SSRIs relative to adults.

AVAILABLE TREATMENTS

The safety and effectiveness of many antidepressants for use in children and adolescents have been extensively studied and reviewed (e.g., Maneeton and Srisurapanont, 2000; Hazell et al., 2002; Courtney, 2004; Hazell, 2004; Whittington et al., 2004; Bhatia and Bhatia, 2007). Fluoxetine is FDA approved for children and adolescents as young as 8 years and older, and escitalopram is approved for children and adolescents age 12 and older. In addition to these, other antidepressants can be prescribed off label. For example, sertraline and fluvoxamine (also SSRIs) are approved to treat obsessive compulsive disorder in children and adolescents and therefore can be prescribed "off label" to treat depression in these young populations (Kodish et al., 2011). The numbers of prescriptions for antidepressants continues to increase in individuals over the age of six with approximately 3% of individuals under 18 years of age being prescribed an antidepressant (Kafali et al., 2018).

Whether these treatments yield clinical benefits remains controversial (see **Table 1**). For example, fluoxetine was originally found to be effective in treating children and adolescents with major depressive disorder (Emslie et al., 1997, 2002), but using fluoxetine as a positive control in later studies examining the efficacy of other SSRIs, the same investigators found no difference between fluoxetine and placebo treated groups (Atkinson et al., 2014; Emslie et al., 2014). As summarized in **Table 1**, randomized controlled trials conducted in children and adolescents have yielded variable results across studies and types of SSRIs, with roughly equal numbers reporting therapeutic benefit versus no difference from placebo. These mixed results are likely attributable, at least in part, to differences in population samples and symptomology scales used, with no clear pattern emerging with regard to dose or duration of treatment. In turn, this highlights a need to refine and standardize the diagnostic criterion for depression in juveniles and adolescents, which likely differs from that used to assess depression in adults. Regardless, based on current clinical literature it is difficult to conclude whether or not SSRIs are beneficial in the treatment of depressive disorders in children and adolescents. To this end, preclinical studies are beginning to provide insights into the utility of SSRIs as antidepressants in these young populations.

LESSONS LEARNED FROM PRECLINICAL STUDIES

Preclinical studies using murine models afford well-controlled conditions to study normal brain development, as well as the neural and behavioral effects of SSRIs, and other classes of antidepressants, in juveniles and adolescents. While prior to 2008 only a handful of studies had examined antidepressant-like activity of SSRIs in adolescent rodents (and none in juveniles), research in this arena has been steadily increasing during the past decade (see **Table 2**). These studies are discussed in the following sections.

TABLE 1 | Clinical studies examining the efficacy of antidepressant medications in children and adolescents.

Age (years)	SSRI	Dose and duration	Outcome	Reference
13–18	Citalopram	10 mg/day 12 weeks	No difference from placebo	von Knorring et al., 2006
7–17	Citalopram	20–40 mg/day 8 weeks	Improved treatment compared to placebo	Wagner et al., 2004
7–17	Duloxetine	60–120 mg/day 36 weeks	No difference from placebo	Atkinson et al., 2014
7–17	Duloxetine	30, 60, 90, or 120 mg/day 36 weeks	No difference from placebo	Emslie et al., 2014
12–17	Escitalopram	10 or 20 mg/day 8 weeks	Improved treatment compared to placebo	Emslie et al., 2009
12–17	Escitalopram	10–20 mg/day 24 weeks	Improved treatment compared to placebo	Findling et al., 2013
6–17	Escitalopram	10–20 mg/day 8 weeks	No difference from placebo	Wagner et al., 2006
7–17	Fluoxetine	20 mg/day 8 weeks	Improved treatment compared to placebo	Emslie et al., 1997
8–18	Fluoxetine	10 or 20 mg/day 9 weeks	Improved treatment compared to placebo	Emslie et al., 2002
12–17	Fluoxetine	10–40 mg/day 12 weeks	Improved treatment compared to placebo	March et al., 2004
13–18	Fluoxetine	20, 40, or 60 mg/day 7 weeks	No difference from placebo	Simeon et al., 1990
13–18	Paroxetine	20–40 mg/day 12 weeks	No difference from placebo	Berard et al., 2006
7–17	Paroxetine	10–50 mg/day 8 weeks	No difference from placebo	Emslie et al., 2006
12–18	Paroxetine	20–40 mg/day 8 weeks	Improved treatment compared to placebo	Keller et al., 2001
6–17	Sertraline	50–200 mg/day 6 weeks	Improved treatment compared to placebo	Alderman et al., 1998
6–17	Sertraline	50–200 mg/day 10 weeks	Improved treatment compared to placebo	Wagner et al., 2003
12–17	Vilazodone	15 or 30 mg/day 8 weeks	No difference from placebo	Durgam et al., 2018

Antidepressant-Like Efficacy of SSRIs in Juvenile and Adolescent Rodents

In rodents, postnatal days (P) 21–27 are considered the juvenile period, P28–42 early adolescence and P44–56 late adolescence (Spear, 2000; Bylund and Reed, 2007; Hefner and Holmes, 2007; Pechnick et al., 2008; Reed et al., 2008). Although there are still relatively few publications investigating antidepressant-like efficacy of SSRIs in juvenile and adolescent rodents, the literature is growing, and revealing some interesting patterns, discussed below (**Table 2**).

Acute Antidepressant-Like Effects of SSRIs

Studies examining the acute effects of antidepressant drugs have used the forced swim test (FST) and/or tail suspension test (TST), the gold standards for assaying antidepressant-like activity in rodents (Cryan and Holmes, 2005; Castagné et al., 2011). While there are some inconsistencies among reports (**Table 2A**), the most common finding is that SSRIs are less effective in juvenile and adolescent rodents, than in adults (Bourin et al., 1998; Mason et al., 2009; Mitchell et al., 2013, 2017). This is in line with clinical reports (Tsapakis et al., 2008; Hetrick et al., 2010, 2012), and underscores the utility of these preclinical tests as predictors of antidepressant-like efficacy in humans.

Interestingly, Mason et al. (2009) found that age-dependent differences in antidepressant-like response were also dependent on mouse strain and test used. For example, in the TST fluoxetine (20 mg/kg) produced an antidepressant-like effect in juvenile (P35) and adult C57BL/6J and F2 mice, but not in BALB/C mice. However, when using the FST, juvenile and adult C57BL/6 mice were insensitive to fluoxetine, but F2 and BALB/C mice responded. This is not surprising in view of elegant studies from Lucki's group using adult mice to show clear strain-dependent differences in response to fluoxetine in the FST (Lucki et al., 2001) and to citalopram in the TST (Crowley et al., 2005). Regardless, in the Mason study, when antidepressant effects were detectable they were less in juveniles than in adults when using the FST, and less than or equivalent to those of adults when using the TST.

While the majority of studies have used mice, one study used rats (Reed et al., 2008). Using the FST, these investigators found that the SSRIs fluoxetine and escitalopram produced

TABLE 2 | Preclinical studies examining the antidepressant-like effects of SSRIs (2A), effect of chronic SSRI treatment on SERT expression (2B), and age-dependent shifts in SERT expression (2C) in juvenile and adolescent rodents, compared to adults.

A: Behavioral outcomes from studies of antidepressant-like activity

Age	Species	Strain	Sex	Drug	Dose/duration	Assay	Outcome	Reference
Behavioral studies – acute administration								
P21 compared with adult (age not specified)	Rat	Sprague-Dawley	Male	Escitalopram (ESC) Desipramine (DMI) Fluoxetine (FLX) Imipramine (IMI)	1–20 mg/kg 1–20 mg/kg 0.1–10 mg/kg 0.1–10 mg/kg Drugs given ip 23 h, 5 h, and 1 h prior to FST	FST	FLX and ESC produced similar, dose-dependent antidepressant-like effects in P21 and adults rats, however, DMI and IMI were without effect in both ages. Both ESC and DMI produced antidepressant-like effects in adult rats (FLX and IMI were not tested in adults).	Reed et al., 2008
P21 and P28 compared with P90	Mouse	C57BL/6J	Male and Female	Escitalopram (ESC) Desipramine (DMI)	10 mg/kg, sc 32 mg/kg, ip Drugs given 30 min prior to test	TST	Antidepressant-like effects were observed in all ages. However, ESC was less effective in P21 mice compared to P28 and P90 mice. No sex differences.	Mitchell et al., 2013
P21 and P28 compared with P90	Mouse	C57BL/6J SERT+/+ SERT+/– SERT–/–	Male and Female	Escitalopram (ESC)	0.32–10 mg/kg, sc, given 30 min prior to test	TST	Antidepressant-like effects were observed in all ages. Maximal effects were less in P21 mice than in P90 mice, and more so in SERT+/– mice. The potency for ESC to produce antidepressant-like effects in SERT+/+ and SERT+/– mice was greater in P21 and P28 mice than in adults. No effect of ESC in SERT–/– mice. No sex differences.	Mitchell et al., 2016
P28 compared with P84, P168, and P280	Mouse	Swiss	Male	Citalopram (CIT) Paroxetine (PRX) Imipramine (IMI) Desipramine (DMI) Bupropion (BUP) Moclobemide (MOC)	2–32 mg/kg 1–8 mg/kg 4–32 mg/kg 2–16 mg/kg 4–64 mg/kg 8–128 m/kg Drugs given ip 30 min prior to test	FST	At low doses, CIT and PRX reduced immobility time in P280 mice, but not younger mice. At higher doses, CIT and PRX were inactive in P280 mice, but active in younger mice. IMI, DMI and BUP reduced immobility in all age groups. MOC reduced immobility only at the highest dose, and only in P84 mice.	Bourin et al., 1998
P28 compared with P280	Mouse	Swiss	Male	Fluvoxamine (FLV) Sertraline (SER) Venlafaxine (VEN) Imipramine (IMI) Maprotiline (MAP)	4–16 mg/kg 8–32 mg/kg 4–16 mg/kg 8–32 mg/kg 8–32 mg/kg Drugs given ip 30 min prior to test	FST	At higher doses, all antidepressants were effective in both ages, with the exception of FLV in P280 mice. Reduction in time spent immobile was greater in P28 mice than P280 mice following SER, IMI and MAP.	David et al., 2001
P35 compared with P84–91	Mouse	C57BL/6J F2 BALB/cJ	Male	Fluoxetine (FLX) Imipramine (IMI)	10 and 20 mg/kg 10 and 30 mg/kg Drugs given ip 30 min prior to TST, and 24 h, 18 h, and 1 h prior to FST.	TST and FST	FLX produced an antidepressant-like effect in F2 and C57 P35 and adult mice but not BALB/cJ P35 and adult mice in the TST. In the FST, FLX produced an antidepressant-like effect in adult mice of all strains, however, only in F2 juvenile mice. IMI produced an antidepressant-like effect across all strains and ages of mice in both tests.	Mason et al., 2009

(Continued)

TABLE 2 | Continued

A: Behavioral outcomes from studies of antidepressant-like activity

Age	Species	Strain	Sex	Drug	Dose/duration	Assay	Outcome	Reference
Behavioral studies – chronic administration								
P21 and P28 compared with adult (age not specified)	Rat	Sprague-Dawley	Male	Escitalopram (ESC) Desipramine (DMI)	10 mg/kg 3–15 mg/kg All drugs administered ip twice per day for 7 days	Inescapable shock and shuttle box test	ESC produced an antidepressant-like effect in all ages. DMI did not produce an effect in P21 rats, but did in P28 and adult rats.	Reed et al., 2009
P25 compared with P65	Rat	Wistar	Male	Fluoxetine (FLX)	5 mg/kg, ip for 3 weeks with a 1 week washout	FST	No antidepressant-like effect in either age.	Bouet et al., 2012
P25–49 compared with P67–88 at start of treatment	Rat	Wistar	Male	Fluoxetine (FLX)	12 mg/kg, oral gavage for 21 days	FST, 10 days after last dose.	FLX increased immobility time in adolescents, and had no effect in adults.	Homberg et al., 2011
P28–49 compared with P70–91	Rat	Wistar	Male	Paroxetine (PRX)	5 and 10 mg/kg, drinking water for 18 days	FST	PRX did not produce an antidepressant-like effect in adolescent rats, however, it did in adult rats.	Karanges et al., 2011

B: SERT expression after chronic SSRI treatment

Age	Species	Strain	Sex	Drug	Dose and Duration	Assay	Outcome	Reference
P25 compared with P50	Rat	Wistar	Male	Fluoxetine (FLX)	5 mg/kg, drinking water for 2 weeks	[^{3}H]paroxetine binding assay	Increase in SERT expression in the frontal cortex in rats treated with FLX starting at P25, however, not in rats treated with FLX starting at P50.	Wegerer et al., 1999
P25 compared with P65	Rat	Wistar	Male	Fluoxetine (FLX)	5 mg/kg, ip for 3 weeks with a 1 week washout	*Ex vivo* binding assay with [^{123}I]β-citalopram and *in vivo* pharmacological MRI (phMRI)	In P25 rats, there was an increase in binding after FLX treatment in the prefrontal cortex and hippocampus. In P65 rats, there was a decrease in binding in the occipital and cingulate cortex after treatment with FLX. phMRI did not indicate changes in level of activation of brain areas after treatment with FLX in either P25 or P65 rats.	Bouet et al., 2012
P28–49 compared with P70–91	Rat	Wistar	Male	Paroxetine (PRX)	5 and 10 mg/kg, drinking water for 18 days	Autoradiography with [^{125}I]RTI-55	SERT density in the basolateral amygdala was increased in adolescent rats treated with PRX compared to control, but not in adults. There were no differences in SERT density in the CA3 of the hippocampus between rats treated with PRX and control in adolescent and adult rats.	Karanges et al., 2011
Two-year old*	Monkey	Rhesus	Male	Fluoxetine (FLX)	3 mg/kg/day for 1 year in mashed banana, with a 1.5 year washout	Positron emission tomography (PET) with [^{11}C]DASB	SERT expression was increased in neocortex, hippocampus, lateral temporal and cingulate cortices.	Shrestha et al., 2014

(Continued)

TABLE 2 | Continued

C: Post-natal ontogeny of SERT

Age	Species	Strain	Sex	Assay	Outcome	Reference
P0, P7, P14, P21, P28, P70	Rat	Wistar	Male	Quantitative autoradiography with [^{3}H]*N,N*-dimethyl-2-(2-amino-4-methylphenylthio) benzylamine (MADAM)	In terminal regions such as amygdala and hypothalamus, expression peaked around P21, decreased at P28 and plateaued through P70.	Galineau et al., 2004
P21 and P28 compared with P90	Mouse	C57BL/6J	Male and female	[^{3}H]Citalopram saturation binding in hippocampal homogenates	There was no difference in [^{3}H]citalopram maximal binding or affinity across ages. However, there was significantly greater variability in affinity for [^{3}H]citalopram binding in P21 mice compared to P28 and P90 mice.	Mitchell et al., 2013
P21 and P28 compared with P90	Mouse	C57BL/6J	Male and female	Quantitative autoradiography with [^{3}H]citalopram	SERT expression increased with age in terminal regions and decreased with age in cell body regions.	Mitchell et al., 2016
P24–32 and P40–41 compared with 3–5 months and 12–14 months	Rat	Wistar	Male	Immunofluorescence	Prepubertal (P24–32) and pubertal (P40–41) rats exhibited lower SERT immunoreactivity in the lateral septum and dorsal raphe compared to young adult (3–5 months) rats with no difference in SERT immunoreactivity compared to middle age rats.	Ulloa et al., 2014
P25 compared with P65	Rat	Wistar	Male	*Ex vivo* binding assay with [^{123}I]β-citalopram and phMRI	[^{123}I]β-citalopram binding in the prefrontal cortex and cingulate cortex was lower in P25 rats compared to P65 rats, however, it was higher in the raphe nuclei of P25 rats compared to P65 rats.	Bouet et al., 2012
P28–49 compared with P70–91	Rat	Wistar	Male	Autoradiography with [^{125}I]RTI-55	There were no significant differences between adolescents and adults in [^{125}I]RTI-55 binding in either BLA or CA3 region of hippocampus.	Karanges et al., 2011

**Note that puberty occurs in Rhesus macaques between 2.5 and 4.5 years (Colman et al., 2009).*

antidepressant-like effects that were of a similar magnitude in P21 and adult rats (**Table 2A**). Interestingly, they found that the tricyclic antidepressants, desipramine and imipramine, were ineffective in P21 rats, but produced robust antidepressant-like effects in adults. While the mechanistic underpinnings for these results are yet to be determined, a common view is that delayed maturation of the norepinephrine neurotransmitter system relative to serotonin is a factor (Bylund and Reed, 2007). However, in contrast to the rat study, mouse studies generally found tricyclic antidepressants to be either equally effective in producing antidepressant-like effects in the TST and FST in juveniles, adolescents, and adults (Bourin et al., 1998; Mason et al., 2009; Mitchell et al., 2013), or more efficacious in juveniles and adolescents than in adults (David et al., 2001; Mitchell et al., 2017), suggesting that in mice, the norepinephrine neurotransmitter system matures faster than in the rat. In humans, tricyclics are not prescribed for children and adolescents due to poor tolerability (e.g., vertigo, tremors, low blood pressure, and dry mouth; Andersen et al., 2009; Dell'Osso et al., 2011; Hazell and Mirzaie, 2013). However, preclinical findings from mouse studies indicate that the utility of NET, and/or dual NET and SERT blockers in the treatment of juvenile and adolescent depression warrants further investigation. It is possible that the development of new NET (and NET/SERT) targeting drugs that have fewer side effects than currently available drugs may be better suited to the treatment of pediatric depression (Mitchell et al., 2017).

Clearly, differing rates of development of noradrenergic and serotonin systems, and the development of systems that impinge on them (e.g., histamine, see Munari et al., 2015) may also factor into the magnitude of antidepressant-response as a function of age. Rates of maturation also differ among species, and is a consideration when relating findings from rodents to outcomes in humans. The reader is directed to an excellent review by Murrin et al. (2007) for information on differential rates of development of noradrenergic and serotonergic systems among species. While studies in human are relatively scant, those that exist, together with studies in non-human primates, suggest that the serotonin system matures faster than the noradrenergic system (Murrin et al., 2007). It will be of interest for future studies to precisely map treatment response to different classes of antidepressant on developmental stage of the noradrenergic and serotonergic systems in humans.

Lastly, one study included constitutive SERT heterozygote mice as a murine model of humans carrying a common variant of the SERT gene, which confers reduced SERT functionality, and who respond less well to SSRIs than those without this variant (Kronenberg et al., 2007). Consistent with clinical literature, the maximal effect of escitalopram in juvenile SERT heterozygote mice was less than that in juvenile SERT wildtype mice. Moreover, in both SERT genotypes the maximal effect in juveniles was less than that in adults (Mitchell et al., 2016). These findings again underscore the utility of preclinical models of antidepressant-like activity in probing mechanisms contributing to age- and genotype-dependent responses to antidepressants (but see section "Caveats to Currently Available Behavioral Assays for Identifying Drugs With Antidepressant Potential").

In sum, the most common finding from studies of the acute antidepressant-like effects of SSRIs in preclinical studies is that they are less effective in juveniles and adolescents than in adults, though some studies do report no difference in response among ages. With proper attention to mouse strain, and behavioral test employed, such preclinical models provide valuable tools to study the mechanistic basis for SSRIs being less effective in treating pediatric depression than in adults, as will be discussed further.

Chronic Antidepressant-Like Effects

While the TST and FST are currently the best tests available to quickly screen for drugs with antidepressant potential, for drugs to be useful clinically they must be effective after chronic administration. Numerous groups have reported antidepressant-like effects following chronic SSRI treatment in adult mice and rats, using tests such as the FST, TST, and novelty-induced hypophagia (Detke et al., 1997; Dulawa et al., 2004; Cryan et al., 2005; Holick et al., 2008; Miller et al., 2008; Balu et al., 2009; Jiao et al., 2011; Roohi-Azizi et al., 2018), with only one group reporting no effect (Griebel et al., 1999). However, only four studies have examined the antidepressant-like effect of chronic SSRI administration in juveniles and/or adolescents. All have used rats (**Table 2A**). Three used the FST, and though different SSRIs were used (fluoxetine in two studies [Homberg et al., 2011; Bouet et al., 2012] and paroxetine in the other [Karanges et al., 2011]), the overall findings were in general agreement. In the two studies where there was no washout period, chronic SSRI treatment either had no effect in young rats (Karanges et al., 2011), or increased immobility time (i.e., pro-depressive-like behavior; Homberg et al., 2011), whereas in adults there was either no effect (Homberg et al., 2011), or a robust antidepressant-like effect (Karanges et al., 2011), in agreement with published literature using adult rodents. These results are consistent with clinical literature reporting that children and adolescents respond poorly to SSRIs compared with adults, if at all (Tsapakis et al., 2008; Hetrick et al., 2010, 2012). In the one study where a 1-week washout period was allowed before testing in the FST, no antidepressant-like effect of fluoxetine was observed in either juvenile/adolescent or adult rats (Bouet et al., 2012).

Another study utilized inescapable shock and shuttle box tests to assay antidepressant-like activity following a week of twice daily injections of escitalopram or desipramine in rats. Escitalopram produced similar antidepressant-like effects in juveniles, adolescents, and adults, but desipramine was only effective in adolescent and adult rats, and ineffective in juvenile rats (Reed et al., 2009). This result is consistent with findings reported by the same group following acute administration of these drugs (Reed et al., 2008; see discussion above).

Overall, results from studies examining the chronic effects of SSRIs in rats again support the utility of preclinical assays such as these. However, given that mice seem to more reliably parallel human clinical literature, at least in the acute tests described above, it will be of interest to implement mice in studies of chronic SSRI administration as well.

Caveats to Currently Available Behavioral Assays for Identifying Drugs With Antidepressant Potential

Though the FST and TST are the current gold standards for assaying antidepressant-like activity in rodents (Cryan and Holmes, 2005), it is important to note that while the vast majority of antidepressant drugs reduce immobility time in these tests, not all drugs that reduce immobility time are antidepressants. Thus, these tests are valuable when comparing the sensitivity to drugs across ages, but they do not necessarily predict therapeutic effectiveness in the treatment of depression. In this realm, the field is in need of new tests (be they behavioral or biochemical) that can reliably predict therapeutic efficacy of novel drugs for the treatment of depression, while screening out false positives.

In addition, to date studies of juvenile and adolescent rodents in tests for antidepressant-like activity of drugs have used only naïve animals. Ideally, such tests should be performed in animal models of depression, such as those afforded by unpredictable chronic mild stress, or social defeat paradigms, to name a few (see O'Leary and Cryan, 2013 for review). While these models have been frequently applied to studies of depression in adult rodents, their application to juveniles (in particular), as well as adolescents, is impractical due to the shortness of these developmental periods (e.g., 7-day juvenile period, 14-day early adolescent, and 14-day late adolescent periods; Spear, 2000; Bylund and Reed, 2007; Hefner and Holmes, 2007; Pechnick et al., 2008; Reed et al., 2008). This is further compounded when the effects of chronic drug treatment need to be studied. While it is not within the scope of this focused review to discuss the pros and cons of existing models for depression research, the reader should be aware that there are limitations to those currently available (see O'Leary and Cryan, 2013 for review). Of course, recapitulating the heterogeneous nature of human depression, without yet fully understanding the etiologies of this complex disorder, makes developing improved animal models a challenging undertaking. However, as we learn more about the complexities of this disorder, new knowledge can be applied to the development of improved models to study depression and to screen novel compounds for therapeutic efficacy in its treatment. Importantly, models that can be applied to the study of juveniles and adolescents are sorely needed.

Expression and Function of SERT in Juvenile and Adolescent Rodents

Understanding how SERT expression and function varies across juvenile, adolescent and adult ages, and how chronic treatment with SSRIs influences SERT expression and function across these ages may lend insight into age-dependent differences in antidepressant-like responses to SSRIs. Studies investigating this subject are discussed below.

Effect of Chronic SSRI Treatment on SERT Expression and Function

In adult rodents, chronic treatment with SSRIs downregulates SERT expression (Benmansour et al., 1999; Gould et al., 2003, 2007; Mirza et al., 2007; Lin et al., 2017), which is associated with putatively reduced functionality of SERT, as demonstrated by *in vivo* chronoamperometry studies measuring clearance of serotonin from extracellular fluid in hippocampus (Benmansour et al., 1999). These decreases were not associated with reduced SERT gene expression or neurotoxicity (Benmansour et al., 1999). Several lines of evidence suggest that SSRI-induced downregulation of SERT function is attributable, at least in part, to internalization of SERT to the cytosolic compartment. For example, *in vitro* studies using Caco-2 cells transfected with human (h) SERT show that long-term exposure to fluoxetine causes internalization of hSERT, leaving less hSERT on the plasma membrane (Iceta et al., 2007). These studies showed no effect of fluoxetine treatment on either total hSERT protein or mRNA. Studies in rats found that chronic, but not acute fluoxetine treatment causes internalization of SERT in both cell bodies and terminals (Descarries and Riad, 2012). Similarly, translational approaches using stem cell-derived serotonergic neurons and a transgenic mouse expressing hSERT found that citalopram dose-dependently causes internalization of hSERT in both models (Matthäus et al., 2016). Such studies underscore the utility of complementary/translational approaches to understanding antidepressant response on a cellular and molecular level. While it remains to be determined if internalization of SERT following chronic SSRI treatment occurs in humans, and is temporally synced with therapeutic benefit, studies in adult rodents, and *in vitro*, suggests that downregulation of SERT, putatively by trafficking away from the plasma membrane, is at least in part required for ultimate therapeutic benefit following chronic treatment with SSRIs. Moreover, that it takes chronic exposure to SSRIs for this downregulation of SERT to occur, provides a potential mechanistic basis for the delay to onset of therapeutic benefit.

Currently there is no literature reporting effects of chronic SSRI treatment in juvenile rodents. This is almost certainly due to the very narrow juvenile window in rodents (i.e., P21–27). There is remarkably sparse literature on the effect of chronic SSRI treatment on SERT expression in adolescent rodents (reviewed in Daws and Gould, 2011). However, a consistent finding is that chronic treatment with SSRIs, beginning at either P25 or P28 in rodents, *increases* SERT expression in a number of brain regions (Wegerer et al., 1999; Karanges et al., 2011; Bouet et al., 2012) (**Table 2B**). These findings in rodents are further supported by a study in juvenile rhesus macaque monkeys, which found that chronic treatment with fluoxetine increased SERT expression in several brain regions, including neocortex and hippocampus (Shrestha et al., 2014) (**Table 2B**). With more SERT putatively becoming available to take up serotonin as SSRI treatment continues, the increases in extracellular serotonin that are thought to be needed to trigger the downstream cascade of events leading to ultimate therapeutic benefit could be greatly diminished. Thus, the clinical implications of increased SERT expression in children and adolescents could include a need to increase dose of SSRI in order to fully occupy SERT. It is interesting to note that initiating dosing of SSRIs for pediatric depression is generally less than that for adults. For example, initiating low doses of fluoxetine and citalopram to 5- to 12-year olds is less than 10 mg/d, whereas in adults (18–64 years) the dose is less than 20 mg/d (Bushnell et al., 2016). Before the

black-box warning on all antidepressants was issued by the FDA in 2004, the percent of patients initiating low dose SSRI treatment was similar across ages (∼15%), with the exception of 10- to 12-year olds where the percent was much lower (5%). In contrast, following the black-box warning the percent of patients initiating low dose SSRI *(relevant to their age)* increased markedly (5–9 years to ∼28%, 13–17 years to ∼38%, 18–24 years to ∼25%, 25–64 years to ∼20%, and 10–12 years to ∼11%; see Bushnell et al., 2016). In the context of the present review, this raises the possibility that increasing initial dosing in pediatric depression may be therapeutically beneficial, though clearly with this comes the risk of increased, potentially life-threatening, side-effects. It must also be kept in mind that of the clinical studies summarized in **Table 1**, SSRI doses much higher than these initiating low doses (e.g., 60 mg/day for fluoxetine; 200 mg/d sertraline) yielded mixed outcomes, some reporting therapeutic efficacy, and others no difference from placebo.

Clearly it is difficult to draw conclusions from existing literature. The important take home message here is that *increased* SERT expression following chronic SSRI treatment in children and adolescents could account, at least in part, for their therapeutic efficacy being less than that in adults. Though there are no studies of SERT in brain of children and adolescents, one study examining platelet SERT expression before and after treatment with the SSRI sertraline, provides support for this premise. Sertraline treatment was found to decrease [^{3}H]paroxetine binding affinity. Notably *increased* maximal binding of [^{3}H]paroxetine to SERT was associated with non-response (Sallee et al., 1998). Further study into the benefits of more rapidly ramping SSRI dosing in pediatric depression may therefore be warranted.

To stay within the scope of this focused review, we have discussed only the consequences of chronic SSRI treatment for their primary target, SERT. Of course this is an overly simplistic view of the neural consequences of chronic SSRI treatment in juveniles and adolescents, which has also been shown to impact glycogen synthase kinase-3 (GSK3; Beurel et al., 2012), extracellular signal-regulated protein kinase 1/2 (ERK) and cAMP response element binding protein (CREB; Alcantara et al., 2014), brain derived neurotrophic factor (BDNF) and TrkB receptors (Kozisek et al., 2008), genes associated with cell cycle and survival (Tsapakis et al., 2014), and neuroplasticity (Bastos et al., 1999; Norrholm and Ouimet, 2000; Bock et al., 2013; Klomp et al., 2014). Some of the effects of chronic SSRI treatment on these factors in juveniles and adolescents are in parallel with those reported in adults (Bastos et al., 1999), while others are not (Kozisek et al., 2008; Beurel et al., 2012; Bock et al., 2013; Alcantara et al., 2014; Klomp et al., 2014). A deeper focus on SSRI-induced changes that are specific to juveniles and adolescents will help guide future research to develop improved antidepressants for these young populations.

Expression of SERT in Juveniles, Adolescents, and Adults

Prior to 2011, remarkably little was known about the ontogeny of SERT expression during juvenile and adolescent periods (reviewed in Daws and Gould, 2011). The first paper to describe postnatal ontogeny of SERT found that in terminal regions, including amygdala and hypothalamus, expression was maximal around P21, decreased at P28, before rising again to stabilize through P70, the oldest age tested (Galineau et al., 2004; Daws and Gould, 2011). Subsequent studies of SERT expression from P21 through adulthood reported either no change across those ages (Karanges et al., 2011; Mitchell et al., 2013), or a steady increase from P21 through adulthood in terminal fields (Bouet et al., 2012; Ulloa et al., 2014; Mitchell et al., 2016), and a decrease from P21 in cell body regions (Bouet et al., 2012; Mitchell et al., 2016) (**Table 2C**). Of particular interest, Mitchell et al. (2013) using [^{3}H]citalopram saturation binding assays in hippocampal homogenates, provided evidence that SERT in juveniles may be functionally less able to transport serotonin than in adults. The modest variation in results among these studies is likely due to nuanced differences in the assays used, ages studies, and species used. However, taken together current evidence suggests that SERT expression and/or function in terminal fields is lower in juveniles than in adults, whereas expression in cell bodies is greater (**Table 2C**).

It is difficult to speculate on how these postnatal shifts in SERT expression and function ultimately play into therapeutic responsiveness to SSRIs, but this is clearly a line of investigation that warrants further research. Likewise, it will be important to determine the relationship between receptor systems that are well-known to be influenced by SSRIs (e.g., Artigas, 2013; Kulikov et al., 2018), SERT, and therapeutic response in juveniles and adolescents.

Alternative Transporter Targets for Therapeutic Intervention in Juveniles and Adolescents

In recent times, there has been increasing recognition of transporter "promiscuity" (Daws, 2009). High-affinity monoamine transporters are not loyal to their specific monoamine, and will take up other species, sometimes with greater efficiency than their own (Daws, 2009). Moreover, there is increasing awareness of "uptake-2" transporters in monoamine clearance (Gasser and Daws, 2017a,b). These are classified as transporters with low-affinity, but high-capacity to take up monoamines, and include organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT).

Of the three OCT subtypes (OCT1, OCT2, OCT3), OCT2 and OCT3 are most widely expressed in brain (Amphoux et al., 2006; Vialou et al., 2008; Gasser et al., 2009; Couroussé and Gautron, 2015). PMAT is also widely expressed in brain, including limbic regions (Engel et al., 2004; Dahlin et al., 2007). Each of these transporters is capable of clearing serotonin, norepinephrine, and dopamine from extracellular fluid, albeit with varying affinities (Koepsell et al., 2007; Duan and Wang, 2010; Wang, 2016). Emerging evidence indicates that, in adult mice, OCT3 may be a novel target for the development of new antidepressants. For example, the non-selective OCT/PMAT blocker decynium-22 produced robust antidepressant-like effects in adult SERT heterozygous and knockout mice, which have upregulated OCT3 expression, but was without effect in SERT

wildtype mice (Baganz et al., 2008). Subsequently, Horton et al. (2013) showed that decynium-22 augmented the antidepressant-like effect of a sub-effective dose of the SSRI fluvoxamine, and that this effect was attenuated in OCT3 knockout mice. Recently, novel decynium-22 analogs were synthesized and characterized. Unlike decynium-22, which lacks activity at SERT, NET, and DAT, analogs with activity at OCT3, *and modest* activity at SERT, were found to produce stand-alone antidepressant-like effects in wildtype mice. Together with the findings of Horton et al. (2013), these results support the notion that dual SERT/OCT3 blockers may be more effective antidepressants than SSRIs alone (Krause-Heuer et al., 2017; Fraser-Spears et al., 2019). In addition, studies using OCT2 knockout mice provide some evidence that OCT2 may also be a new pharmacological target for mood disorders (Bacq et al., 2012).

These findings in adult mice raise the possibility that OCTs and/or PMAT may play a mechanistic role in poor response of children and adolescents to SSRIs relative to adults. For example, based on SERT expression studies described earlier, it appears that SERT expression and perhaps function is lower in juveniles and adolescents than in adults. Putatively, serotonin clearance might be driven more by OCTs/PMAT during these post-natal periods, or perhaps these "uptake-2" transporters could be transiently expressed at greater functional levels than in adults. Though no data currently exists regarding the ontogeny of OCTs and PMAT in brain, in the periphery, OCT1 mRNA expression reaches adult levels by P15 in kidney and by P22 in liver (Alnouti et al., 2006). While it is difficult to translate these findings to changes that may occur in brain, these data support the idea that in juveniles and adolescents, OCTs and/or PMAT may already be at adult levels, or perhaps transiently expressed at higher levels. In this scenario, OCTs/PMAT could conceivably buffer any increase in serotonin following an SSRI, thereby reducing, or preventing therapeutic benefit (Daws and Gould, 2011). Studies using adult SERT heterozygote mice, which afford a murine model of humans carrying a common low expressing variant of the SERT gene, lend support to this premise. OCT3 expression is increased in these mice, presumably to compensate for reduced SERT expression/function (Baganz et al., 2008). As in humans carrying this low expressing SERT gene variant (Kronenberg et al., 2007), SERT heterozygote mice are less responsive to the antidepressant-like effect of escitalopram than SERT wildtype mice (Mitchell et al., 2016). However, SERT heterozygote mice are responsive to the antidepressant-like effect of decynium-22 (OCT/PMAT blocker), whereas SERT wildtype mice are not.

Although a role for OCTs/PMAT in antidepressant-like response of juveniles and adolescents awaits empirical verification, evidence in hand supports further investigation of these transporters in regulating homeostasis of serotonin neurotransmission (as well as that of other monoamines), during juvenile and adolescent periods.

CONCLUSION

This review of current literature examining clinical and preclinical antidepressant efficacy of SSRIs in juveniles and adolescents reveals many knowledge gaps. These range from a need to better understand the ontogeny of neural systems known to be involved in antidepressant action in adults (in both humans and rodents), to a need for improved preclinical models in which to study antidepressant efficacy (across all ages). Although the utility of SSRIs in the treatment of pediatric depression is not clear from clinical studies, preclinical research (in spite of its current limitations) is providing valuable insights. Results from preclinical studies examining the acute and chronic antidepressant-like effects of SSRIs share remarkable similarities with clinical outcomes, supporting the use of paradigms such as the TST and FST as valuable tools to screen novel compounds for their potential therapeutic utility in these young populations. Studies of SERT expression and function, though few, are revealing a striking dichotomy in the effect of chronic SSRI treatment on SERT expression. In adults SERT is downregulated, whereas in juveniles and adolescents, SERT is upregulated. Moreover, there is growing evidence for a role of OCTs and PMAT in regulating monoamine neurotransmission. Literature reviewed in this focal area of "transporters for serotonin" encourages further research into what appears to be fundamental differences in SERT, and putatively other transporters capable of serotonin uptake, between juvenile and adolescents on the one hand, and adults on the other. In doing so, novel targets for the improved treatment of depression in children and adolescents should be revealed.

AUTHOR CONTRIBUTIONS

LD conceived the content. LD and MB wrote the manuscript.

FUNDING

This work was supported by R01 MH093320, R01 MH106978, and R21 DA046044 to LD.

REFERENCES

Alcantara, L., Warren, B. L., Parise, E. M., Iñiguez, S. D., and Bolaños-Guzmán, C. A. (2014). Effects of psychotropic drugs on second messenger signaling and preference for nicotine in juvenile male mice. *Psychopharmacology* 231, 1479–1492. doi: 10.1007/s00213-014-3434-4

Alderman, J., Wolkow, R., Chung, M., and Johnston, H. F. (1998). Sertraline treatment of children and adolescents with obsessive-compulsive disorder or depression: pharmacokinetics, tolerability, and efficacy. *J. Am. Acad. Child Adolesc. Psychiatry* 37, 386–394. doi: 10.1097/00004583-199804000-00016

Alnouti, Y., Petrick, J. S., and Klaassen, C. D. (2006) Tissue distribution and ontogeny of organic cation transporters in mice. *Drug Metabol. Disposit.* 34, 477–482.

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders,* 5th Edn. Arlington, VA: American Psychiatric Publishing. doi: 10.1176/appi.books.9780890425596

Amphoux, A., Vialou, V., Drescher, E., Brüss, M., La Cour, C. M., and Gautron, S. (2006). Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. *Neuropharmacology* 50, 941–952. doi: 10.1016/j.neuropharm.2006.01.005

Andersen, J., Kirstensen, A. S., Bang-Andersen, B., and Stromgaard, K. (2009). Recent advances in the understanding of the interaction of antidepressant drugs with serotonin and norepinephrine transporters. *Chem. Commun.* 25, 3677–3692. doi: 10.1039/b903035m

Artigas, F. (2013). Serotonin receptors involved in antidepressant effects. *Pharmacol. Therap.* 137, 119–131. doi: 10.1016/j.pharmthera.2012.09.006

Atkinson, S. D., Prakash, A., Zhang, Q., Pangallo, B. A., Bangs, M. E., Emslie, G. J., et al. (2014). A double-blind efficacy and safety study of duloxetine flexible dosing in children and adolescents with major depressive disorder. *J. Child Adolesc. Psychopharmacol.* 24, 180–189. doi: 10.1089/cap.2013.0146

Bacq, A., Balasse, L., Biala, G., Guiard, B., Gardier, A. M., Schinkel, A., et al. (2012). Organic cation transporter 2 controls brain norepinephrine and serotonin clearance and antidepressant response. *Mol. Psychiatry* 17, 926–939. doi: 10.1038/mp.2011.87

Baganz, N. L., Horton, R. E., Calderon, A. S., Owens, W. A., Munn, J. L., Watts, L. T., et al. (2008). Organic cation transporter 3: keeping the brake on extracellular serotonin in serotonin-transporter deficit mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18976–18981. doi: 10.1073/pnas.0800466105

Balu, D. T., Hodes, G. E., Anderson, B. T., and Lucki, I. (2009). Enhanced sensitivity of the MRL/MpJ mouse to the neuroplastic and behavioral effects of chronic antidepressant treatments. *Neuropsychopharmacology* 34, 1764–1773. doi: 10.1038/npp.2008.234

Bastos, E. F., Marcelino, L. S., Amaral, A. R., and Serfaty, C. A. (1999). Fluoxetine-induced plasticity in the rodent visual system. *Brain Res.* 824, 28–35. doi: 10.1016/S0006-8993(99)01184-1

Benmansour, S., Cecchi, M., Morilak, D. A., Gerhardt, G. A., Javors, M. A., Gould, G. G., et al. (1999). Effects of chronic antidepressant treatments on serotonin transporter function, density, and mRNA level. *J. Neurosci.* 19, 10494–10501. doi: 10.1523/JNEUROSCI.19-23-10494.1999

Berard, R., Fong, R., Carpenter, D. J., Thomason, C., and Wilkinson, C. (2006). An international, multicenter, placebo-controlled trial of paroxetine in adolescents with major depressive disorder. *J. Child Adolesc. Psychopharmacol.* 16, 59–75. doi: 10.1089/cap.2006.16.59

Beurel, E., Mines, M. A., Song, L., and Jope, R. S. (2012). Glycogen synthase kinase-3 levels and phosphorylation undergo large fluctuations in mouse brain during development. *Bipolar Disord.* 14, 822–830. doi: 10.1111/bdi.12023

Bhatia, S. K., and Bhatia, S. C. (2007). Childhood and adolescent depression. *Am. Fam. Phys.* 75, 73–80.

Bock, N., Koc, E., Alter, H., Roessner, V., Becker, A., Rothenberger, A., et al. (2013). Chronic fluoxetine treatment changes S100B expression during postnatal rat brain development. *J. Child Adolesc. Psychopharmacol.* 23, 481–489. doi: 10.1089/cap.2011.0065

Bouet, V., Klomp, A., Freret, T., Wylezinska-Arridge, M., Lopez-Tremoleda, J., Dauphin, F., et al. (2012). Age-dependent effects of chronic fluoxetine treatment on the serotonergic system one week following treatment. *Psychopharmacology* 221, 321–339. doi: 10.1007/s00213-011-2580-1

Bourin, M., Colombel, M. C., Redrobe, J. P., Nizard, J., Hascoët, M., and Baker, G. B. (1998). Evaluation of efficacies of different classes of antidepressants in the forced swimming test in mice at different ages. *Progr. Neuro-Psychopharmacol. Biol. Psychiatry* 22, 343–351. doi: 10.1016/S0278-5846(98)00009-8

Bujoreanu, S., Benhayon, D., and Szigethy, E. (2011). Treatment of depression in children and adolescents. *Pediatr. Ann.* 40, 548–555. doi: 10.3928/00904481-20111007-05

Bushnell, G. A., Stürmer, T., Swanson, S. A., White, A., Azrael, D., Pate, V., et al. (2016). Dosing of selective serotonin reuptake inhibitors among children and adults before and after the FDA black-box warning. *Psychiat. Serv.* 67, 302–309. doi: 10.1176/appi.ps.201500088

Bylund, D. B., and Reed, A. L. (2007). Childhood and adolescent depression: why do children and adults respond differently to antidepressant drugs? *Neurochemistry Int.* 51, 246–253. doi: 10.1016/j.neuint.2007.06.025

Castagné, V., Moser, P., Roux, S., and Porsolt, R. D. (2011). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr. Protoc. Neurosci.* 8:10A. doi: 10.1002/0471142301.ns0810as55

Cheung, A. H., Emslie, G. J., and Mayes, T. L. (2005). Review of the efficacy and safety of antidepressants in youth depression. *J. Child Psychol. Psychiatry* 46, 735–754. doi: 10.1111/j.1469-7610.2005.01.01467

Colman, R. J., Anderson, R. M., and Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T., et al. (2009). Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325, 201–204. doi: 10.1126/science.1173635

Couroussé, T., and Gautron, S. (2015). Role of organic cation transporters (OCTs) in the brain. *Pharmacol. Toxicol.* 146, 94–103. doi: 10.1016/j.pharmthera.2014.09.008

Courtney, D. B. (2004). Selective serotonin reuptake inhibitor and venlafaxine use in children and adolescents with major depressive disorder: a systematic review of published randomized controlled trials. *Can. J. Psychiatry* 49, 557–563. doi: 10.1177/070674370404900807

Crowley, J. J., Blendy, J. A., and Lucki, I. (2005). Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology* 183, 257–264. doi: 10.1007/s00213-005-0166-5

Cryan, J. F., and Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat. Rev. Drug Discov.* 4, 775–790. doi: 10.1038/nrd1825

Cryan, J. F., Page, M. E., and Lucki, I. (2005). Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology* 182, 335–344. doi: 10.1007/s00213-005-0093-5

Dahlin, A., Xia, L., Kong, W., Hevner, R., and Wang, J. (2007). Expression and immunolocalization of the plasma membrane monoamine transporter in the brain. *Neuroscience* 146, 1193–1211. doi: 10.1016/j.neuroscience.2007.01.072

David, D. J., Bourin, M., Hascoët, M., Colombel, M. C., Baker, G. B., and Jolliet, P. (2001). Comparison of antidepressant activity in 4- and 40-week-old male mice in the forced swimming test: involvement of 5-HT1A and 5-HT1B receptors in old mice. *Psychopharmacology* 153, 443–449. doi: 10.1007/s002130000588

Daws, L. C. (2009). Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy. *Pharmacol. Ther.* 121, 89–99. doi: 10.1016/j.pharmthera.2008.10.004

Daws, L. C., and Gould, G. G. (2011). Ontogeny and regulation of the serotonin transporter: providing insights into human disorders. *Pharmacol. Ther.* 131, 61–79. doi: 10.1016/j.pharmthera.2011.03.013

Dell'Osso, B., Palazzo, M. C., Oldani, L., and Altamura, A. C. (2011). The noradrenergic action in antidepressant treatments: pharmacological and clinical aspects. *CNS Neurosci. Ther.* 17, 723–732. doi: 10.1111/j.1755-5949.2010.00217.x

Descarries, L., and Riad, M. (2012). Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT1A receptors and SERT. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 367, 2416–2425. doi: 10.1098/rstb.2011.0361

Detke, M. J., Johnson, J., and Lucki, I. (1997). Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. *Exp. Clin. Psychopharmacol.* 5, 107–112. doi: 10.1037/1064-1297.5.2.107

Duan, H., and Wang, J. (2010). Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. *J. Pharmacol. Exp. Ther.* 335, 743–753. doi: 10.1124/jpet.110.170142

Dulawa, S. C., Holick, K. A., Gundersen, B., and Hen, R. (2004). Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology* 29, 1321–1330. doi: 10.1038/sj.npp.1300433

Durgam, S., Chen, C., Migliore, R., Prakash, C., Edwards, J., and Findling, R. L. (2018). A phase 3, double-blind, randomized, placebo-controlled study of vilazodone in adolescents with major depressive disorder. *Paediatr. Drugs* 20, 353–363. doi: 10.1007/s40272-018-0290-4

Emslie, G. J., Heiligenstein, J. H., Wagner, K. D., Hoog, S. L., Ernest, D. E., Brown, E., et al. (2002). Fluoxetine for acute treatment of depression in children and adolescents: a placebo-controlled, randomized clinical trial. *J. Am. Acad. Child Adolesc. Psychiatry* 41, 1205–1215. doi: 10.1097/01.CHI.0000024834.94814.9A

Emslie, G. J., Prakash, A., Zhang, Q., Pangallo, B. A., Bangs, M. E., and March, J. S. (2014). A double-blind efficacy and safety study of duloxetine fixed doses in children and adolescents with major depressive disorder. *J. Child Adolesc. Psychopharmacol.* 24, 170–179. doi: 10.1089/cap.2013.0096

Emslie, G. J., Rush, A. J., Weinberg, W. A., Kowatch, R. A., Hughes, C. W., Carmody, T., et al. (1997). A double-blind, randomized, placebo-controlled trial of fluoxetine in children and adolescents with depression. *Arch. Gen. Psychiatry* 54, 1031–1037. doi: 10.1001/archpsyc.1997.01830230069010

Emslie, G. J., Ventura, D., Korotzer, A., and Tourkodimitris, S. (2009). Escitalopram in the treatment of adolescent depression: a

randomized placebo-controlled multisite trial. *J. Am. Acad. Child Adolesc. Psychiatry* 48, 721–729. doi: 10.1097/CHI.0b013e3181a2b304

Emslie, G. J., Wagner, K. D., Kutcher, S., Krulewicz, S., Fong, R., Carpenter, D. J., et al. (2006). Paroxetine treatment in children and adolescents with major depressive disorder: a randomized, multicenter, double-blind, placebo-controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry* 45, 709–719. doi: 10.1097/01.chi.0000214189.73240.63

Engel, K., Zhou, M., and Wang, J. (2004). Identification and characterization of a novel monoamine transporter in the human brain. *J. Biol. Chem.* 279, 50042–50049. doi: 10.1074/jbc.M407913200

Findling, R. L., Robb, A., and Bose, A. (2013). Escitalopram in the treatment of adolescent depression: a randomized, double-blind, placebo-controlled extension trial. *J. Child Adolesc. Psychopharmacol.* 23, 468–480. doi: 10.1089/cap.2012.0023

Fraser-Spears, R., Krause-Heuer, A. M., Basiouny, M., Mayer, F. P., Manishimwe, R., Wyatt, N. A., et al. (2019). Comparative analysis of novel decynium-22 analogs to inhibit transport by the low-affinity, high-capacity biogenic amine transporters, organic cation transporters 2 and 3, and plasma membrane monoamine transporter. *Eur. J. Pharmacol.* 842, 351–364. doi: 10.1016/j.ejphar.2018.10.028

Fuhrmann, P., Equit, M., Schmidt, K., and von Gontard, A. (2014). Prevalence of depressive symptoms and associated developmental disorders in preschool children: a population-based study. *Eur. Child Adolesc. Psychiatry* 23, 219–224. doi: 10.1007/s00787-013-0452-4

Galineau, L., Kodas, E., Guilloteau, D., Vilar, M. P., and Chalon, S. (2004). Ontogeny of the dopamine and serotonin transporters in the rat brain: an autoradiographic study. *Neurosci. Lett.* 363, 266–271. doi: 10.1016/j.neulet.2004.04.007

Gasser, P. J., and Daws, L. C. (2017a). Editorial for the special issue: monoamine transporters in health and disease. *J. Chem. Neuroanat.* 8, 1–2. doi: 10.1016/j.chemneu.2017.07.008

Gasser, P. J., and Daws, L. C. (2017b). Extending the family: roles of uptake2 transporters in regulation of monoaminergic signaling. *J. Chem. Neuroanat.* 8, 107–108. doi: 10.1016/j.chemneu.2017.07.009

Gasser, P. J., Orchinik, M., Raju, I., and Lowry, C. A. (2009). Distribution of organic cation transporter 3, a corticosterone-sensitive monoamine transporter, in the rat brain. *J. Compar. Neurol.* 512, 529–555. doi: 10.1002/cne.21921

Gould, G. G., Brooks, B. W., and Frazer, A. (2007). [(3)H] citalopram binding to serotonin transporter sites in minnow brains. *Basic Clin. Pharmacol. Toxicol.* 101, 203–210. doi: 10.1111/j.1742-7843.2007.00100.x

Gould, G. G., Pardon, M. C., Morilak, D. A., and Frazer, A. (2003). Regulatory effects of reboxetine treatment alone, or following paroxetine treatment, on brain noradrenergic and serotonergic systems. *Neuropsychopharmacology* 28, 1633–1641. doi: 10.1038/sj.npp.1300236

Griebel, G., Cohen, C., Perrault, G., and Sanger, D. J. (1999). Behavioral effects of acute and chronic fluoxetine in Wistar-Kyoto rats. *Physiol. Behav.* 67, 315–320. doi: 10.1016/S0031-9384(98)00298-4

Hazell, P. (2004). Depression in children and adolescents. *Clin. Evid.* 12, 427–442.

Hazell, P. (2009). Depression in children and adolescents. *Clin. Evid.* 1008, 1–31.

Hazell, P., and Mirzaie, M. (2013). Tricyclic drugs for depression in children and adolescents. *Cochr. Datab. Systemat. Rev.* 18:CD002317. doi: 10.1002/14651858.CD002317.pub2

Hazell, P., O'Connell, D., Heathcote, D., and Henry, D. (2002). Tricyclic drugs for depression in children and adolescents. *Cochr. Datab. Syst. Rev.* 2:CD002317. doi: 10.1002/14651858.CD002317

Hefner, K., and Holmes, A. (2007). Ontogeny of fear-, anxiety-, and depression-related behavior across adolescence in C57BL/6J mice. *Behav. Brain Res.* 176, 210–215. doi: 10.1016/j.bbr.2006.10.001

Hetrick, S. E., McKenzie, J. E., Cox, G. R., Simmons, M. B., and Merry, S. N. (2012). Newer generation antidepressants for depressive disorders in children and adolescents (review). *Cochr. Datab. Syst. Rev.* 11, 1–137. doi: 10.1002/14651858.CD004851.phb3

Hetrick, S. E., McKenzie, J. E., and Merry, S. N. (2010). The use of SSRIs in children and adolescents. *Curr. Opin. Psychiatry* 23, 53–57. doi: 10.1097/yco.0b013e328334bc92

Holick, K. A., Lee, D. C., Hen, R., and Dulawa, S. C. (2008). Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. *Neuropsychopharmacology* 33, 406–417. doi: 10.1038/sj.npp.1301399

Homberg, J. R., Olivier, J. D. A., Blom, T., Arentsen, T., van Brunschot, C., Schipper, P., et al. (2011). Fluoxetine exerts age-dependent effects on behavior and amygdala neuroplasticity in the rat. *PLoS One* 6:e16646. doi: 10.1371/journal.pone.0016646

Horton, R. E., Apple, D. M., Owens, W. A., Baganz, N. L., Cano, S., Mitchell, N. C., et al. (2013). Decynium-22 enhances SSRI-induced antidepressant-like effects in mice: uncovering novel targets to treat depression. *J. Neurosci.* 33, 10534–10543. doi: 10.1523/jneurosci.5687-11.2013

Iceta, R., Mesonero, J. E., and Alcalde, A. I. (2007). Effect of long-term fluoxetine treatment on the human serotonin transporter in Caco-2 cells. *Life Sci.* 80, 1517–1524. doi: 10.1016/j/lfs.2007.01.020

Jiao, J., Nitzke, A. M., Doukas, D. G., Seiglie, M. P., and Dulawa, S. C. (2011). Antidepressant response to chronic citalopram treatment in eight inbred mouse strains. *Psychopharmacology* 213, 509–520. doi: 10.1007/s00213-010-2140-0

Kafali, N., Progovac, A., Hou, S. S., and Cook, B. L. (2018). Long-run trends in antidepressant use among youths after the FDA black box warning. *Psychiatr. Serv.* 69, 389–395. doi: 10.1176/appi.ps.201700089

Karanges, E., Li, K. M., Motbey, C., Callaghan, P. D., Katsifis, A., and McGregor, I. S. (2011). Differential behavioural and neurochemical outcomes from chronic paroxetine treatment in adolescent and adult rats: a model of adverse antidepressant effects in human adolescents? *Int. J. Neuropsychopharmacol.* 14, 491–504. doi: 10.1017/S146114571100006X

Keller, M. B., Ryan, N. D., Strober, M., Klein, R. G., Kutcher, S. P., Birmaher, B., et al. (2001). Efficacy of paroxetine in the treatment of adolescent major depression: a randomized, controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry* 40, 762–772. doi: 10.1097/00004583-200107000-00010

Kessler, R. C., Avenevoli, S., and Ries Merikangas, K. (2001). Mood disorders in children and adolescents: an epidemiologic perspective. *Biol. Psychiatry* 49, 1002–1014. doi: 10.1016/S0006-3223(01)01129-5

Klomp, A., Václavu, L., Meerhoff, G. F., Reneman, L., and Lucassen, P. (2014). Effects of chronic fluoxetine treatment on neurogenesis and tryptophan hydroxylase expression in adolescent and adult rats. *PLoS One* 9:e97603. doi: 10.1371/journal.pone.0097603

Kodish, I., Rockhill, C., and Varley, C. (2011). Pharmacotherapy for anxiety disorders in children and adolescents. *Dial. Clin. Neurosci.* 13, 439–452. doi: 10.1016/j.pcl.2010.10.002

Koepsell, H., Lips, K., and Volk, C. (2007). Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm. Res.* 24, 1227–1251. doi: 10.1007/s11095-007-9254-z

Kozisek, M. E., Middlemas, D., and Bylund, D. B. (2008). The differential regulation of BDNF and TrkB levels in juvenile rats after four days of escitalopram and desipramine treatment. *Neuropharmacology* 54, 251–257. doi: 10.1016/j.neuropharm.2007.08.001

Krause-Heuer, A. M., Fraser-Spears, R., Dobrowolski, J. C., Ashford, M. E., Wyatt, N. A., Roberts, M. P., et al. (2017). Evaluation of the antidepressant therapeutic potential of isocyanine and pseudoisocyanine analogues of the organic cation decynium-22. *Eur. J. Med. Chem.* 137, 476–487. doi: 10.1016/j.ejmech.2017.06.011

Kronenberg, S., Apter, A., Brent, D., Schirman, S., Melhem, N., Pick, N., et al. (2007). Serotonin transporter polymorphism (5-HTTLPR) and citalopram effectiveness and side effects in children with depression and/or anxiety disorders. *J. Child Adolesc. Psychopharmacol.* 17, 741–750. doi: 10.1089/cap.2006.0144

Kulikov, A. V., Gainetdinov, R. R., Ponimaskin, E., Kalueff, A. V., Naumenko, V. S., and Popova, N. K. (2018). Interplay between key proteins of serotonin system in SSRI antidepressant efficacy. *Exp. Opin. Therapeut. Targets* 22, 319–330. doi: 10.1080/14728222.2018.1452912

Lenroot, R. K., and Giedd, J. N. (2006). Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci. Biobehav. Rev.* 30, 718–729. doi: 10.1016/j.neubiorev.2006.06.001

Lin, W. H., Li, X. F., Lin, M. X., Zhou, Y., and Huang, H. P. (2017). Novel insights into the effect of paroxetine administration in pilocarpine-induced chronic epileptic rats. *Mol. Med. Rep.* 16, 8245–8252. doi: 10.3892/mmr.2017.7659

Lucki, I., Dalvi, A., and Mayorga, A. J. (2001). Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology* 155, 315–322. doi: 10.1007/s002130100694

Maneeton, N., and Srisurapanont, M. (2000). Tricyclic antidepressants for depressive disorders in children and adolescents: a meta-analysis of randomized-controlled trials. *J. Med. Assoc. Thailand* 83, 1367–1374.

March, J., Silva, S., Petrycki, S., Curry, J., Wells, K., Fairbank, J., . . .Treatment for Adolescents with Depression Study (TADS) Team (2004). Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: treatment for adolescents with depression study (TADS) randomized controlled trial. *J. Am. Med. Assoc.* 292, 807–820. doi: 10.1001/jama.292.7.807

Mason, S. S., Baker, K. B., Davis, K. W., Pogorelov, V. M., Malbari, M. M., Ritter, R., et al. (2009). Differential sensitivity to SSRI and tricyclic antidepressants in juvenile and adult mice of three strains. *Eur. J. Pharmacol.* 602, 306–315. doi: 10.1016/j.ejphar.2008.11.010

Matthäus, F., Haddjeri, N., Sánchez, C., Martí, Y., Bahri, S., Rovera, R., et al. (2016). The allosteric citalopram binding site differentially interferes with neuronal firing rate and SERT trafficking in serotonergic neurons. *Eur. Neuropsychopharmacol.* 26, 1806–1817. doi: 10.1016/j.euroneuro.2016.09.001

Maughan, B., Collishaw, S., and Stringaris, A. (2013). Depression in childhood and adolescence. *J. Can. Acad. Child Adolesc. Psychiatry* 22, 35–40.

Miller, B. H., Schultz, L. E., Gulati, A., Cameron, M. D., and Pletcher, M. T. (2008). Genetic regulation of behavioral and neuronal responses to fluoxetine. *Neuropsychopharmacology* 33, 1312–1322. doi: 10.1083/sj.npp.1301497

Mirza, N. R., Nielsen, E. O., and Troelsen, K. B. (2007). Serotonin transporter density and anxiolytic-like effects of antidepressants in mice. *Progr. Neuro-psychopharmacol. Biol. Psychiatry* 31, 858–866. doi: 10.1016/j.pnpbp.2007.01.020

Mitchell, N. C., Bowman, M. A., Gould, G. G., Koek, W., and Daws, L. C. (2017). Ontogeny of norepinephrine transporter expression and antidepressant-like response to desipramine in wild-type and serotonin transporter mutant mice. *J. Pharmacol. Exp. Ther.* 360, 84–94. doi: 10.1124/jpet.116.237305

Mitchell, N. C., Gould, G. G., Koek, W., and Daws, L. C. (2016). Ontogeny of SERT expression and antidepressant-like response to escitalopram in wild-type and SERT mutant mice. *J. Pharmacol. Exp. Ther.* 358, 271–281. doi: 10.1124/jpet.116.233338

Mitchell, N. C., Gould, G. G., Smolik, C. M., Koek, W., and Daws, L. C. (2013). Antidepressant-like drug effects in juvenile and adolescent mice in the tail suspension test: relationship with hippocampal serotonin and norepinephrine transporter expression and function. *Front. Pharmacol.* 4:131. doi: 10.3389/fphar.2013.00131

Mojtabai, R., Olfson, M., and Han, B. (2016). National trends in the prevalence and treatment of depression in adolescents and young adults. *Pediatrics* 138:e20161878. doi: 10.1542/peds.2016-1878

Munari, L., Provensi, G., Passani, M. B., Galeotti, N., Cassano, T., Benetti, F., et al. (2015). Brain histamine is crucial for selective serotonin reuptake inhibitors' behavioral and neurochemical effects. *Int. J. Neuropsychopharmacol.* 18:pyv045. doi: 10.1093/ijnp/pyv045

Murrin, L. C., Sanders, J. D., and Bylund, D. B. (2007). Comparison of the maturation of the adrenergic and serotonergic neurotransmitter systems in the brain: implications for differential drug effects on juveniles and adults. *Biochem. Pharmacol.* 73, 1225–1236. doi: 10.1016/j.bcp.2007.01.028

Norrholm, S. D., and Ouimet, C. C. (2000). Chronic fluoxetine administration to juvenile rats prevents age-associated dendritic spine proliferation in hippocampus. *Brain Res.* 883, 205–215. doi: 10.1016/S0006-8993(00)02909-7

O'Leary, O. F., and Cryan, J. F. (2013). Towards translational rodent models of depression. *Cell Tissue Res.* 354, 141–153. doi: 10.1007/s00441-013-1587-9

Pechnick, R. N., Bresee, C. J., Manalo, C. M., and Poland, R. E. (2008). Comparison of the effects of desmethylimipramine on behavior in the forced swim test in peripubertal and adult rats. *Behav. Pharmacol.* 19, 81–84. doi: 10.1097/fbp.0b013e3282f3d07f

Reed, A. L., Anderson, J. C., Bylund, D. B., Petty, F., Refaey, H. E., and Happe, H. K. (2009). Treatment with escitalopram but not desipramine decreases escape latency times in a learned helplessness model using juvenile rats. *Psychopharmacology* 205, 249–259. doi: 10.1007/s00213-009-1535-2

Reed, A. L., Happe, H. K., Petty, F., and Bylund, D. B. (2008). Juvenile rats in the forced-swim test model the human response to antidepressant treatment for pediatric depression. *Psychopharmacology* 197, 433–441. doi: 10.1007/s00213-007-1052-0

Roohi-Azizi, M., Torkaman-Boutorabi, A., Akhondzadeh, S., Nejatisafa, A. A., Sadat-Shirazi, M. S., and Zarrindast, M. R. (2018). Influence of citicoline on citalopram-induced antidepressant activity in depressive-like symptoms in male mice. *Physiol. Behav.* 195, 151–157. doi: 10.1016/j.physbeh.2018.08.002

Sallee, F. R., Hilal, R., Dougherty, D., Beach, K., and Nesbitt, L. (1998). Platelet serotonin transporter in depressed children and adolescents: 3H-paroxetine platelet binding before and after sertraline. *J. Am. Acad. Child Adolesc. Psychiatry* 37, 777–784. doi: 10.1097/00004583-199807000-00018

Shrestha, S. S., Nelson, E. E., Liow, J. S., Gladding, R., Lyoo, C. H., Noble, P. L., et al. (2014). Fluoxetine administration to juvenile monkeys: effects on the serotonin transporter and behavior. *Am. J. Psychiatry* 171, 323–331. doi: 10.1176/appi.ajp.2013.13020183

Simeon, J. G., Dinicola, V. F., Ferguson, H. B., and Copping, W. (1990). Adolescent depression: a placebo-controlled fluoxetine treatment study and follow-up. *Progr. Neuro-psychopharmacol. Biol. Psychiatry* 14, 791–795. doi: 10.1016/0278-5846(90)90050-Q

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463. doi: 10.1016/S0149-7634(00)00014-2

Thapar, A., Collishaw, S., Pine, D. S., and Thapar, A. K. (2012). Depression in adolescence. *Lancet* 379, 1056–1067. doi: 10.1016/S0140-6736(11)60871-4

Tsapakis, E. M., Fernandes, C., Moran-Gates, T., Basu, A., Sugden, K., Aitchison, K. J., et al. (2014). Effects of antidepressant drug exposure on gene expression in the developing cerebral cortex. *Synapse* 68, 209–220. doi: 10.1002/syn.21732

Tsapakis, E. M., Soldani, F., Tondo, L., and Baldessarini, R. J. (2008). Efficacy of antidepressants in juvenile depression: meta-analysis. *Br. J. Psychiatry* 193, 10–17. doi: 10.1192/bjp.bp.106/031088

Ulloa, R.-E., Díaz-Valderrama, A., Herrera-Pérez, J., León-Olea, M., and Martínez-Mota, L. (2014). Age differences in the impact of forced swimming test on serotonin transporter levels in lateral septum and dorsal raphe. *Behav. Brain Funct.* 10, 1–8. doi: 10.1186/1744-9081-10-3

Vialou, V., Balasse, L., Callebert, J., Launay, J.-M., Giros, B., and Gautron, S. (2008). Altered aminergic neurotransmission in the brain of organic cation transporter 3-deficit mice. *J. Neurochemistry* 106, 1471–1482. doi: 10.1111/j.1471-4159.2008.05506.x

von Knorring, A. L., Olsson, G. I., Thomsen, P. H., Lemming, O. M., and Hultén, A. (2006). A randomized, double-blind, placebo-controlled study of citalopram in adolescents with major depressive disorder. *J. Clin. Psychopharmacol.* 26, 311–315. doi: 10.1097/01.jcp.0000219051.40632.d5

Wagner, K. D., Ambrosini, P., Rynn, M., Wohlberg, C., Yang, R., Greenbaum, M. S., et al. (2003). Efficacy of sertraline in the treatment of children and adolescents with major depressive disorder: two randomized controlled trials. *J. Am. Med. Assoc.* 290, 1033–1041. doi: 10.1001/jama.290.8.1033

Wagner, K. D., Jonas, J., Findling, R. L., Ventura, D., and Saikali, K. (2006). A double-blind, randomized, placebo-controlled trial of escitalopram in the treatment of pediatric depression. *J. Am. Acad. Child Adolesc. Psychiatry* 45, 280–288. doi: 10.1097/01.chi.0000192250.38400.9e

Wagner, K. D., Robb, A. S., Findling, R. L., Jin, J., Gutierrez, M. M., and Heydorn, W. E. (2004). A randomized, placebo-controlled trial of citalopram for the treatment of major depression in children and adolescents. *Am. J. Psychiatry* 161, 1079–1083. doi: 10.1176/appi.ajp.161.6.1079

Wang, J. (2016). The plasma membrane monoamine transporter (PMAT): structure, function, and role in organic cation disposition. *Clin. Pharmacol. Therapeut.* 100, 489–499. doi: 10.1002/cpt.442

Wegerer, V., Moll, G. H., Bagli, M., Rothenberger, A., Rüther, E., and Huether, G. (1999). Persistently increased density of serotonin transporters in the frontal cortex of rats treated with fluoxetine during early juvenile life. *J. Child Adolesc. Psychopharmacol.* 9, 13–24. doi: 10.1089/cap.1999.9.13

Whittington, C. J., Kendall, T., Fonagy, P., Cottrell, D., Cotgrove, A., and Boddington, E. (2004). Selective serotonin reuptake inhibitors in childhood depression: systematic review of published versus unpublished data. *Lancet* 363, 1341–1345. doi: 10.1016/S0140-6736(04)16043-1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Bowman and Daws. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

REVIEW
published: 04 March 2019
doi: 10.3389/fnins.2019.00032

Responses of Plasma Catecholamine, Serotonin, and the Platelet Serotonin Transporter to Cigarette Smoking

Curtis Lee Lowery III[1], *Donna Woulfe*[2] *and Fusun Kilic*[1]*

[1] Departments of Biochemistry and Molecular Biology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, United States, [2] Department of Biological Sciences, University of Delaware, Newark, DE, United States

Cigarette smoking is one of the major causes of coronary heart disease with a thirty percent mortality rate in the United States. Cigarette smoking acting on the central nervous system (CNS) to stimulate the sympathetic nervous system (SNS) through, which facilitates the secretion of serotonin (5-HT) and catecholamines to supraphysiological levels in blood. The enhanced levels of 5-HT and catecholamines in smokers' blood are associated with increases in G protein-coupled receptor signaling and serotonylation of small GTPases, which in turn lead to remodeling of cytoskeletal elements to enhance granule secretion and promote unique expression of sialylated *N*-glycan structures on smokers' platelets. These mechanisms enhance aggregation and adhesion of smokers' platelets relative to those of non-smokers. This review focuses on the known mechanisms by which 5-HT and SERT, in coordinated signaling with catecholamines, impacts cigarette smokers' platelet biology.

Keywords: cigarette smoking, serotonin, catecholamine, platelet aggregation, serotonin transporter

OPEN ACCESS

Edited by:
Nasser Haddjeri,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Clinton Canal,
Mercer University, United States
Santiago J. Ballaz,
Yachay Tech University, Ecuador

***Correspondence:**
Fusun Kilic
fusunkilic@yahoo.com

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *30 October 2018*
Accepted: *14 January 2019*
Published: *04 March 2019*

Citation:
Lowery CL III, Woulfe D and Kilic F (2019) Responses of Plasma Catecholamine, Serotonin, and the Platelet Serotonin Transporter to Cigarette Smoking. Front. Neurosci. 13:32. doi: 10.3389/fnins.2019.00032

INTRODUCTION

Serotonin (5-HT) is secreted from the enterochromaffin cells of the intestine to the blood and taken into the platelets by a specific transporter, SERT (SLC6A4) via a saturable reuptake mechanism (Rudnick, 1977; Lesch et al., 1993; McNicol and Israels, 1999; McNicol and Israels, 2003). Platelets do not synthesize 5-HT, yet they are the major biological storage pool of circulating 5-HT. Platelet surface located SERT tightly regulates the free 5-HT level in the blood. In platelets, vesicular monoamine transporter (VMAT) on the dense granules takes free 5-HT from the platelet cytoplasm into the dense granule which is sequestered there to millimolar concentrations, while free concentrations in the blood are in the low nanomolar range (McNicol and Israels, 2003; Brenner et al., 2007).

5-HT is a multifunctional signaling molecule, growth factor, and endocrine hormone or paracrine messenger (Maroteaux and Kilic, 2018) while it plays key roles in a variety of psychiatric diseases as a neurotransmitter, it also has extra-cerebral roles as a potent vasoconstrictor as well as a weak agonist of platelet activation, both roles serve to modulate cardiovascular disease (Rapport et al., 1984; Sjoerdsma and Palfreyman, 1990).

In various pathological conditions including hypertension and thrombosis, plasma 5-HT level is elevated (Biondi et al., 1986; Kerr et al., 1999; Vikens et al., 1999; Brenner et al., 2007; Davis et al., 2013; Fraer and Kilic, 2015; Lowery et al., 2017). The cause of 5-HT elevation, its effect on SERT, and the contribution to platelet physiology were studied in preclinical models, rat (Homberg et al., 2006) and mouse (Bengel et al., 1998) with SERT knock-out (KO) gene.

These studies demonstrated that platelets were almost completely depleted of 5-HT in SERT-KO rodent models confirming that 5-HT in dense granules are also filled by platelet SERT through an uptake mechanism. Although the source of the initial elevation in plasma 5-HT levels remains controversial it has been proposed that it may originate from an increased rate of 5-HT synthesis or secretion from the enterochromaffin cells of the intestine due to the initial stage of a disease (Gershon and Tack, 2007). Studies with the blood samples collected from hypertensive subjects showed that there is a biphasic relationship with the plasma concentration of 5-HT and the density of SERT on platelet surface (Brenner et al., 2007). The level of SERT proteins on the surface of platelet and the 5-HT uptake rates of platelets initially rise as plasma 5-HT levels are increased, but then fall below normal as the plasma 5-HT level continues to rise (Brenner et al., 2007). These findings indicate a biphasic relationship between plasma 5-HT-level and the platelet SERT density on the plasma membrane, more specifically, down-regulation of platelet SERT in the presence of high level of 5-HT in plasma. We hypothesized that the elevated plasma 5-HT levels downregulate the 5-HT uptake of platelets via decreasing the density of SERT molecules on platelet surface. This hypothesis switches the cellular roles of 5-HT and SERT and proposes that 5-HT controls its own concentration in plasma by modulating the uptake properties of platelet SERT rather than SERT controls the plasma 5-HT levels. Further studies correlated the impact of 5-HT signaling on the membrane trafficking of SERT (Ahmed et al., 2008, 2009; Mercado and Kilic, 2010; Mercado et al., 2011).

In general, translocation of proteins from/to the plasma membrane is mediated by other proteins that facilitate their movement between the surface membrane and intracellular compartments. 5-HT signaling acts on the membrane trafficking of SERT molecules in two independent pathways: (1) on the exocytosis of SERT via acting on small GTPases (Ahmed et al., 2008; Li et al., 2016; Lowery et al., 2017; Mercado and Kilic, 2010; Mercado et al., 2011; Ziu et al., 2012); and (2) on the rate of internalization of SERT via acting on cytoskeletal proteins such as myosin IIa and vimentin (Ozaslan et al., 2003; Ahmed et al., 2009; Mercado and Kilic, 2010). The translocation of SERT from/to platelet plasma membrane is controlled by the 5-HT signaling-dependent pathways.

Studies in the platelets of mice lacking the gene for tryptophan hydroxylase (TPH1), the rate-limiting enzyme in the synthesis of 5-HT in peripheral cells, demonstrated that intracellular 5-HT acts on the exocytosis of the dense and α-granules during platelet activation (Walther et al., 2003; Ziu et al., 2012, 2014; Lowery et al., 2017). These studies in isolated platelets indicated that 5-HT-stimulation accelerated the exocytosis of granules, which secrete their contents, 5-HT, ADP, and procoagulant molecules, such as fibrinolytic regulators, growth factors, chemokines, immunologic modulators, P-selectin, von Willebrand factor, thrombospondin, fibrinogen, and fibronectin (Shirakawa et al., 2000; Walther et al., 2003; Ziu et al., 2012; Lowery et al., 2017). These findings specifically showed that 5-HT signaling manipulates the exocytosis mechanism of dense and α-granules in platelets. As explained in the following sections, the 5-HT signaling pathway controls the movement of SERT from/to plasma membrane of platelets (Ozaslan et al., 2003; Ahmed et al., 2008, 2009; Mercado and Kilic, 2010; Mercado et al., 2011; Ziu et al., 2012; Li et al., 2016; Lowery et al., 2017). This will elevate plasma 5-HT concentration to supraphysiological levels as seen during smoking cigarette (Lowery et al., 2017). However, smoking also elevates the blood plasma catecholamine levels (Siess et al., 1982; Grassi et al., 1994; Narkiewicz et al., 1998; Parati and Esler, 2012; Lowery et al., 2017). Together with 5-HT circulating catecholamines are associated with the increased risk of arterial thrombosis. Yet, in the absence of cardiovascular disease, elevated blood 5-HT level does not increase blood pressure, suggesting that the elevation in plasma 5-HT level could be a consequence rather than a cause of hypertension (Singh et al., 2013). Thus, the mechanisms by which elevated concentrations of 5-HT may lead to thrombosis in cigarette smokers is an active area of research that may yield improved development and application of anti-thrombotic therapy. In particular, the mechanisms by which 5-HT potentiates platelet aggregation may play a contributing role in the acutely increased risk of arterial thrombosis associated with cigarette smoking. The main goal of this review is to summarize a novel mechanism by which 5-HT and SERT in coordination with catecholamines impact cigarette smokers' platelet biology.

Cigarette Smoking and Clinical Relevance

Cigarette smoking has significant roles in the development of various cardiovascular diseases (CVD) through inhalation exposure of smoked tobacco and the secondary effects of tobacco products on circulating hormone levels. Even passive smoking (second hand smoke), with a smoke exposure about 1/10th that of active smoking, is associated with an approximate 30% increase of coronary artery disease (CAD), compared with an 80% increase in active smokers (Willett et al., 1987; He et al., 1999; Barnoya and Glantz, 2005). Despite increasing social and legal pressure to restrict tobacco use, an estimated 36.5 million adults in the United States still smoke tobacco cigarettes (Crow et al., 2018). Although awareness of these health risks has reduced the prevalence of smoking, a large portion of the population continues to use cigarettes and will suffer adverse cardiovascular events attributable to tobacco use.

While the exact toxic components of cigarette smoke and the mechanisms involved in cigarette-related long term cardiovascular dysfunction have not been fully elucidated, it has been repeatedly demonstrated that the acute effects (minutes to hours) of cigarette smoke are linked to plasma concentrations of nicotine (Powell, 1998; Mendelson et al., 2005; Tweed et al., 2012). Nicotine, a naturally occurring alkaloid found in the tobacco plant, appears to be the primary addictive and bioactive agent in cigarette smoke (Goldberg et al., 1991; Doolittle et al., 1995; Corrigall et al., 2001; Rabinoff et al., 2007). Inhalation results in rapid absorption of nicotine through the lungs into the blood stream. Nicotine crosses the blood-brain barrier and reaches the central nervous system (CNS) within 7 s of inhalation, where it stimulates nicotinic acetylcholine receptors (Doolittle et al., 1995; Mendelson et al., 2005; Sherva et al., 2008).

Nicotinic CNS stimulation activates the sympathetic nervous system (SNS) to promote release of many chemical messengers including acetylcholine, adrenocorticotropin hormone (ACTH) norepinephrine (NE), epinephrine (E), arginine vasopressin, 5-HT, and dopamine (DA) (Koob and Le Moal, 2001; Kimes et al., 2003; Sherva et al., 2008; McKee et al., 2011) into blood plasma. Elevations in blood levels of these compounds are associated with the systemic cardiovascular effects of cigarette smoking (Ambrose and Barua, 2004; Mendelson et al., 2005).

Chronic cigarette smoking predisposes the individual to multiple atherosclerotic syndromes as well as peripheral atherosclerosis and aortic aneurysms (Winniford et al., 1987). Long term effects on lungs, blood vessels, and heart are well studied and have repeatedly shown that smoking cessation decreases the risk of all-cause mortality with an exponential decline approaching the risk of non-smokers at 5 years (Rosenberg et al., 1985; Fusegawa et al., 1999) Data also demonstrate an immediate reduction in thrombotic events following smoking cessation indicating important acute effects altering blood and platelet function (FitzGerald et al., 1988; US Department of Health and Human Services, 1990; Samet, 1991).

Biological Mechanisms of Cigarette Smoking-Associated Thrombosis Risk

Thrombosis is the dysregulated formation of a thrombus, comprising the combination of platelet aggregates, and blood clot within blood vessels, such that the flow of blood through the circulatory system is obstructed. Under normal physiological conditions, regulatory mechanisms such as circulating levels of prostacyclin and endothelial expression of ecto-ADPase prevent thrombus formation. Without injury or other insult endothelial cells of intact vessels prevent blood clotting by secreting a heparin-like molecule and thrombomodulin. Additionally, the vascular endothelial cells prevent platelet aggregation and vasospasm by secreting nitric oxide. After vascular damage, thrombus formation is initiated to prevent blood loss after injury to a blood vessel. While acute injury results in hemostasis, which is the localized vascular response to prevent blood loss, thrombosis is a pathological response, typically initiated in the presence of atherosclerotic lesion. While hemostasis and thrombosis share similar initiating mechanisms, thrombosis proceeds to complete vessel occlusion, while physiological hemostasis stems blood loss, but allows maintenance of blood flow.

Hemostasis can be conceived as occurring via a three-step process (Levy et al., 2010). The first step is vascular spasm (vasoconstriction), caused by contraction of vascular smooth muscle cells. This local constriction of blood vessels results in decreased blood flow through the area and limits blood loss. At the area of damage, blood is also exposed to collagen in the subendothelial matrix. Collagen promotes adherence and activation of platelets localized at the site of injury. Once activated, platelets secrete dense and alpha granules, releasing platelet-activating factors, such as ADP and P-selectin, and causing the localized production of thromboxane A2 and thrombin on platelet and associated membrane surfaces. These factors activate other nearby platelets causing them to release their contents leading to a cascade effect (O'Connell, 2013). The activated platelets alter their shape through cytoskeletal remodeling, enhancing their adhesion to endothelial and other platelet surfaces and aggregate to form a platelet plug. Cytoskeletal remodeling is achieved predominantly through the modification of small GTP-binding proteins, resulting in downstream shape changes to a more spiny form with projecting filopodia. The increased surface area and alteration of surface proteins causes platelets to adhere to one another and to the exposed collagen in the damaged vessel wall. Signaling interactions then promote the activation of integrin alphaIIb-beta3 and subsequent binding of circulating fibrinogen, allowing formation of a stable platelet plug (Farndale et al., 2004). Coincident with these processes, coagulation is initiated on local membrane surfaces, allowing consequent formation of a clot. There is evidence that both platelet aggregation and clotting are excessively activated after acute exposure to cigarette smoke. Therefore, it is important to elucidate the mechanisms by which cigarette smoking induces platelets to become increasingly activated and increases thrombotic risk.

Cigarette smoking enhances the formation of thrombosis by which studies demonstrate that stopping smoking decreases the risk of the reoccurring of myocardial infarction (US Department of Health and Human Services, 1990). Also, epidemiologic evidence indicates that the acute effects of cigarette smoking produce CNS-mediated activation of the SNS (Siess et al., 1982; Peterson et al., 1983; Porchet et al., 1987; Narkiewicz et al., 1998; Parati and Esler, 2012), which stimulates secretion of pro-thrombotic molecules, such as catecholamines (E, NE, and DA) and 5-HT into the blood at supraphysiological levels (Koob and Le Moal, 2001; Kimes et al., 2003; Sherva et al., 2008; McKee et al., 2011). Recent studies from our laboratories demonstrate that smoking results in several-fold increases in plasma levels of 5-HT and catecholamines (Lowery et al., 2017). In platelets, 5-HT and catecholamine signaling are mediated, respectively, through 5-HT2A, β2-, and α2-adrenergic receptors. Activation of each of these receptors directly act on platelet aggregation (Woulfe, 2005; Millan et al., 2008; Smyth et al., 2009; Dowal and Flaumenhaft, 2010) via stimulating the exocytosis of the granules which are the storages of the several procoagulant molecules as well as 5-HT and catecholamine (McNicol and Israels, 1999, 2003).

Once bound to platelet surfaces, 5-HT activates a 5-HT-specific G protein-coupled receptor, 5-HT2A. Although the Gq-dependent signals leading to platelet activation are well-accepted, less appreciated is the coordinate interaction of these 5-HT and catecholamine-initiated signals with 5-HT uptake into the platelet cytoplasm. In this process, SERT, on the platelet plasma membrane, plays a major role in regulating extracellular vs intracellular 5-HT concentration. When 5-HT is removed from blood plasma into the platelet, it is stored in dense granules through VMAT (Brunk et al., 2006). Upon the saturation of dense granules with 5-HT, transport via VMAT shuts down and 5-HT appears in the cytoplasm in free, unbound form. The elevated plasma 5-HT level also activates platelet surface-localized 5-HT receptors, which initiates signaling through Gq, activates phospholipase C (PLC) results in the hydrolysis of

phosphatidylinositol 4,5-biphosphate (PIP2) to inositol-1,4,5-triphosphate (IP3) (Jin and Kunapuli, 1998; McNicol and Israels, 1999; Yang et al., 2002; Quinton et al., 2004; Woulfe, 2005; Millan et al., 2008; Smyth et al., 2009; Dowal and Flaumenhaft, 2010; Bynagari-Settipalli et al., 2012). Formation of IP3 activates the serine/threonine protein kinase C (PKC) family and facilitates the secretion of Ca^{2+} from intracellular compartments to cytoplasm. Catecholamine (NE/E) activates α2-adrenoceptor which initiates signaling through Gi, inhibits adenylyl cyclase (AC) while activating phosphoinositide 3-kinases (PI3K), leading to the aggregation of platelets in a biphasic manner (Dowal and Flaumenhaft, 2010). Additionally, NE signaling also acts on Src family kinases (SFK) (McNicol and Israels, 2003; Walther et al., 2003; Senis et al., 2014) to allow PLC-dependent hydrolysis of PIP_2 to IP3 and the secretion of Ca^{2+} from the granules (Walther et al., 2003; Ziu et al., 2012). Particularly, related to the role of 5-HT signaling in platelet pathology, elevation of the cytoplasmic free Ca^{2+} concentration activates transglutaminase (TGase), which transamidates unbound/free cytoplasmic 5-HT to small GTPases; a reaction known as serotonylation (Shirakawa et al., 2000; Walther et al., 2003; Ziu et al., 2012; Senis et al., 2014).

The action of E/NE signaling on platelet via β2-receptor is a controversial topic. Yet, E/NE activates AC through the Gs, upregulates the intracellular concentration of the second messenger cAMP, a powerful inhibitor of platelet aggregation. The cellular act of cAMP reorganizes the actin/myosin cytoskeletal network in a Rho-GTP dependent manner via stimulating Protein kinase A (PKA) (Woulfe, 2005; Millan et al., 2008; Smyth et al., 2009). Thus, transamidation of Rho- and Rab-GTPases with 5-HT are important for platelet cytoskeletal reorganization and secretory behavior, respectively. We hypothesize that following cigarette smoking, IP_3 is activated by 5-HT2A and α2-receptor signals to alter the cytoskeletal network in platelets and counteract the tonic inhibitory effect of β2-receptor signaling elevated cAMP level in platelets (**Figure 1**).

In the last decade, we reported several studies related with the impact of SNS-activated secretion of 5-HT into the blood (Brenner et al., 2007; Mercado and Kilic, 2010; Fraer and Kilic, 2015; Lowery et al., 2017), on platelet aggregation via activation of the 5-HT receptor (5-HT2A) (Ziu et al., 2014), on the secretion of granules as a result of serotonylation of small GTPases (Ahmed et al., 2008; Mercado et al., 2011; Ziu et al., 2012; Li et al., 2016), alteration of the cytoskeletal network (Ahmed et al., 2009; Li et al., 2016), and alteration of the structure and composition of glycans on the surface of mouse platelets (Mercado et al., 2013). Based on these studies, we proposed that 5-HT-mediated signaling and serotonylation of small GTPases accelerates the secretion rates of granule components such as ADP and P-selectin, remodeling of cytoskeletal network also permits altered localization of enzymes regulating glycosylation. The structures of glycans, specifically the terminal positions, act as ligands to the receptors. The best examples are P- and E-selectins which regulate various cell–cell adhesion in occurrence of vascular pathophysiology (Kelm and Schauer, 1997). Together, these studies show that the platelet surface glycans' structures and distributions were altered by 5-HT treatment.

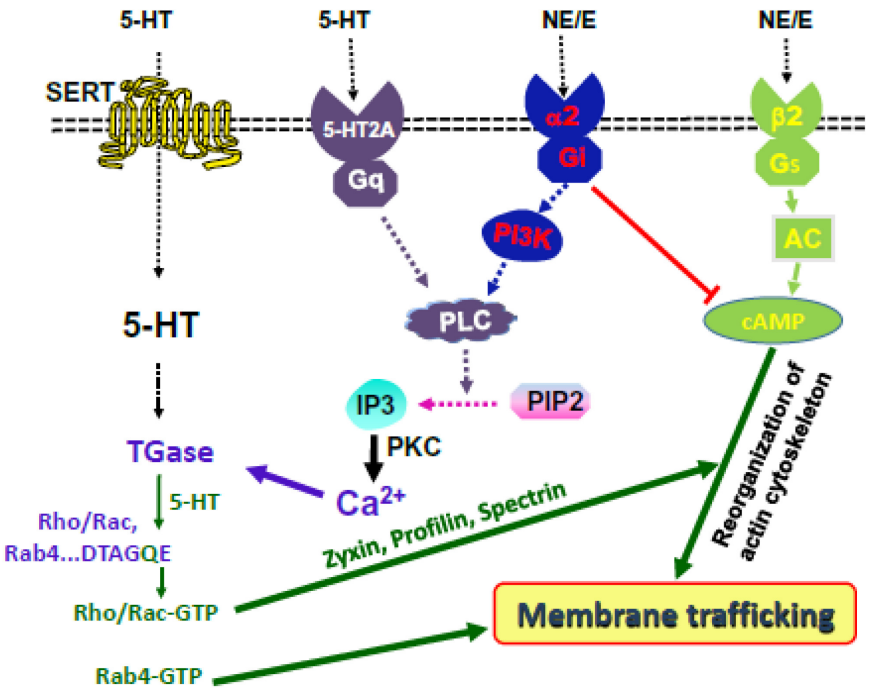


FIGURE 1 | Hypothesis at a glance. 5-HT and NE signaling activate IP3 and elevate the level of cytoplasmic Ca^{2+}. TGase serotonylates small GTPases. 5-HT and NE control platelet membrane-trafficking dynamics by enabling Rab4-GTP formation and Rho/Rac-GTP-mediated cytoskeletal rearrangement, downstream of cAMP/PKA.

Our recently reported studies demonstrate that cigarette smoking induces specific changes to surface glycans and that these changes are associated with a potentiating effect on platelet aggregation (Lowery et al., 2017). Proteomic and glycomic analyses by Dr. Richard Jones at MS Bioworks and Dr. Parastoo Azadi at CCRC identified differences in both the number and specification of core proteins and glycans eluted from platelet plasma membranes isolated from nonsmokers versus smokers' blood. Cigarette smoking acutely changes the glycan structure from high mannose to sialylated *N*-glycan structures expressed on platelet surfaces. Of particular functional relevance, removing the *N*-glycans from the surfaces of smokers' platelets counteracted the smoking-mediated enhancements in platelet aggregation (Lowery et al., 2017), indicating that remodeling the platelet surface with *N*-glycan may establish a more adhesive environment and provide a mechanism by which smoking contributes to platelet activation and thrombosis. However, it remains to be investigated whether the high mannose structures present exclusively on resting (nonsmokers') platelets protect against platelet aggregation. Specifically, proteins involved in the GTPase-activating, and associated with the actin cytoskeletal network were differentially and significantly altered on the surface of the smokers' platelet compared to those of non-smokers.

These findings suggest that plasma 5-HT and catecholamine levels influence the alteration of glycans on platelet surfaces; however, the mechanisms by which 5-HT/catecholamine specifically induce surface glycan alteration is still not known. The abundance of oligomannose glycans on platelets suggests an attenuated N-glycan maturation pathway at the megakaryocyte level; however, the alteration in glycans on smokers' platelet surfaces occurs within 15 min. This glycan structural alteration is too rapid to be explained by traditional glycan modification in Golgi apparatus after protein synthesis and subsequent completion of the membrane trafficking process (Lowery et al., 2017). Therefore, we propose that the involvement of 5-HT/catecholamine signaling in alteration of surface glycans could be through the membrane trafficking of several existing glycoproteins to the plasma membrane. In support of this proposal, *in vitro* studies of 5-HT/catecholamine-exposed nonsmokers' platelets show an elevation in plasma glycan level and percent platelet aggregation rates to the levels found in smokers' plasma, indicating a rapid additive effect of 5-HT and adrenergic receptors' signaling. Furthermore, pharmacologic blockade of 5-HT and adrenergic receptors reduces both plasma glycan and platelet aggregation levels to that of non-smokers' plasma (Lowery et al., 2017).

In Summary

We propose that following cigarette smoking-activated 5-HT as well as α2-receptors downstream elements, such as IP_3 hydrolysis-associated cytoplasmic level of free Ca and the cytoskeletal network in a cooperative manner to counteract the inhibitory effect of cAMP in platelet activation. Therefore, we propose that smoking-associated high levels of 5-HT and catecholamine in blood plasma make platelets prone to aggregation, in part, by changing the cytoskeletal network to accelerate the movement of *N*-glycan to the platelet surface.

AUTHOR CONTRIBUTIONS

This review article is a short summary of the studies performed by CL in FK's laboratories during his Ph.D. program. DW has involved in the project through her expertise in G-protein coupled receptors on platelet.

REFERENCES

Ahmed, B., Bukhari, I. A., Jeffus, B. C., Harney, J. T., Thyparambil, S., Ziu, E., et al. (2009). The cellular distribution of serotonin transporter is impeded on serotonin-altered vimentin network. *PLoS One* 4:e4730. doi: 10.1371/journal.pone.0004730

Ahmed, B. A., Jeffus, B., Harney, J. T., Bukhari, I. S., Unal, R., Lupashin, V. V., et al. (2008). Serotonin transamidates Rab4 and facilitates binding to the C-terminus of hSERT. *J. Biol. Chem.* 283, 9388–9398. doi: 10.1074/jbc.M706367200

Ambrose, J., and Barua, R. (2004). The pathophysiology of cigarette smoking and cardiovascular disease. *J. Am. Coll. Cardiol.* 43, 1731–1737. doi: 10.1016/j.jacc.2003.12.047

Barnoya, J., and Glantz, S. A. (2005). Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation* 111, 2684–2698. doi: 10.1161/CIRCULATIONAHA.104.492215

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Heils, A., et al. (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ('ecstasy') in serotonin transporter deficient mice. *Mol. Pharmacol.* 53, 649–655. doi: 10.1124/mol.53.4.649

Biondi, M. L., Agostoni, A., and Marasini, B. (1986). Serotonin levels in hypertension. *J. Hypertens.* 4, S39–S41.

Brenner, B., Harney, J. T., Ahmed, B. A., Jeffus, B. C., Unal, R., Mehta, J. L., et al. (2007). Plasma serotonin level and the platelet serotonin transporter. *J. Neurochem.* 102, 206–216. doi: 10.1111/j.1471-4159.2007.04542.x

Brunk, I., Blex, C., Rachakonda, S., Höltje, M., Winter, S., Pahner, I., et al. (2006). The first luminal domain of vesicular monoamine transporters mediates G-protein-dependent regulation of transmitter uptake. *J. Biol. Chem.* 281, 33373–33385. doi: 10.1074/jbc.M603204200

Bynagari-Settipalli, Y. S., Lakhani, P., Jin, J., Bhavaraju, K., Rico, M. C., Kim, S., et al. (2012). Protein Kinase C isoform epsilon negatively regulates ADP-induced calcium mobilization and thromboxane generation in platelets. *Arterioscler. Thromb. Vasc. Biol.* 32, 1211–1219. doi: 10.1161/ATVBAHA.111.242388

Corrigall, W. A., Zack, M., Eissenberg, T., Belsito, L., and Scher, R. (2001). Acute subjective and physiological responses to smoking in adolescents. *Addiction* 96, 1409–1417. doi: 10.1046/j.1360-0443.2001.961014095.x

Crow, R. S., Lohman, M. C., Titus, A. J., Bruce, M. L., Mackenzie, T. A., Bartels, S. J., et al. (2018). Mortality risk along the frailty spectrum: data from the National Health and Nutrition Examination Survey 1999 to 2004. *J. Am. Geriatr. Soc.* 66, 496–502. doi: 10.1111/jgs.15220

Davis, R. P., Szasz, T., Garver, H., Burnett, R., Tykocki, N. R., and Watts, S. W. (2013). One-month serotonin infusion results in a prolonged fall in blood pressure in the deoxycorticosterone acetate (DOCA) salt hypertensive rat. *ACS Chem. Neurosci.* 4, 141–148. doi: 10.1021/cn300114a

Doolittle, D. J., Winegar, R., Lee, C. K., Caldwell, W. S., Hayes, A. W., and deBethizy, J. D. (1995). The genotoxic potential of nicotine and its major metabolites. *Mutat. Res.* 344, 95–102. doi: 10.1016/0165-1218(95)00037-2

Dowal, L., and Flaumenhaft, R. (2010). Targeting platelet G-protein coupled receptors (GPCRs): looking beyond conventional GPCR antagonism. *Curr. Vasc. Pharmacol.* 8, 140–154. doi: 10.2174/15701611079088 6938

Farndale, R. W., Sixma, J. J., Barnes, M. J., and De Groot, P. G. (2004). The role of collagen in thrombosis and hemostasis. *J. Thromb. Haemost.* 2, 561–573. doi: 10.1111/j.1538-7836.2004.00665.x

FitzGerald, G. A., Oates, J. A., and Nowak, J. (1988). Cigarette smoking and hemostatic function. *Am. Heart J.* 115(1 Pt 2), 267–271. doi: 10.1016/0002-8703(88)90648-5

Fraer, M., and Kilic, F. (2015). Serotonin: a different player in hypertension-induced thrombosis. *Hypertension* 65, 942–948. doi: 10.1161/HYPERTENSIONAHA.114.05061

Fusegawa, Y., Goto, S., Handa, S., Kawada, T., and Ando, Y. (1999). Platelet spontaneous aggregation in platelet-rich plasma is increased in habitual smokers. *Thromb. Res.* 93, 271–278. doi: 10.1016/S0049-3848(98)00184-4

Gershon, M. D., and Tack, J. (2007). The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 132, 397–414. doi: 10.1053/j.gastro.2006.11.002

Goldberg, R. J., Bengtson, J., Chen, Z. Y., Anderson, K. M., Locati, E., and Levy, D. (1991). Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham Heart Study experience). *Am. J. Cardiol.* 67, 55–58. doi: 10.1016/0002-9149(91)90099-7

Grassi, G., Seravalle, G., Calhoun, D. A., Bolla, G. B., Giannattasio, C., Marabini, M., et al. (1994). Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation* 90, 248–253. doi: 10.1161/01.CIR.90.1.248

He, J., Vupputuri, S., Allen, K., Prerost, M. R., Hughes, J., and Whelton, P. K. (1999). Passive smoking and the risk of coronary heart disease–a meta-analysis of epidemiologic studies. *N. Engl. J. Med.* 340, 920–926. doi: 10.1056/NEJM199903253401204

Homberg, J. H., Mudde, J. M., Braam, B., Ellenbroek, B., and Cuppen, E. (2006). Blood pressure in mutant rats lacking the 5-hydroxytryptamine transporter. *Hypertension* 48, e115–e116. doi: 10.1161/01.HYP.0000246306.61289.d8

Jin, J., and Kunapuli, S. P. (1998). Coactivation of two different G protein-coupled receptors is essential for ADP-induced plateletaggregation. *Proc. Natl. Acad. Sci. U.S.A.* 95, 8070–8074. doi: 10.1073/pnas.95.14.8070

Kelm, S., and Schauer, R. (1997). Sialic acids in molecular and cellular interactions. *Int. Rev. Cytol.* 175, 137–240. doi: 10.1016/S0074-7696(08)62127-0

Kerr, S., Brosnan, M. J., McIntyre, M., Reid, J. L., Dominiczak, A. F., and Hamilton, C. A. (1999). Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. *Hypertension* 33, 1353–1358. doi: 10.1161/01.HYP.33.6.1353

Kimes, A. S., Horti, A. G., London, E. D., Chefer, I., Contoreggi, C., Ernst, M., et al. (2003). 2-[18F]F-A-85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans. *FASEB J.* 10, 1331–1333. doi: 10.1096/fj.02-0492fje

Koob, G. F., and Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24, 97–129. doi: 10.1016/S0893-133X(00)00195-0

Lesch, K. P., Wolozin, B. L., Murphy, D. L., and Riderer, P. (1993). Primary structure of the human platelet 5-HT uptake site: identity with the brain 5-HT transporter. *J. Neurochem.* 60, 2319–2322. doi: 10.1111/j.1471-4159.1993.tb03522.x

Levy, J. H., Dutton, R. P., Hemphill, J. C., Shander, A., Cooper, D., Paidas, M. J., et al. (2010). Multidisciplinary approach to the challenge of hemostasis. *Anesth. Analg.* 110, 354–364. doi: 10.1213/ANE.0b013e3181c84ba5

Li, Y., Hadden, C., Cooper, A., Ahmed, A., Wu, H., Lupashin, V. V., et al. (2016). Sepsis-induced elevation in plasma serotonin facilitates endothelial hyperpermeability. *Sci. Rep.* 9:22747. doi: 10.1038/srep22747

Lowery, C. L. I. I. I., Elliott, C., Cooper, A., Hadden, C., Sonon, R. N., Azadi, P., et al. (2017). Cigarette smoking-associated alterations in serotonin/adrenalin signaling pathways of platelets. *J. Am. Heart Assoc.* 6:e005465. doi: 10.1161/JAHA.116.005465

Maroteaux, L., and Kilic, F. (2018). Frontiers of serotonin beyond the brain. *Pharmacol. Res.* doi: 10.1016/j.phrs.2018.10.022 [Epub ahead of print].

McKee, S. A., Sinha, R., Weinberger, A. H., Sofuoglu, M., Harrison, E. L., Lavery, M., et al. (2011). Stress decreases the ability to resist smoking and potentiates smoking intensity and reward. *J. Psychopharmacol.* 25, 490–502. doi: 10.1177/0269881110376694

McNicol, A., and Israels, S. J. (1999). Platelet dense granules: structure, function and implications for haemostasis. *Thromb. Res.* 95, 1–18. doi: 10.1016/S0049-3848(99)00015-8

McNicol, A., and Israels, S. J. (2003). Platelets and anti-platelet therapy. *J. Pharmacol. Sci.* 93, 381–396. doi: 10.1254/jphs.93.381

Mendelson, J. H., Sholar, M. B., Goletiani, N., Siegel, A. J., and Mello, N. K. (2005). Effects of low- and high-nicotine cigarette smoking on mood states and the HPA axis in men. *Neuropsychopharmacology* 30, 1751–1763. doi: 10.1038/sj.npp.1300753

Mercado, C., and Kilic, F. (2010). Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. *Mol. Interv.* 10, 231–241. doi: 10.1124/mi.10.4.6

Mercado, C., Ziu, E., and Kilic, F. (2011). Communication between 5-HT and small GTPases. *Curr. Opin. Pharmacol.* 11, 23–28. doi: 10.1016/j.coph.2011.01.006

Mercado, C. P., Quintero, M. V., Li, Y., Singh, P., Byrd, A. K., Talabnin, K., et al. (2013). A serotonin-induced N-glycan switch regulates platelet aggregation. *Sci. Rep.* 30:2795. doi: 10.1038/srep02795

Millan, M. J., Marin, P., Bockaert, J., and Mannoury la Cour, C. (2008). Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. *Trends Pharmacol. Sci.* 29, 454–464. doi: 10.1016/j.tips.2008.06.007

Narkiewicz, K., van de Borne, P. J., Hausberg, M., Cooley, R. L., Winniford, M. D., Davison, D. E., et al. (1998). Cigarette smoking increases sympathetic outflow in humans. *Circulation* 98, 528–534. doi: 10.1161/01.CIR.98.6.528

O'Connell, N. (2013). Practical hemostasis and thrombosis. *J. Chem. Inform. Model.* 53:328.

Ozaslan, D., Wang, S., Ahmed, B., Bene, A., Kocabas, A. M., and Kilic, F. (2003). Glycosyl modification facilitates homo- and hetero-oligomerization of serotonin transporter. A specific role for the sialic acid residues. *J. Biol. Chem.* 278, 43991–44000. doi: 10.1074/jbc.M306360200

Parati, G., and Esler, M. (2012). The human sympathetic nervous system: its relevance in hypertension and heart failure. *Eur. Heart J.* 33, 1058–1066. doi: 10.1093/eurheartj/ehs041

Peterson, D. F., Coote, J. H., Gilbey, M. P., and Futuro-Neto, H. A. (1983). Differential pattern of sympathetic outflow during upper airway stimulation with smoke. *Am. J. Physiol.* 245, 433–437. doi: 10.1152/ajpregu.1983.245.3.R433

Porchet, H. C., Benowitz, N. L., Sheiner, L. B., and Copeland, J. R. (1987). Apparent tolerance to the acute effect of nicotine results in part from distribution kinetics. *J. Clin. Invest.* 80, 1466–1471. doi: 10.1172/JCI113227

Powell, J. T. (1998). Vascular damage from smoking: disease mechanisms at the arterial wall. *Vasc. Med.* 3, 21–28. doi: 10.1177/1358836X9800300105

Quinton, T. M., Murugappan, S., Kim, S., Jin, J., and Kunapuli, S. P. (2004). Different G protein-coupled signaling pathways are involved in alpha granule release from human platelets. *J. Thromb. Haemost.* 2, 978–984. doi: 10.1111/j.1538-7836.2004.00741.x

Rabinoff, M., Caskey, N., Rissling, A., and Park, C. (2007). Pharmacological and chemical effects of cigarette additives. *Am. J. Public Health* 97, 1981–1991. doi: 10.2105/AJPH.2005.078014

Rapport, M. M., Green, A. A., and Page, I. H. (1984). Serum vasoconstrictor, serotonin; isolation and characterization. *J. Biol. Chem.* 176, 1243–1251.

Rosenberg, L., Kaufman, D. W., Helmrich, S. P., Miller, D. R., Stolley, P. D., and Shapiro, S. (1985). Myocardial infarction and cigarette smoking in women younger than 50 years of age. *JAMA* 253, 2965–2969. doi: 10.1001/jama.1985.03350440043028

Rudnick, G. (1977). Active transport of 5-hydroxytryptamine by plasma membrane vesicles isolated from human blood platelets. *J. Biol. Chem.* 252, 2170–2174.

Samet, J. M. (1991). Health benefits of smoking cessation. *Clin. Chest. Med.* 12, 669–679.

Senis, Y. A., Mazharian, A., and Mori, J. (2014). Src family kinases: at the forefront of platelet activation. *Blood* 124, 2013–2024. doi: 10.1182/blood-2014-01-453134

Sherva, R., Wilhelmsen, K., Pomerleau, C. S., Chasse, S. A., Rice, J. P., Snedecor, S. M., et al. (2008). Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. *Addiction* 103, 1544–1552. doi: 10.1111/j.1360-0443.2008.02279.x

Shirakawa, R., Yoshioka, A., Horiuchi, H., Nishioka, H., Tabuchi, A., and Kita, T. (2000). Small GTPase Rab4 regulates Ca2+-induced α-granule secretion in platelets. *J. Biol. Chem.* 275, 33844–33849. doi: 10.1074/jbc.M002834200

Siess, W., Lorenz, R., Roth, P., and Weber, P. C. (1982). Plasma catecholamines, platelet aggregation and associated thromboxane formation after physical exercise, smoking or norepinephrine infusion. *Circulation* 66, 44–48. doi: 10.1161/01.CIR.66.1.44

Singh, P., Fletcher, T. W., Li, Y., Rusch, N. J., and Kilic, F. (2013). Serotonin uptake rates in platelets from angiotension II-induced hypertensive mice. *Health* 5, 31–39. doi: 10.4236/health.2013.54A005

Sjoerdsma, A., and Palfreyman, M. G. (1990). History of serotonin and serotonin disorders. *Ann. N. Y. Acad. Sci.* 600, 1–7. doi: 10.1111/j.1749-6632.1990.tb16869.x

Smyth, S. S., Smyth, S. S., Woulfe, D. S., Weitz, J. I., Gachet, C., Conley, P. B., et al. (2009). G-protein-coupled receptors as signaling targets for antiplatelet therapy. *Arterioscler. Thromb. Vasc. Biol.* 29, 449–457. doi: 10.1161/ATVBAHA.108.176388

Tweed, J. O., Hsia, S. H., Lutfy, K., and Friedman, T. C. (2012). The endocrine effects of nicotine and cigarette smoke. *Trends Endocrinol. Metab.* 7, 334–342. doi: 10.1016/j.tem.2012.03.006

US Department of Health and Human Services (1990). *The Health Benefits of Smoking Cessation A Report of the Surgeon General*. Atlanta: US Department of Health and Human Services.

Vikens, K., Farstad, M., and Nordrehaug, J. E. (1999). Serotonin is associated with coronary artery disease and cardiac events. *Circulation* 100, 483–489. doi: 10.1161/01.CIR.100.5.483

Walther, D. J., Peter, J. U., Winter, S., Holtje, M., Paulmann, N., Grohmann, M., et al. (2003). Serotonylation of small GTPases is a signal transduction pathway that triggers platel etalpha-granule release. *Cell* 26, 851–862. doi: 10.1016/S0092-8674(03)01014-6

Willett, W. C., Green, A., Stampfer, M. J., Speizer, F. E., Colditz, G. A., Rosner, B., et al. (1987). Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. *New Engl. J. Med.* 317, 1303–1309. doi: 10.1056/NEJM198711193172102

Winniford, M. D., Jansen, D. E., Reynolds, G. A., Apprill, P., Black, W. H., and Hillis, L. D. (1987). Cigarette smoking-induced coronary vasoconstriction in atherosclerotic coronary artery disease and prevention by calcium antagonists and nitroglycerin. *Am. J. Cardiol.* 59, 203–207. doi: 10.1016/0002-9149(87)90785-5

Woulfe, D. S. (2005). Platelet G protein-coupled receptors in hemostasis and thrombosis. *J. Thromb. Haemost.* 310, 2193–2200. doi: 10.1111/j.1538-7836.2005.01338.x

Yang, J., Wu, J., Jiang, H., Mortensen, R., Austin, S., Manning, D. R., et al. (2002). Signaling through Gi family members in platelets. Redundancy and specific regulation of adenylyl cyclase and other effectors. *J. Biol. Chem.* 277, 46035–46042. doi: 10.1074/jbc.M208519200

Ziu, E., Freyaldenhoven, S., Mercado, C. P., Ahmed, B. A., Preeti, P., Lensing, S., et al. (2012). Down-regulation of the serotonin transporter in hyperreactive platelets counteracts the pro-thrombotic effect of serotonin. *J. Mol. Cell. Cardiol.* 52, 1112–1121. doi: 10.1016/j.yjmcc.2012.02.004

Ziu, E., Hadden, C., Li, Y., Lowery, C. L. I. I. I., Singh, P., Ucer, S. S., et al. (2014). Effect of serotonin on platelet function in cocaine exposed blood. *Sci. Rep.* 4:5945. doi: 10.1038/srep05945

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Lowery, Woulfe and Kilic. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 23 April 2019
doi: 10.3389/fnins.2019.00362

In vivo Hippocampal Serotonin Dynamics in Male and Female Mice: Determining Effects of Acute Escitalopram Using Fast Scan Cyclic Voltammetry

Rachel A. Saylor[1†], Melinda Hersey[1,2†], Alyssa West[1], Anna Marie Buchanan[1,2], Shane N. Berger[1], H. Frederik Nijhout[3], Michael C. Reed[4], Janet Best[5] and Parastoo Hashemi[1]*

[1] Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, United States, [2] Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, United States, [3] Department of Biology, Duke University, Durham, NC, United States, [4] Department of Mathematics, Duke University, Durham, NC, United States, [5] Department of Mathematics, The Ohio State University, Columbus, OH, United States

OPEN ACCESS

Edited by:
Nasser Haddjeri,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Albert Adell,
Spanish National Research Council (CSIC), Spain
Lynn G. Kirby,
Temple University, United States

***Correspondence:**
Parastoo Hashemi
hashemi@mailbox.sc.edu

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: 22 December 2018
Accepted: 29 March 2019
Published: 23 April 2019

Citation:
Saylor RA, Hersey M, West A, Buchanan AM, Berger SN, Nijhout HF, Reed MC, Best J and Hashemi P (2019) In vivo Hippocampal Serotonin Dynamics in Male and Female Mice: Determining Effects of Acute Escitalopram Using Fast Scan Cyclic Voltammetry. Front. Neurosci. 13:362. doi: 10.3389/fnins.2019.00362

Depression is a highly prevalent psychiatric disorder, impacting females at a rate roughly twice that of males. This disparity has become the focus of many studies which are working to determine if there are environmental or biological underpinnings to depression pathology. The biology of depression is not well understood, but experts agree that a key neurotransmitter of interest is serotonin. Most research on basic serotonin neurochemistry, by us and others, has predominantly focused on male models. Thus, it is now critical to include female models to decipher possible fundamental differences between the sexes that may underlie this disorder. In this paper, we seek to determine any such differences using fast-scan cyclic voltammetry (FSCV) and fast-scan controlled adsorption voltammetry. These techniques allow us to probe the serotonergic system *via* measurement of evoked and ambient serotonin at carbon fiber microelectrodes (CFMs). Our data reveal no statistical differences, in the hippocampus, in female serotonin chemistry during the different stages of the estrous cycle compared to the mean female response. Furthermore, no difference was observed in evoked serotonin release and reuptake, nor ambient extracellular serotonin levels between male and female mice. We applied a previously developed mathematical model that fits our serotonin signals as a function of several synaptic processes that control the extracellular levels of this transmitter. We used the model to study potential system differences between males and females. One hypothesis brought fourth, that female mice exhibit tighter autoreceptor control of serotonin, is validated *via* literature and methiothepin challenge. We postulate that this tight regulation may act as a control mechanism against changes in the serotonin signal mediated by estrogen spikes. Importantly, this safety mechanism has no consequence for acutely administered escitalopram's (ESCIT's) ability to increase extracellular serotonin between the sexes.

This work demonstrates little fundamental differences in *in vivo* hippocampal serotonin between the sexes, bar control mechanisms in female mice that can be observed under extraneous circumstances. We thus highlight the importance of considering sex as a biological factor in determining pharmacodynamics for personalized medical treatments that involve targeting serotonin receptors.

Keywords: serotonin, hippocampus, SSRI, FSCV, FSCAV, sex, depression

INTRODUCTION

Clinical depression is more than twice as prevalent in adult females than males (Weissman and Klerman, 1977; Kessler et al., 1993; Hankin et al., 1998; Cyranowski et al., 2000; Piccinelli and Wilkinson, 2000; Grigoriadis and Robinson, 2007) and antidepressants exert varying degrees of efficacy by sex (Frackiewicz et al., 2000; Sramek and Cutler, 2011). Interestingly, most studies show this sex-dependent increased risk of depression only emerges post-puberty (Anderson et al., 1987; McGee et al., 1992; Nolen-Hoeksema and Girgus, 1994). This disparity in adult depression rates has been explained *via* environmentally induced (i.e., societal stress) or innate (fundamental biology) phenomena (Sullivan et al., 2000; Eley et al., 2004). The idea that there are inherent biological underpinnings to depression is well-debated and hypotheses that have been brought forth over the years have not had unanimous acceptance (Asberg et al., 1976a,b; Owens and Nemeroff, 1994). Historically, preclinical and basic research has not encompassed both sexes equally, with a strong bias toward male models. This approach was formed under the preconception that the estrous cycle confounds experimental data, however, as stated in NOT-OD-15-102 in 2015, "An overreliance on male animals and cells may obscure understanding of key sex influences on health processes and outcomes" (National Institutes of Health, 2015). Henceforth, there is a strong emphasis on including male and female mice in all basic and preclinical studies.

We are in a good position to study intrinsic neurochemical differences that may drive depression and antidepressant actions in male and female animal models since we can monitor sub-second changes in serotonin in real time, *in vivo*. Serotonin is a well-established transmitter of interest to depression and antidepressant activity in both sexes (Piccinelli and Wilkinson, 2000; Sramek and Cutler, 2011). We monitor serotonin dynamics *in vivo* using voltammetric techniques, fast-scan cyclic voltammetry (FSCV) and fast-scan controlled adsorption voltammetry (FSCAV) at carbon fiber microelectrodes (CFMs). FSCV enables the *in vivo* monitoring of the release and reuptake of serotonin on a sub-second timescale (Hashemi et al., 2009; Wood and Hashemi, 2013; Wood et al., 2014) and FSCAV quantifies ambient serotonin concentrations on the order of tens of seconds (Abdalla et al., 2017).

In this article, we study the *in vivo* serotonin chemistry in the CA2 region of the hippocampus of male and female mice. We chose to start this study with the hippocampus because of this brain region's heavy association with depression and antidepressant actions. For example, decreased hippocampal volume is found in human and animal models of depression (Magarinos and McEwen, 1995; Sheline et al., 1996; Bremner et al., 2000; Videbech and Ravnkilde, 2004). Furthermore, selective serotonin reuptake inhibitors (SSRIs) are shown to change hippocampal architecture *via* neurogenesis (Czeh et al., 2001; Malberg and Duman, 2003; Jayatissa et al., 2006). In our cohort of female mice, statistical differences are not found in serotonin signals between the overall female mean and the different stages of the estrous cycle. Importantly, evoked serotonin release, reuptake, and ambient serotonin in 23 female (all cycle stages) and 23 male mice are not statistically different. Potential functional differences are investigated by mathematically modeling the averaged male and female responses as a measure of several synaptic processes that regulate extracellular serotonin. Specifically, the evoked signal in male mice is postulated to have higher input and the signal in female mice is hypothesized to undergo tighter autoreceptor control. We provide validation of the model's autoreceptor hypothesis in female mice *via* administration of methiothepin, a non-selective serotonin receptor antagonist with high affinity for the serotonin autoreceptors. We put forth that this stronger autoreceptor control may act as a safety mechanism against serotonin-mediating estrogen spikes. To understand whether these autoreceptor effects are consequential for SSRI response, we compare administration of acute doses of the SSRI, escitalopram (ESCIT) to male and female cohorts of mice, some of which are pretreated with methiothepin. The percent change in serotonin reuptake is less dramatic across all doses of ESCIT in females but this effect is independent of autoreceptor antagonism.

In summary, serotonin chemistry, in the hippocampus, during the different stages of the estrous cycle is not different from the mean in female mice and the control evoked release, reuptake, and ambient serotonin are not statistically different between the sexes. On the microanalysis level, differences in serotonin regulation between male and female mice may lie, in part, in autoreceptor regulation. This finding is especially useful when considering pharmacodynamics for personalized medical treatments that involve serotonin receptors.

MATERIALS AND METHODS

Chemicals and Reagents

Calibration solutions were prepared by dissolving serotonin hydrochloride (Sigma–Aldrich Co., St. Louis, MO, United States) in Tris buffer to produce solution concentration of 10, 25, 50, and 100 nM. Tris buffer consisted of: 15 mM $H_2NC(CH_2OH)_2$ HCl, 140 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM

$NaH_2PO_4.H_2O$, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4 (Sigma–Aldrich Co., St. Louis, MO, United States) in deionized water and pH adjusted to 7.4. ESCIT oxalate (≥98, HPLC) (3, 10, or 30 mg kg^{-1}) from Sigma–Aldrich (St. Louis, MO, United States) and methiothepin mesylate salt (≥98, HPLC) also from Sigma–Aldrich (St. Louis, MO, United States) were individually dissolved in sterile saline (Hospira, Lake Forest, IL, United States) and administered *via* intraperitoneal (i.p.) injection at a volume of 5.0 ml kg^{-1} of animal weight. Liquion (LQ-1105, 5% by weight Nafion^{TM}) was purchased from Ion Power Solutions (New Castle, DE, United States).

Electrode Fabrication

Voltammetric analysis of serotonin was performed as described previously (Hashemi et al., 2009; Wood and Hashemi, 2013; Wood et al., 2014). Briefly, CFMs were constructed by aspirating 7 μm carbon fibers (Goodfellow Corporation, Coraopolis, PA, United States) into glass capillaries (0.4 mm internal diameter, 0.6 mm outer diameter, AM Systems, Carlsborg, WA, United States). A vertical pipette puller (Narishige Group, Tokyo, Japan) was employed to create a carbon-glass seal. Subsequently, the exposed carbon fiber was cut to 150 μm and silver paint was used to forge an electrical connection to a connection pin. Finally, electrodes were electrodeposited with Nafion^{TM} as described previously (Hashemi et al., 2009).

Animal and Surgical Procedures

All animal procedures and protocols were performed in accordance with regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina, which operates with accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Male and female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, United States), 6–12 weeks old and weighing 18–25 g, were group housed, had constant access to food and water, and were kept on a 12 h light/dark cycle (lights off at 7:00 and on at 19:00). We chose to include mice from this broad age range since we found no statistical differences in mice aged 6–8 and 9–12 weeks (**Supplementary Figure S1**). Female mice were selected at random, without regard to their estrous cycle. In the estrous cycle experiments, vaginal smears were collected after the conclusion of the experiment and cycle determine according to Caligioni (**Supplementary Figure S2**) (Caligioni, 2009). Estrous cycle determination was limited to a single-day cell analysis at the end of the neurochemical analysis in order to limit stress to the animal that would likely alter FSCV/FSCAV serotonergic responses. To induce and maintain anesthesia, 25% w/v urethane [Sigma–Aldrich Co., dissolved in 0.9% NaCl solution (Hospira)] was injected i.p. (7 μl/g of body weight). Mouse body temperature was maintained using a heating pad (Braintree Scientific, Braintree, MA, United States). Stereotaxic surgery (David Kopf Instruments, Tujunga, CA, United States) was performed, and all coordinates were taken in reference to bregma. A Nafion^{TM}-modified CFM was lowered into the CA2 region of the hippocampus (AP: −2.91, ML: +3.35, DV: −2.5 to −3.0) (Franklin, 2013) or into the medial prefrontal cortex (mPFC) (AP: +1.7, ML: −0.2, DV: −2.2 to −2.9) (Franklin, 2013) and adjusted in the dorsal/ventral plane until a serotonin signal was observed. A stimulating electrode (insulated stainless steel, diameter: 0.2 mm, untwisted, Plastics One, Roanoke, VA, United States) was placed into the medial forebrain bundle (AP: −1.58, ML: +1.00, DV: −4.8) (Franklin, 2013) and a pseudo Ag/AgCl reference electrode, created by electroplating chloride (30 s in 0.1 M HCl at 5 V) onto a silver wire, was placed into the contralateral hemisphere.

Data Collection

FSCV and FSCAV were performed using a Dagan potentiostat (Dagan Corporation, Minneapolis, NM, United States), WCCV 3.06 software (Knowmad Technologies LLC, Tucson, AZ, United States) and either a Dagan or Pine Research headstage (Pine Research Instrumentation, Durham, NC, United States). For FSCV collection, the "Jackson" serotonin waveform (Jackson et al., 1995) was applied to the electrode at a scan rate of 1000 V s^{-1} and at a frequency of 10 Hz. To evoke serotonin release, a biphasic stimulation was applied through a linear constant current stimulus isolator (NL800A Neurolog, Medical Systems Corp, Great Neck, NY, United States) with the following parameters: 60 Hz, 360 μA each, 2 ms in width, and 2 s in length. Upon completion of data collection, a high voltage was applied to the working electrode to lesion the tissue surrounding the electrode for electrode placement verification using histology.

For basal experiments, control evoked files were collected followed by the methodology being switched to FSCAV. For FSCAV collection, the serotonin waveform was applied at 100 Hz for 2 s, followed by a period of controlled adsorption where the potential was held at 0.2 V for 10 s, lastly the serotonin waveform was reapplied at 100 Hz, as described in Abdalla et al. (2017). Thirty files (at one file per minute) were collected as control files. Following control files, an i.p. injection of saline was administered and 30 more files of FSCAV were collected. Animals were then administered ESCIT (10 mg kg^{-1}) i.p. and 60 files post-ESCIT were collected. The system was then switched back to traditional FSCV and four post-basal stimulation files were collected. Electrodes were then removed and underwent a post calibration in which 10 files were collected with the electrode in solutions of 10, 25, 50, and 100 nM solutions of serotonin. A dose response was also conducted using FSCV, as previously described, in which male and female mice were administered either 3, 10, or 30 mg kg^{-1} ESCIT and four control files averaged together were then compared with stimulated release 30 min post-ESCIT.

Data Analysis and Statistics

Digital filtering (zero phase, Butterworth, 5 kHz low-pass) was accomplished within the WCCV software. For FSCV analysis, signals were smoothed using WCCV software, the cyclic voltammogram (CV) taken for serotonin identification, and the current vs. time (IT) trace extracted to visualize the release and reuptake of serotonin. Four evoked events, with 10 min between each event, were averaged for each individual mouse to establish a control evoked signal. A previously established calibration factor (49.5 ± 10.2 nA/μM) was used to convert current into concentration. For FSCAV analysis, the third CV after the reapplication of the waveform was selected for quantification, and

the peak occurring approximately between 0.4 and 0.85 V was integrated to determine the charge value (pC). Post calibrations of each electrode, plotting charge (pC) vs. [serotonin] (nM), were used to determine basal concentration.

For FSCV data, four IT curves were averaged for each animal to establish a control. The average for each individual animal was then combined with the other animals in the group to determine an overall group average. The standard error of the mean (SEM) was calculated using the average IT for each animal (n = # animals). To determine significance between two points, a two-tailed student's t-test was utilized ($p < 0.05$).

To determine the $t_{1/2}$, a code was custom written in excel to fit the reuptake component of the curve and calculate the time taken to reach half of the maximum amplitude. The number of files with a concentration of less than zero was used to quantify the "dip" below baseline, associated with autoreceptors, which will be covered in more detail below. Determination of the percent reuptake change following ESCIT is explained in Section "Modeling."

Data were excluded based on the criteria outlined herein. For all experiments, the evoked signal CV was compared to well-established *in vivo* and *in vitro* serotonin CVs and signals in which the CVs did not contain the characteristic serotonin redox peaks were excluded. Animals which did not survive the full experiment or whose drug injection did not elicit a response were removed. Signals which did not return to baseline or were otherwise unstable, as well as those which were outside the normal range, as determined by a Q-test, were removed. Data which contained a peak resulting from the stimulation electrode touching the skull that masked, delayed, or minimized the serotonin response (stimulation glitch) were excluded. According to these criteria, 10 animals were excluded, which accounts for 8% of the total number of animals used. All other data were included and all raw evoked data are shown in **Figure 2**.

To determine the number of animals required for observing significant differences in serotonin signals, we employed a power analysis (Charan and Kantharia, 2013). The following formula was used to calculate a quantitative endpoint for the sample size required to compare two groups.

$$\text{Sample size} = 2\ \text{SD}^2\ (\text{Z}^{\alpha/2} + \text{Z}^{\beta})^2/\text{d}^2$$

The pooled standard deviation from the sample data was 0.83 and Cohen's d was calculated as 1.76. The $\text{Z}^{\alpha/2}$ term was 1.96 (from Z table) as a type 1 error of 5% and the Z^{β} was 0.842 (from Z table) at 80% power. This power analysis resulted in a n = 3.5. The sample size corrected for exclusion was calculated using n = 3.5 and a percent loss of animals as 8%, showing that about 3.8 animals were required.

Modeling

A previously presented mathematical model was used to model the average male and female evoked responses:

$$\frac{d[S(t)]}{dt} = R(t)(1 - A(t)) - \alpha\frac{V_{\text{max1}}[S(t)]}{k_{\text{m1}} + [S(t)]} - \beta\frac{V_{\text{max2}}[S(t)]}{k_{\text{m2}} + [S(t)]}$$

$S(t)$ is the concentration of serotonin in the extracellular space, $R(t)$ is the release rate of the serotonin neurons in the hippocampus near the electrode that rises briefly after stimulation and then returns to baseline, and $A(t)$ represents the strength of the autoreceptor effect caused by rising serotonin in the extracellular space [the higher $A(t)$ the more serotonin release is inhibited] (Wood et al., 2014). The first negative term represents reuptake resulting from Uptake 1 transporters, the serotonin transporters (SERTs), with V_{max1} = 19.25 nM s^{-1} and K_{m1} = 5 nM. The second term represents reuptake *via* Uptake 2 transporters [dopamine transporters (DATs), norepinephrine transporters (NETs), and organic cation transporters (OCTs)] with V_{max2} = 780 nM s^{-1} and K_{m2} = 170 nM. We modeled and discussed Uptake 1 and Uptake 2 in detail previously (Wood et al., 2014). Briefly, Uptake 1 is high affinity but low efficiency serotonin transport (Shaskan and Snyder, 1970) while Uptake 2 is low affinity, high efficiency serotonin transport (Daws et al., 2013; Horton et al., 2013). Thus, at serotonin concentrations well above the basal level, Uptake 2 is primarily responsible for serotonin removal from the extracellular space, but low concentrations, closer to the steady state, Uptake 2 has little effect on reuptake of serotonin. For the purpose of our simulations, we assume the basal steady state is 60 nM, roughly the mean of measured basal levels (see below), and the parameter beta decreases from 0.05 above 82 nM linearly to zero at 62 nM, reflecting the properties of Uptake 2. In some simulations, the concentration cutoffs 82 and 62 nM are slightly varied to fit the experimental data. In all our simulations, $\alpha = 1$.

We believe that stimulation of the MFB causes antidromic spikes that stimulate the dorsal raphe nucleus (DRN). The increased firing of DRN neurons increases the release rate, $R(t)$, in the hippocampus. Before stimulation, we assume that $R(t)$ is a constant, R_0, chosen so that the basal steady state is 60 nM. After stimulation, $R(t)$ rises linearly for 1 s followed by decay back to R_0 linearly over 2 s. This value can be varied to reflect the different release rates produced by slight differences in the stimulation of the MFB. The parameter r scales how high above R_0 the release rate goes, with $r = 1$ indicating an increase of release rate of 40 nM s^{-1}. We previously showed that the autoreceptor effects are long-lasting (up to 30 s) and continue after both $R(t)$ and $S(t)$ have returned to baseline (Wood et al., 2014). This longer lasting autoreceptor effect drives the serotonin concentration below baseline after most stimulations.

The model was further used to calculate estimates of the percentage decrease of V_{max2} caused by different doses of ESCIT in male mice and female mice (**Figure 4**). These calculations were carried out assuming that most of the initial decrease of serotonin in the extracellular space is caused by the Uptake 2 transporters. This assumption is supported by our previous work (Wood et al., 2014). The experimental data give us the evoked serotonin response before ESCIT (control curve) and the evoked serotonin response 30 min following the administration of ESCIT (dose curve). As the K_{m2} of Uptake 2 is known, the control signal was used to calculate the value of the effective V_{max2} for the control signal. The process was repeated for the ESCIT signal to calculate the effective V_{max2} for the ESCIT signal. **Figure 4** reports the percentage change from our estimate of

V_{max2} for control to our estimate of V_{max2} 30 min following the administration of ESCIT.

RESULTS

Serotonin During the Different Stages of the Estrous Cycle

Evoked and basal serotonin was measured during each stage of the estrous cycle in the CA2 region of the hippocampus in female mice (images verifying stage of cycle are in **Supplementary Figure S2**). The basal serotonin concentrations were added to the respective animals' evoked response and are displayed in the colored traces in **Figure 1** for each stage of the estrous cycle (n = 3–4). The gray traces in **Figure 1** are the averaged female responses (blinded for stage of estrous cycle, n = 10). When comparing the data from each stage of the cycle to the average, we found no significant differences in evoked release amplitude and $t_{1/2}$ of serotonin clearance (table in **Figure 1**).

Evoked and Basal Serotonin in Male and Female Mice

Evoked serotonin was measured in male and female mice (regardless estrous cycle stage). Results are shown in **Figure 2** where **Figure 2A** shows the average serotonin concentration ([serotonin]) vs. time traces in male and female mice and **Figure 2B** shows the raw data of each individual making up the averages shown in **Figure 2A**. The results of FSCAV experiments in male and female mice are seen in **Figure 2C**. The values for the maximum amplitude of serotonin release, the $t_{1/2}$ of serotonin clearance, and the average ambient [serotonin] are in the table in **Figure 2C** (middle). These results demonstrate no significant difference in the evoked release amplitude (35.0 ± 3.3 nM in males and 32.2 ± 4.3 nM in females, p = 0.61, two-tailed student's t-test), $t_{1/2}$ of serotonin clearance (2.1 ± 0.2 s in males and 2.1 ± 0.2 s in females and p = 0.93, two-tailed student's t-test), or average ambient serotonin levels [62.5 ± 1.8 nM in males and 60.4 ± 1.8 nM in females (p = 0.43, two-tailed student's t-test)]. To determine if these results are specific to the hippocampus, we explored an additional brain region, the mPFC (**Supplementary Figure S3**). No statistical difference was found in this region between the male and female mice.

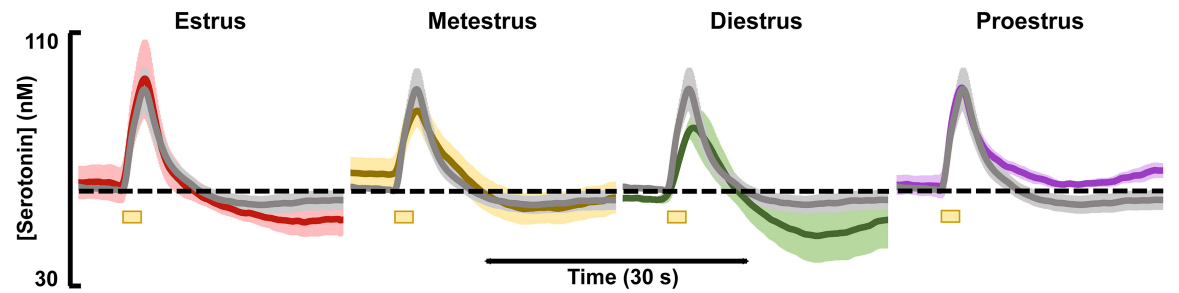
Mathematically Modeling Male and Female Serotonin Signals

Figure 3 shows the results of fitting the averaged male and female serotonin responses with a model that we previously developed that captures experimental data in the context of the synaptic mechanisms that control extracellular serotonin. In these animals that we reported evoked release, ambient serotonin data were not available. Thus, to give basis to the model, an average concentration from a subset of mice in this work (60 nM) was added, arbitrarily, to each signal. The amplitude of the signal in male mice was higher than in the female mice, which was modeled *via* a larger input term to the terminal (r = 0.54 in males, r = 0.43 in females). Additionally, there was a larger dip below baseline after stimulation in female mice, which was modeled *via* a larger value for the autoreceptor term in the model. We described this autoreceptor phenomenon at length previously (Wood et al., 2014).

Serotonin Response to ESCIT

An acute ESCIT dose, was administered *via* i.p. injection, to male and female mice, separately at 3, 10, and 30 mg kg^{-1} and the evoked and basal serotonin responses were monitored in separate cohorts of mice per dose. The evoked responses, seen in **Figure 4**, are shown for each dose along with the percent change in reuptake. At every dose, the female mice had a lower percent change in reuptake compared to the males.

The basal, steady-state serotonin concentration response to ESCIT is shown in **Figure 5** in male and female mice. Control files were collected for 30 min, after saline injection, files were taken for 30 min, finally ESCIT was administered and files were taken for an additional hour. Serotonin was not altered



	Average	p-value	Average	p-value	Average	p-value	Average	p-value
Amp_{max}	95.7 nM	0.86	86.5 nM	0.46	81.7 nM	0.19	92.7 nM	0.96
$t_{1/2}$	1.6 s	0.23	2.4 s	0.74	2.4 s	0.69	1.80	0.48

FIGURE 1 | Serotonin signals, incorporating respective basal levels, from each of the four estrous cycles in female mice are shown. The average from female mice in the estrus (red, n = 4), metestrus (yellow, n = 4), diestrus (green, n = 3), and proestrus (purple, n = 3) stages are shown. A table showing the average amplitude and $t_{1/2}$ for each stage of the female mouse cycle along with the p-value resulting from a two-tailed t-test is seen below. Significance was defined as $p < 0.05$.

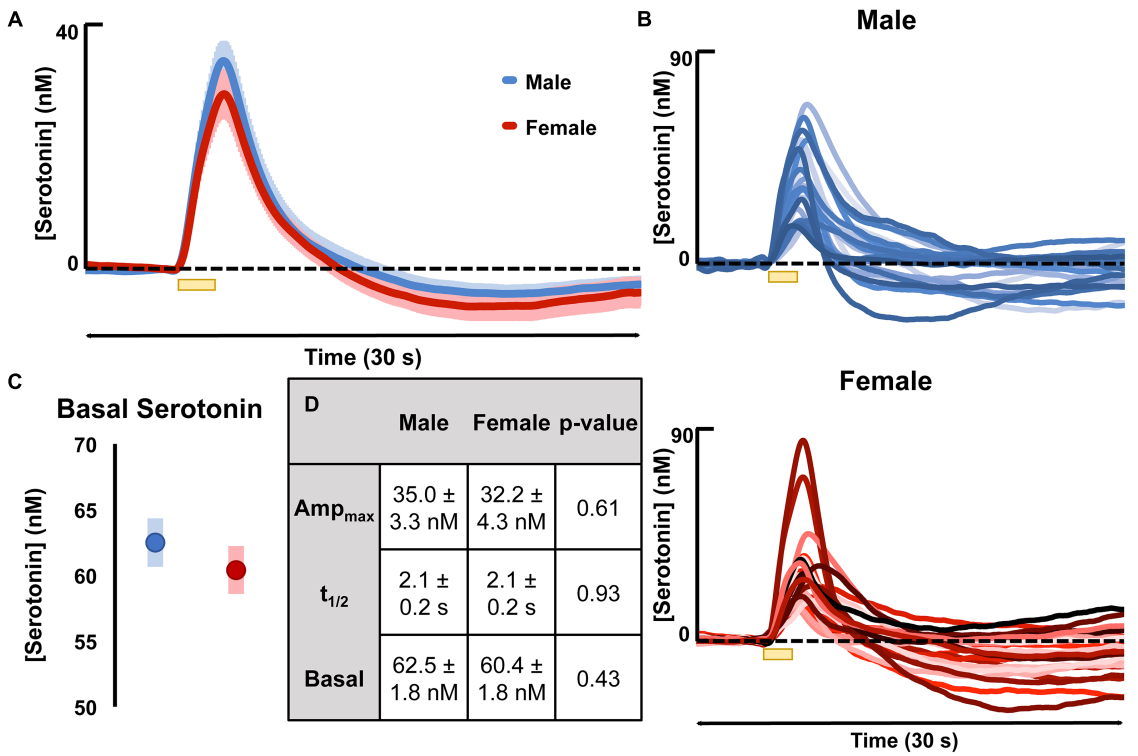


FIGURE 2 | (A) The average evoked serotonin for male mice (blue, $n = 23$) and female mice (red, $n = 23$) with SEM shown in a lighter shade around each. Serotonin was evoked *via* a short stimulation (yellow bar). **(B)** The average basal serotonin concentration found in male ($n = 17$, shown in blue) and female (red, $n = 18$) animals is shown, along with the SEM of each. **(C)** The table details male and female average maximum evoked [serotonin] amplitude, the average $t_{1/2}$ of serotonin clearance and the average basal [serotonin]. **(D)** The raw data for each individual mouse used to generate the average evoked serotonin signal are shown in varying shades of blue (male) and red (female). Significance was defined as $p < 0.05$.

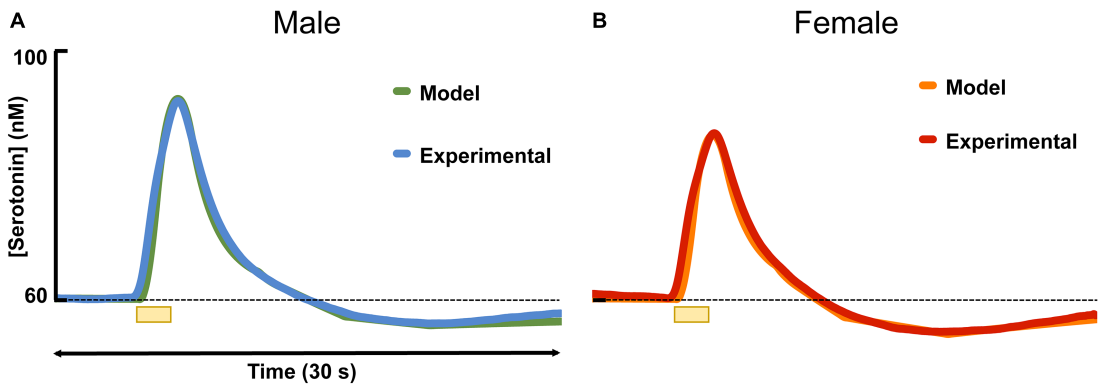


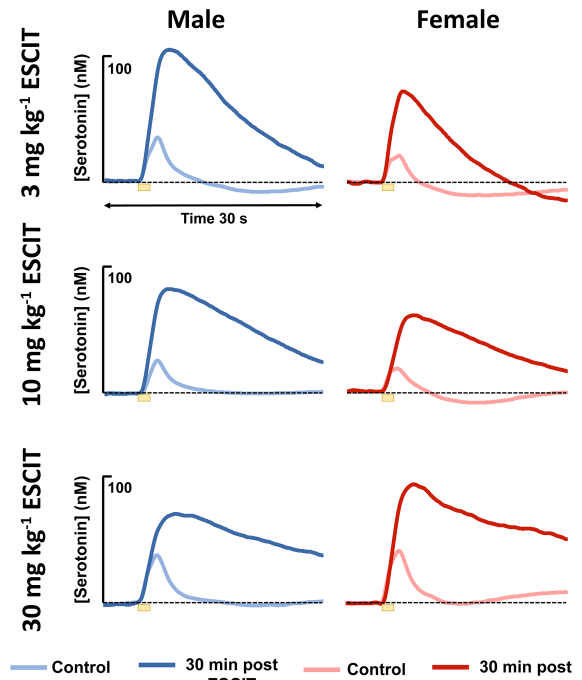
FIGURE 3 | The average experimental serotonin signal from male **(A)** and female **(B)** mice is shown plotted with the modeled serotonin signal. An average basal value of 60 nM has been incorporated into the male and female responses.

by saline, while roughly 10 min after ESCIT, the signal rose ∼43–46%. This represented a significant change in serotonin in both sexes ($p < 0.01$, two-tailed student's t-test) but no significant difference between the two sexes ($p = 0.96$, two-tailed student's t-test). Likewise, the basal serotonin concentrations before and after ESCIT were not significantly different between males and females ($p = 0.244$ and $p = 0.220$, respectively, two-tailed student's t-test).

DISCUSSION

Control Serotonin Chemistry Is Not Significantly Different Between the Sexes in the Hippocampus

The possible inherent biological underpinnings of depression are not well understood and hypotheses that have been brought forth



Percent Decrease of Uptake

Dose	Male	Female
3 mg kg^{-1}	48%	23%
10 mg kg^{-1}	76%	50%
30 mg kg^{-1}	86%	80%

FIGURE 4 | The male (blue) and female (red) evoked serotonin signals are shown (n = 4 for each). A lighter color is shown to indicate control release and a darker shade is used to denote response 30 min following i.p. ESCIT administration (3, 10, and 30 mg kg^{-1}). The table lists percent decrease of reuptake in males and females at the three doses.

over the years have not had unanimous recognition. It is well established that alterations in serotonin chemistry likely underlie the behavioral phenotypes of depression (Asberg et al., 1976a,b; Owens and Nemeroff, 1994; Stockmeier, 2003). We are in a good position to investigate the chemistry of the serotonin system from a fundamental perspective. Our techniques, FSCV and FSCAV, allow us to probe the essence of synaptic release and reuptake of serotonin *via* the many parallel systems that regulate this transmitters' extracellular concentration.

There is a belief that neurochemistry in female rodents is intrinsically more variable due to the estrous cycle (Beery and Zucker, 2011). Accordingly, the majority of studies on serotonin's role in depression and antidepressant effects (including our own) have so far only been in male mice (Borsini and Meli, 1988; Stahl, 1998; Wood and Hashemi, 2013; Abdalla et al., 2017). Here, we perform the first FSCV serotonin measurements in female mice and investigate whether the different stages of the estrous cycle alter the characteristics of serotonin neurochemistry. The estrous cycle in rodents is approximately 4–5 days long and is divided into four stages: proestrus, estrus, metestrus, and diestrus. These stages can be determined through looking at the difference in cell types in a vaginal smear under a standard light microscope and correlating those differences to the stage of estrous cycle (Caligioni, 2009; Byers et al., 2012) (**Supplementary Figure S2**). The phase of the estrous cycle for each female mouse was recorded and the responses of the mice in each respective cycle were averaged. **Figure 1** shows that none of the averaged responses between the cycles differ statistically from the mean of 10 females blinded for stage of cycle. Estrogen is thought to modulate serotonin (Biegon et al., 1980; Rubinow et al., 1998), although it is unclear to which extent this phenomenon extends during the different stages of the cycle (Gundlah et al., 1998; Maswood et al., 1999; Bethea et al., 2002) Overall, our data do not have enough significance to support this notion in the hippocampus. Additional data from male and female mice in the mPFC (**Supplementary Figure S3**) also show no significant differences between the sexes. An important caveat here is that, while the hippocampus and mPFC are brain regions heavily implicated in depression and antidepressant actions (Goldapple et al., 2004; Kodama et al., 2004; Drevets et al., 2008), our study has not included other brain regions thought to be important in the pathology depression, which may show differences. An additional point of note is the age range of animals used here since there is evidence that depression rates vary with age (Anderson et al., 1987; McGee et al., 1992; Hankin et al., 1998; Piccinelli and Wilkinson, 2000). We utilized a broad age range (6–12 weeks) to maximize potential observable differences. In **Supplementary Figure S1**, we found no statistical differences between young adult (6–8 week old) and adult (9–12 week old) mice.

Scientists have found a variety of sex differences in rodents related to serotonin including: metabolism (Curzon and Bridges, 1970; Rosecrans, 1970; Kennett et al., 1986), synthesis (Nishizawa et al., 1997), receptor binding (Fischette et al., 1983; Arango et al., 1995; Parsey et al., 2002; Jovanovic et al., 2008), transporter functions (McQueen et al., 1997; Jovanovic et al., 2008), extracellular levels (Gundlah et al., 1998), and many other processes (Biegon et al., 1980; Rubinow et al., 1998; Dominguez et al., 2003). However, parallel studies have found little to no differences in other aspects of the serotonin system (Rosecrans, 1970; Kunimura et al., 2015). This incongruity makes it very difficult to ascertain whether the serotonin system plays a fundamental role in the disparity of depression between male and females.

In **Figure 2A**, we see that evoked release and reuptake of serotonin is remarkably reproducible in male vs. female mice. A point of note is the high precision of these experimental data. These data (**Figure 2D**) are essentially raw data (two analysis steps include smoothing the data and conversion of current to concentration). These data are highly reproducible, in contrast to other chemical data that often needs to be normalized and shown as a % change from baseline (Kaminska et al., 2018; Wojcieszak et al., 2018). Also, compared to dopamine voltammetry studies, where significant heterogeneity manifests as "hotspots" of dopamine activity (May and Wightman, 1989; Wightman et al., 2007), these serotonin data are much more uniform.

In **Figure 2B**, in the interest of scientific transparency, we purposefully show all of our raw data that were used to generate the average curves illustrated in the bottom panel. The SEM of the maximum amplitude of the average female curve is

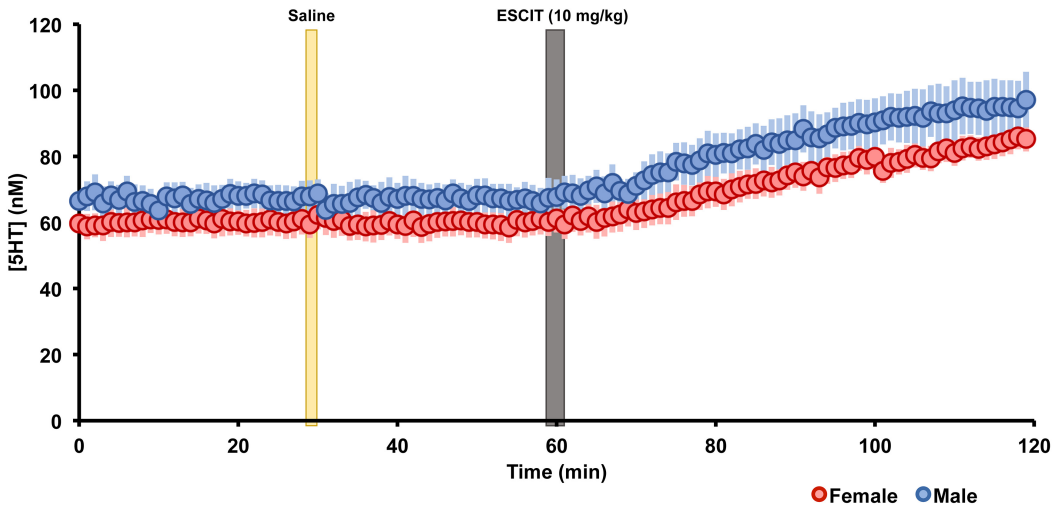


FIGURE 5 | The male (blue, n = 5) and female (red, n = 5) basal serotonin levels were collected for 30 min. A saline injection (marked by a yellow bar) preceded another 30 min of basal serotonin data was collected. At the gray bar, escitalopram (ESCIT, 10 mg kg^{-1}) was administered and 60 min of basal serotonin data was collection. SEM is shown as a lighter bar behind.

4.3 nM. This small error is in accordance with our long-standing hypothesis that the serotonin system is a profoundly regulated one (Hashemi et al., 2009; Wood et al., 2014). Standard statistics showed no difference in the amplitude or $t_{1/2}$ and of evoked release nor in basal [serotonin] (**Figure 2**). These statistical findings emphasize both the high reproducibility of our data and the tightly controlled *in vivo* serotonin system. To summarize these data: there are no statistical differences between the stages of the cycle and the mean in females. Critically, the averaged responses between male and female mice are not different in the CA2 region of the hippocampus.

Modeling Serotonin Signals Reveals Potential Regulation Differences

We previously developed a model that deconstructs the chemical signal as a function of the many synaptic processes that control extracellular serotonin. Thus, modeling the experimental data can provide specific information about the components of system (Wood et al., 2014). As a starting point, we fit the averaged male and averaged female experimental data; the model hypothesized two differences between the male and female evoked response. First, the evoked response in males necessitated a larger model input term to the terminal. Second, a stronger autoreceptor term was necessary to model the female data. This finding is insightful but requires further verification since the model was based on the average, and not individual data. To further verify these notions, we hypothesized potential biochemical processes that underlie the findings.

Larger Model Input Term to the Terminal in Males

To model the experimental data, a larger input term was required in male mice to fit the larger amplitude of evoked serotonin release. We hypothesize three biochemical reasons for this larger input. First, a higher amplitude response could be the result of more axons. However, in most investigations, there have not been significant differences found in the number of serotonin axons between male and female mice (Jitsuki et al., 2009; Kunimura et al., 2015; Rajkowska et al., 2017). Second, we postulate that axons in male mice have a lower stimulation threshold than in female mice. The literature has come to no consensus on this front (Yang et al., 2015; Strupp-Levitsky et al., 2016). Third, we put forth that more vesicular serotonin is released in response to stimulation in male mice. This could be a result of a variety of changes within the synapse ranging from vesicle number, Ca^{2+} dependent vesicular release, amount of serotonin released with each vesicle fusion, or catabolism of released serotonin; again these possibilities remain inconclusive based on current literature (Mermelstein et al., 1996; Nishizawa et al., 1997; Rehavi et al., 1998).

Larger Model Autoreceptor Term in Females

Previously, we showed that the dip below baseline after stimulation was a fall in serotonin levels (Wood et al., 2014). We modeled and pharmacologically verified that this decrease in ambient serotonin level was due to prolonged autoreceptor control. In these data, the model captured a larger dip below baseline in females by necessitating a larger autoreceptor term. We postulate that this is a function of a higher density of, or higher functionality of autoreceptors in females. A significant amount of current research suggests sex may affect quantity and function of autoreceptors (Jones and Lucki, 2005; Goswami et al., 2010; Goel et al., 2014). Jones and Lucki (2005), in particular, determined that 5HT1B autoreceptor knockout mice exhibited a sex-dependent increase in baseline hippocampal serotonin present only in females. Goswami et al. (2010) found increased

levels of 5HT1D autoreceptor mRNA in serotonin neurons in the dorsal raphe of females with major depressive disorder compared to control females, a trend that did not persist in male subjects.

The literature does not enable verification of the model's two hypotheses, yet it is important to verify whether these fundamental differences in serotonin chemistry exist in the context of depression and/or antidepressant efficacy in males vs. females. As a pharmacological means to verify these hypotheses, we study acute methiothepin (non-selective serotonin receptor antagonist, with high affinity for the autoreceptors) and ESCIT administration.

Escitalopram Induces Differences in Serotonin Uptake but Not Basal Concentrations Between Sexes

With the increasing global rate of depression, the issue of antidepressant efficacy is brought to the forefront. SSRIs have been shown to have different effects in male and female patients in clinical trials (Kornstein et al., 2000; Khan et al., 2005). In rodents, SSRIs have differential effects by sex. For example, male and female rodents respond differently in the forced swim test (FST) (Cryan et al., 2002; Drossopoulou et al., 2004; Petit-Demouliere et al., 2005; Bogdanova et al., 2013). The FST is a behavioral test; among other uses, it has been used to screen for antidepressant efficacy after acute i.p. SSRI injections in naive animals (Borsini and Meli, 1988; Willner, 1990).

In the context of antidepressant effects in male vs. female mice, we sought to test the two hypotheses brought forth by the model by adhering to the traditional SSRI screening procedure (acute SSRI i.p. injection). The first hypothesis was that there is a higher input to the serotonin terminals in male mice resulting in higher amplitude of evoked release. For the hypothesis to hold, this amplitude difference is expected to persist after SSRI. We used three different doses of ESCIT and presented averaged control evoked responses (n = 4 animals) and the averaged responses 30 min after SSRI administration. ESCIT administration slowed the reuptake of serotonin, as predicted by the mechanism of action of this agent. Notably, following ESCIT, there was a systematic increase in the signal amplitude; however, there was no consistency in amplitude change between the doses in the two sexes. Specifically, at 30 mg kg^{-1}, the female ESCIT response amplitude is higher than that of the males. This finding nullifies the model's first hypothesis, since the higher amplitude in males did not persist across all doses.

In addressing the second hypothesis brought forth by our model, that there is stronger autoreceptor regulation in females, we found that acute ESCIT was less effective at decreasing the rate of serotonin reuptake at all doses in female mice. We propose that this phenomenon could be due to increased functionality of the serotonin autoreceptors, a notion supported in the literature (Jones and Lucki, 2005; Goswami et al., 2010; Goel et al., 2014). Increased autoreceptor activity could counteract SERT-mediated effects of the SSRI, especially since 5HT1-B autoreceptors are G-protein coupled to the SERTs and have previously been

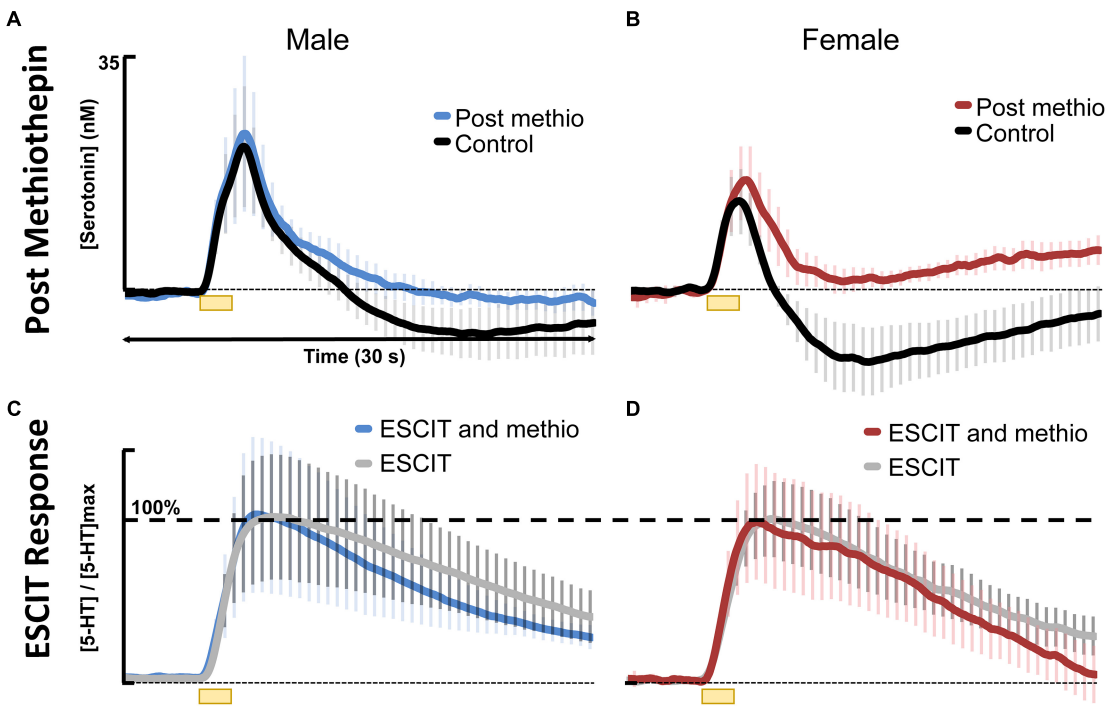


FIGURE 6 | Control stimulated 5HT is shown in black for **(A)** males (n = 4) and **(B)** females (n = 4) and 30 min following 0.1 mg kg^{-1} methiothepin (methio) are shown for **(A)** males (n = 4) in blue and **(B)** females (n = 4) in red. Post methiothepin pretreatment ESCIT (10 mg kg^{-1}) response is shown for **(C)** males in blue and **(D)** females in red. These are normalized to ESCIT responses seen in **Figure 4**, shown in gray for **(C)** males and **(D)** females.

found to mediate serotonin reuptake (Montanez et al., 2014). A potential rationale for increased autoreceptor control in female mice is the modulation of serotonin by estrogen in the brain (Biegon et al., 1980; Lu et al., 1999; Bethea et al., 2002; Abraham et al., 2003; Sheng et al., 2004; Cornil et al., 2006; Barth et al., 2015; Kunimura et al., 2015), a complex relationship previously examined in in-depth reviews (Rubinow et al., 1998; Borrow and Cameron, 2014). Estrogen is thought to modulate serotonin in both male and female mice on both a slower, ambient level as well as rapid, transient effects which can alter intracellular signaling (Abraham et al., 2003; Cornil et al., 2006). The rapid changes in estrogen are more commonly associated with female models (Hansel and Convey, 1983). To protect against these "spikes" in estrogen, we propose the stronger activity of autoreceptors in female mice serve as a control mechanism. High levels of extracellular serotonin are neurotoxic (Boyer and Shannon, 2005). In accordance with this, other researchers have found increased density of 5HT1 autoreceptors and a higher rate of serotonin turnover in female vs. male mice, mediated by estrogen (Biegon et al., 1980; Carlsson et al., 1985). To test this notion, we pretreated male and female mice with methiothepine (0.1 mg kg^{-1}) before ESCIT administration (**Figures 6A,B**). We were limited to using this small methiothepin dose because when administered with SSRI, receptor antagonists induce the fatal serotonin syndrome pathology. Statistically, we found that the "dip" below baseline, that we previously attributed to serotonin autoreceptors (Wood et al., 2014), is diminished in both sexes, but to a larger extent in female mice (191 $\pm$ 13 data points in the controls to 64.8 $\pm$ 45 files after methiothepin treatment, $p = 0.048$). This could point toward the fact that the female serotonin signal is under greater autoreceptor control since the same dose creates a larger effect (less of a correction is needed to prevent the "dip" in males). While these data are good evidence for stronger autoreceptor regulation in females, we find no effects of methiothepin pretreatment on the $t_{1/2}$ of the ESCIT response (**Figures 6C,D** where responses are normalized from animals with and without methiothepin treatment). We thus reject the notion that autoreceptors are responsible for the lesser effect of ESCIT on the reuptake curve of serotonin in female mice. Other hypotheses that may account for this sex specific response to acute SSRI include metabolic effects (Curzon and Bridges, 1970; Rosecrans, 1970; Kennett et al., 1986), SERT trafficking (Malison et al., 1998; Joensuu et al., 2007), or promiscuous reuptake by other monoamine transporters (Fox et al., 2008; Daws, 2009). These effects will be the focus of future studies.

It is, however, important to note that the large electrical stimulations necessary to collect FSCV files cause aphysiological serotonin release. The stimulation allows us to test the nuances of the regulation of the serotonin system; however, it is unlikely that endogenous serotonin levels would spike to the levels we observe in our evoked experiments. Thus, we measured the ambient hippocampal serotonin response to ESCIT in male and female mice using FSCAV. We selected the intermediary dose of 10 mg kg^{-1}, administered acutely. Interestingly, following administration of ESCIT, basal serotonin increased in both male and female mice with no detectable differences between the sexes (**Figure 4**).

Taken together, these data suggest that sex-mediated differences in serotonin reuptake following ESCIT in the context of stronger autoreceptor control in females is present only in response to the large electrical stimulation used with FSCV. This aphysiological stimulation allows us to delve into the fine biochemical differences that may exist within the synapse of male and female mice. These differences are in place to protect against potential variation in modulation between the sexes but are only activated under unusual circumstances. Therefore, on a physiological level, ESCIT appears to have consistent effects across the sex lines in mice.

CONCLUSION

No distinction was found between the different stages of the estrous cycle, in the hippocampus, in female mice. Furthermore, the average male and female control serotonin signals and ambient levels were not significantly different from each other. Modeling the average male and female serotonin evoked signals revealed a larger autoreceptor effect in female mice, which was confirmed *via* methiothepin administration. It is important to note that these autoreceptor differences may only be visible under aphysiological conditions (such as electrical stimulation or disease). Nonetheless, we showed that autoreceptor effects were not responsible for our finding that acute SSRI are lesser able to slow reuptake in female mice. Therefore, while there is evidence that under physiological conditions, there are no differences in hippocampal serotonin; the data presented here emphasize considering sex as an important biological factor when evaluating disease states and personalized treatment options.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina, which operates with accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The protocol was approved by IACUC.

AUTHOR CONTRIBUTIONS

RS, MH, AB, and SB contributed to the collection of the data presented in this paper. MH, PH, AW, RS, MR, JB, and FN participated in writing the manuscript. MH, AW, and PH each worked on data processing and analysis. AW and MH created the figures and completed the necessary statistics for the data. MR, JB, and FN carried out the mathematical modeling of the data.

FUNDING

This work was supported by the National Institutes of Health R01MH106563 (PH).

ACKNOWLEDGMENTS

The authors would like to acknowledge the other members of the Hashemi lab for their support in various aspects including electrode fabrication, experimental assistance, and input to constructive discussions. The authors would also like to thank Jan Ostermann for help with data analysis and Edsel Pena for help with statistical analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00362/full#supplementary-material

FIGURE S1 | (A) The raw data for evoked serotonin response in young adult mice (aged 6–8 weeks, n = (11 females, 3 males) = 14 mice total) and **(B)** adult mice (aged 9–12 weeks, n = (3 females, 5 males) = 8 mice total) are shown in purple and green respectively. **(C)** A table with the average maximum amplitude and $t_{1/2}$ are shown, neither of which are statistically between the two age groups.

FIGURE S2 | A representative cell sample pictomicrograph is shown for each of the estrous cycles: **(A)** estrus, **(B)** metestrus, **(C)** diestrus, and **(D)** proestrus. Estrus contains anucleated cornified cells, metestrus contains cornified, nucleated epithelial cells, and leukocytes, diestrus contains leukocytes and proestrus contains nucleated epithelial cells. Methods and analysis were completed as described in Caligioni, 2009.

FIGURE S3 | Female (red) serotonin responses (n = 5) in the medial prefrontal cortex (mPFC) are compared to previously reported male (blue) serotonin response (n = 20). Error bars for every tenth file are shown in similar corresponding colors. The wide error bar range results from single and double peak signals being averaged together as well as the low n size in the case of the females. Nonetheless, the error bars suggest that there is no difference in male and female serotonin signals in the mPFC.

REFERENCES

Abdalla, A., Atcherley, C. W., Pathirathna, P., Samaranayake, S., Qiang, B., Pena, E., et al. (2017). In vivo ambient serotonin measurements at carbon-fiber microelectrodes. *Anal. Chem.* 89, 9703–9711. doi: 10.1021/acs.analchem.7b01257

Abraham, I. M., Han, S. K., Todman, M. G., Korach, K. S., and Herbison, A. E. (2003). Estrogen receptor beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. *J. Neurosci.* 23, 5771–5777. doi: 10.1523/JNEUROSCI.23-13-05771.2003

Anderson, J. C., Williams, S., Mcgee, R., and Silva, P. A. (1987). DSM-III disorders in preadolescent children. Prevalence in a large sample from the general population. *Arch. Gen. Psychiatry* 44, 69–76. doi: 10.1001/archpsyc.1987.01800130081010

Arango, V., Underwood, M. D., Gubbi, A. V., and Mann, J. J. (1995). Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res.* 688, 121–133. doi: 10.1016/0006-8993(95)00523-S

Asberg, M., Thoren, P., Traskman, L., Bertilsson, L., and Ringberger, V. (1976a). "Serotonin depression"–a biochemical subgroup within the affective disorders? *Science* 191, 478–480.

Asberg, M., Traskman, L., and Thoren, P. (1976b). 5-HIAA in the cerebrospinal fluid. a biochemical suicide predictor? *Arch. Gen. Psychiatry* 33, 1193–1197.

Barth, C., Villringer, A., and Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front. Neurosci.* 9:37. doi: 10.3389/fnins.2015.00037

Beery, A. K., and Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci. Biobehav. Rev.* 35, 565–572. doi: 10.1016/j.neubiorev.2010.07.002

Bethea, C. L., Lu, N. Z., Gundlah, C., and Streicher, J. M. (2002). Diverse actions of ovarian steroids in the serotonin neural system. *Front. Neuroendocrinol.* 23, 41–100. doi: 10.1006/frne.2001.0225

Biegon, A., Bercovitz, H., and Samuel, D. (1980). Serotonin receptor concentration during the estrous cycle of the rat. *Brain Res.* 187, 221–225. doi: 10.1016/0006-8993(80)90509-0

Bogdanova, O. V., Kanekar, S., D'anci, K. E., and Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. *Physiol. Behav.* 118, 227–239. doi: 10.1016/j.physbeh.2013.05.012

Borrow, A. P., and Cameron, N. M. (2014). Estrogenic mediation of serotonergic and neurotrophic systems: implications for female mood disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 54, 13–25. doi: 10.1016/j.pnpbp.2014.05.009

Borsini, F., and Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology* 94, 147–160.

Boyer, E. W., and Shannon, M. (2005). The serotonin syndrome. *N. Engl. J. Med.* 352, 1112–1120. doi: 10.1056/NEJMra041867

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., and Charney, D. S. (2000). Hippocampal volume reduction in major depression. *Am. J. Psychiatry* 157, 115–118. doi: 10.1176/ajp.157.1.115

Byers, S. L., Wiles, M. V., Dunn, S. L., and Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PLoS One* 7:e35538. doi: 10.1371/journal.pone.0035538

Caligioni, C. S. (2009). Assessing reproductive status/stages in mice. *Curr. Protoc. Neurosci.* 48, A.4I.1-A.4I.8. doi: 10.1002/0471142301.nsa04is48

Carlsson, M., Svensson, K., Eriksson, E., and Carlsson, A. (1985). Rat brain serotonin: biochemical and functional evidence for a sex difference. *J. Neural. Transm.* 63, 297–313. doi: 10.1007/BF01252033

Charan, J., and Kantharia, N. D. (2013). How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* 4, 303–306. doi: 10.4103/0976-500X.119726

Cornil, C. A., Ball, G. F., and Balthazart, J. (2006). Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? *Brain Res.* 1126, 2–26. doi: 10.1016/j.brainres.2006.07.098

Cryan, J. F., Markou, A., and Lucki, I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol. Sci.* 23, 238–245. doi: 10.1016/S0165-6147(02)02017-5

Curzon, G., and Bridges, P. K. (1970). Tryptophan metabolism in depression. *J. Neurol. Neurosurg. Psychiatry* 33, 698–704. doi: 10.1136/jnnp.33.5.698

Cyranowski, J. M., Frank, E., Young, E., and Shear, M. K. (2000). Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. *Arch. Gen. Psychiatry* 57, 21–27. doi: 10.1001/archpsyc.57.1.21

Czeh, B., Michaelis, T., Watanabe, T., Frahm, J., De Biurrun, G., Van Kampen, M., et al. (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12796–12801. doi: 10.1073/pnas.211427898

Daws, L. C. (2009). Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy. *Pharmacol. Ther.* 121, 89–99. doi: 10.1016/j.pharmthera.2008.10.004

Daws, L. C., Koek, W., and Mitchell, N. C. (2013). Revisiting serotonin reuptake inhibitors and the therapeutic potential of "uptake-2" in psychiatric disorders. *ACS Chem. Neurosci.* 4, 16–21. doi: 10.1021/cn3001872

Dominguez, R., Cruz-Morales, S. E., Carvalho, M. C., Xavier, M., and Brandao, M. L. (2003). Sex differences in serotonergic activity in dorsal and median raphe nucleus. *Physiol. Behav.* 80, 203–210. doi: 10.1016/j.physbeh.2003.07.012

Drevets, W. C., Price, J. L., and Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118. doi: 10.1007/s00429-008-0189-x

Drossopoulou, G., Antoniou, K., Kitraki, E., Papathanasiou, G., Papalexi, E., Dalla, C., et al. (2004). Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. *Neuroscience* 126, 849–857. doi: 10.1016/j.neuroscience.2004.04.044

Eley, T. C., Sugden, K., Corsico, A., Gregory, A. M., Sham, P., Mcguffin, P., et al. (2004). Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol. Psychiatry* 9, 908–915. doi: 10.1038/sj.mp.4001546

Fischette, C. T., Biegon, A., and Mcewen, B. S. (1983). Sex differences in serotonin 1 receptor binding in rat brain. *Science* 222, 333–335. doi: 10.1126/science.6623080

Fox, M. A., Jensen, C. L., French, H. T., Stein, A. R., Huang, S. J., Tolliver, T. J., et al. (2008). Neurochemical, behavioral, and physiological effects of pharmacologically enhanced serotonin levels in serotonin transporter (SERT)-deficient mice. *Psychopharmacology* 201, 203–218. doi: 10.1007/s00213-008-1268-7

Frackiewicz, E. J., Sramek, J. J., and Cutler, N. R. (2000). Gender differences in depression and antidepressant pharmacokinetics and adverse events. *Ann. Pharmacother.* 34, 80–88. doi: 10.1345/aph.18465

Franklin, K. (2013). *Paxinos and Franklin's The mouse brain in stereotaxic coordinates*, 4th edn. Cambridge: Academic Press.

Goel, N., Innala, L., and Viau, V. (2014). Sex differences in serotonin (5-HT) 1A receptor regulation of HPA axis and dorsal raphe responses to acute restraint. *Psychoneuroendocrinology* 40, 232–241. doi: 10.1016/j.psyneuen.2013.11.020

Goldapple, K., Segal, Z., Garson, C., Lau, M., Bieling, P., Kennedy, S., et al. (2004). Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. *Arch. Gen. Psychiatry* 61, 34–41. doi: 10.1001/archpsyc.61.1.34

Goswami, D. B., May, W. L., Stockmeier, C. A., and Austin, M. C. (2010). Transcriptional expression of serotonergic regulators in laser-captured microdissected dorsal raphe neurons of subjects with major depressive disorder: sex-specific differences. *J. Neurochem.* 112, 397–409. doi: 10.1111/j.1471-4159.2009.06462.x

Grigoriadis, S., and Robinson, G. E. (2007). Gender issues in depression. *Ann. Clin. Psychiatry* 19, 247–255. doi: 10.1080/10401230701653294

Gundlah, C., Simon, L. D., and Auerbach, S. B. (1998). Differences in hypothalamic serotonin between estrous phases and gender: an in vivo microdialysis study. *Brain Res.* 785, 91–96. doi: 10.1016/S0006-8993(97)01391-7

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., Mcgee, R., and Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *J. Abnorm. Psychol.* 107, 128–140. doi: 10.1037/0021-843X.107.1.128

Hansel, W., and Convey, E. M. (1983). Physiology of the estrous cycle. *J. Anim. Sci.* 57(Suppl. 2), 404–424.

Hashemi, P., Dankoski, E. C., Petrovic, J., Keithley, R. B., and Wightman, R. M. (2009). Voltammetric detection of 5-hydroxytryptamine release in the rat brain. *Anal. Chem.* 81, 9462–9471. doi: 10.1021/ac9018846

Horton, R. E., Apple, D. M., Owens, W. A., Baganz, N. L., Cano, S., Mitchell, N. C., et al. (2013). Decynium-22 enhances SSRI-induced antidepressant-like effects in mice: uncovering novel targets to treat depression. *J. Neurosci.* 33, 10534–10543. doi: 10.1523/JNEUROSCI.5687-11.2013

Jackson, B. P., Dietz, S. M., and Wightman, R. M. (1995). Fast-scan cyclic voltammetry of 5-hydroxytryptamine. *Anal. Chem.* 67, 1115–1120. doi: 10.1021/ac00102a015

Jayatissa, M. N., Bisgaard, C., Tingstrom, A., Papp, M., and Wiborg, O. (2006). Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology* 31, 2395–2404. doi: 10.1038/sj.npp.1301041

Jitsuki, S., Kimura, F., Funabashi, T., Takahashi, T., and Mitsushima, D. (2009). Sex-specific 24-h profile of extracellular serotonin levels in the medial prefrontal cortex. *Brain Res.* 1260, 30–37. doi: 10.1016/j.brainres.2008.12.084

Joensuu, M., Tolmunen, T., Saarinen, P. I., Tiihonen, J., Kuikka, J., Ahola, P., et al. (2007). Reduced midbrain serotonin transporter availability in drug-naive patients with depression measured by SERT-specific [(123)I] nor-beta-CIT SPECT imaging. *Psychiatry Res.* 154, 125–131. doi: 10.1016/j.pscychresns.2006.08.001

Jones, M. D., and Lucki, I. (2005). Sex differences in the regulation of serotonergic transmission and behavior in 5-HT receptor knockout mice. *Neuropsychopharmacology* 30, 1039–1047. doi: 10.1038/sj.npp.1300664

Jovanovic, H., Lundberg, J., Karlsson, P., Cerin, A., Saijo, T., Varrone, A., et al. (2008). Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *Neuroimage* 39, 1408–1419. doi: 10.1016/j.neuroimage.2007.10.016

Kaminska, K., Gorska, A., Noworyta-Sokolowska, K., Wojtas, A., Rogoz, Z., and Golembiowska, K. (2018). The effect of chronic co-treatment with risperidone and novel antidepressant drugs on the dopamine and serotonin levels in the rats frontal cortex. *Pharmacol. Rep.* 70, 1023–1031. doi: 10.1016/j.pharep.2018.04.009

Kennett, G. A., Chaouloff, F., Marcou, M., and Curzon, G. (1986). Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin. *Brain Res.* 382, 416–421. doi: 10.1016/0006-8993(86)91355-7

Kessler, R. C., Mcgonagle, K. A., Swartz, M., Blazer, D. G., and Nelson, C. B. (1993). Sex and depression in the national comorbidity survey. i: lifetime prevalence, chronicity and recurrence. *J. Affect. Disord.* 29, 85–96. doi: 10.1016/0165-0327(93)90026-G

Khan, A., Brodhead, A. E., Schwartz, K. A., Kolts, R. L., and Brown, W. A. (2005). Sex differences in antidepressant response in recent antidepressant clinical trials. *J. Clin. Psychopharmacol.* 25, 318–324. doi: 10.1097/01.jcp.0000168879.03169.ce

Kodama, M., Fujioka, T., and Duman, R. S. (2004). Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. *Biol. Psychiatry* 56, 570–580. doi: 10.1016/j.biopsych.2004.07.008

Kornstein, S. G., Schatzberg, A. F., Thase, M. E., Yonkers, K. A., Mccullough, J. P., Keitner, G. I., et al. (2000). Gender differences in treatment response to sertraline versus imipramine in chronic depression. *Am. J. Psychiatry* 157, 1445–1452. doi: 10.1176/appi.ajp.157.9.1445

Kunimura, Y., Iwata, K., Iijima, N., Kobayashi, M., and Ozawa, H. (2015). Effect of sex steroid hormones on the number of serotonergic neurons in rat dorsal raphe nucleus. *Neurosci. Lett.* 594, 127–132. doi: 10.1016/j.neulet.2015.03.060

Lu, N. Z., Shlaes, T. A., Gundlah, C., Dziennis, S. E., Lyle, R. E., and Bethea, C. L. (1999). Ovarian steroid action on tryptophan hydroxylase protein and serotonin compared to localization of ovarian steroid receptors in midbrain of guinea pigs. *Endocrine* 11, 257–267. doi: 10.1385/ENDO:11:3:257

Magarinos, A. M., and McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* 69, 89–98. doi: 10.1016/0306-4522(95)00259-L

Malberg, J. E., and Duman, R. S. (2003). Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* 28, 1562–1571. doi: 10.1038/sj.npp.1300234

Malison, R. T., Price, L. H., Berman, R., Van Dyck, C. H., Pelton, G. H., Carpenter, L., et al. (1998). Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. *Biol. Psychiatry* 44, 1090–1098. doi: 10.1016/S0006-3223(98)00272-8

Maswood, S., Truitt, W., Hotema, M., Caldarola-Pastuszka, M., and Uphouse, L. (1999). Estrous cycle modulation of extracellular serotonin in mediobasal hypothalamus: role of the serotonin transporter and terminal autoreceptors. *Brain Res.* 831, 146–154. doi: 10.1016/S0006-8993(99)01439-0

May, L. J., and Wightman, R. M. (1989). Heterogeneity of stimulated dopamine overflow within rat striatum as observed with in vivo voltammetry. *Brain Res.* 487, 311–320. doi: 10.1016/0006-8993(89)90835-4

McGee, R., Feehan, M., Williams, S., and Anderson, J. (1992). DSM-III disorders from age 11 to age 15 years. *J. Am. Acad. Child Adolesc. Psychiatry* 31, 50–59. doi: 10.1097/00004583-199201000-00009

McQueen, J. K., Wilson, H., and Fink, G. (1997). Estradiol-17 beta increases serotonin transporter (SERT) mRNA levels and the density of SERT-binding sites in female rat brain. *Brain Res. Mol. Brain Res.* 45, 13–23. doi: 10.1016/S0169-328X(96)00233-1

Mermelstein, P. G., Becker, J. B., and Surmeier, D. J. (1996). Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci.* 16, 595–604. doi: 10.1523/JNEUROSCI.16-02-00595.1996

Montanez, S., Munn, J. L., Owens, W. A., Horton, R. E., and Daws, L. C. (2014). 5-HT1B receptor modulation of the serotonin transporter in vivo: studies using KO mice. *Neurochem. Int.* 73, 127–131. doi: 10.1016/j.neuint.2013.11.004

National Institutes of Health (2015). *Consideration of Sex as a Biological Variable in NIH-Funded Research: NOT-OD-15-102*. Available at: https://grants.nih.gov/grants/guide/notice-files/not-od-15-102.html (accessed November 11, 2018).

Nishizawa, S., Benkelfat, C., Young, S. N., Leyton, M., Mzengeza, S., De Montigny, C., et al. (1997). Differences between males and females in rates of

serotonin synthesis in human brain. *Proc. Natl. Acad. Sci. U.S.A.* 94, 5308–5313. doi: 10.1073/pnas.94.10.5308

Nolen-Hoeksema, S., and Girgus, J. S. (1994). The emergence of gender differences in depression during adolescence. *Psychol. Bull.* 115, 424–443. doi: 10.1037/0033-2909.115.3.424

Owens, M. J., and Nemeroff, C. B. (1994). Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clin. Chem.* 40, 288–295.

Parsey, R. V., Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., et al. (2002). Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res.* 954, 173–182. doi: 10.1016/S0006-8993(02)03243-2

Petit-Demouliere, B., Chenu, F., and Bourin, M. (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology* 177, 245–255. doi: 10.1007/s00213-004-2048-7

Piccinelli, M., and Wilkinson, G. (2000). Gender differences in depression. *Crit. Rev. Br. J. Psychiatry* 177, 486–492. doi: 10.1192/bjp.177.6.486

Rajkowska, G., Mahajan, G., Legutko, B., Challagundla, L., Griswold, M., Albert, P. R., et al. (2017). Length of axons expressing the serotonin transporter in orbitofrontal cortex is lower with age in depression. *Neuroscience* 359, 30–39. doi: 10.1016/j.neuroscience.2017.07.006

Rehavi, M., Goldin, M., Roz, N., and Weizman, A. (1998). Regulation of rat brain vesicular monoamine transporter by chronic treatment with ovarian hormones. *Brain Res. Mol. Brain Res.* 57, 31–37. doi: 10.1016/S0169-328X(98)00058-8

Rosecrans, J. A. (1970). Differences in brain area 5-hydroxytryptamine turnover and rearing behavior in rats and mice of both sexes. *Eur. J. Pharmacol.* 9, 379–382. doi: 10.1016/0014-2999(70)90239-6

Rubinow, D. R., Schmidt, P. J., and Roca, C. A. (1998). Estrogen-serotonin interactions: implications for affective regulation. *Biol. Psychiatry* 44, 839–850. doi: 10.1016/S0006-3223(98)00162-0

Shaskan, E. G., and Snyder, S. H. (1970). Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. *J. Pharmacol. Exp. Ther.* 175, 404–418.

Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G., and Vannier, M. W. (1996). Hippocampal atrophy in recurrent major depression. *Proc. Natl. Acad. Sci. U.S.A.* 93, 3908–3913. doi: 10.1073/pnas.93.9.3908

Sheng, Z., Kawano, J., Yanai, A., Fujinaga, R., Tanaka, M., Watanabe, Y., et al. (2004). Expression of estrogen receptors (alpha, beta) and androgen receptor in serotonin neurons of the rat and mouse dorsal raphe nuclei; sex and species differences. *Neurosci. Res.* 49, 185–196. doi: 10.1016/j.neures.2004.02.011

Sramek, J. J., and Cutler, N. R. (2011). The impact of gender on antidepressants. *Curr. Top Behav. Neurosci.* 8, 231–249. doi: 10.1007/7854_2010_118

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors. serotonin receptors and pathways mediate therapeutic effects and side effects. *J. Affect. Disord.* 51, 215–235. doi: 10.1016/S0165-0327(98)00221-3

Stockmeier, C. A. (2003). Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J. Psychiatr. Res.* 37, 357–373. doi: 10.1016/S0022-3956(03)00050-5

Strupp-Levitsky, M., Miller, J. M., Rubin-Falcone, H., Zanderigo, F., Milak, M. S., Sullivan, G., et al. (2016). Lack of association between the serotonin transporter and serotonin 1A receptor: an in vivo PET imaging study in healthy adults. *Psychiatry Res. Neuroimag.* 255, 81–86. doi: 10.1016/j.pscychresns.2016.08.002

Sullivan, P. F., Neale, M. C., and Kendler, K. S. (2000). Genetic epidemiology of major depression: review and meta-analysis. *Am. J. Psychiatry* 157, 1552–1562. doi: 10.1176/appi.ajp.157.10.1552

Videbech, P., and Ravnkilde, B. (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *Am. J. Psychiatry* 161, 1957–1966. doi: 10.1176/appi.ajp.161.11.1957

Weissman, M. M., and Klerman, G. L. (1977). Sex differences and the epidemiology of depression. *Arch. Gen. Psychiatry* 34, 98–111. doi: 10.1001/archpsyc.1977.01770130100011

Wightman, R. M., Heien, M. L., Wassum, K. M., Sombers, L. A., Aragona, B. J., Khan, A. S., et al. (2007). Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur. J. Neurosci.* 26, 2046–2054. doi: 10.1111/j.1460-9568.2007.05772.x

Willner, P. (1990). Animal models of depression: an overview. *Pharmacol. Ther.* 45, 425–455. doi: 10.1016/0163-7258(90)90076-E

Wojcieszak, J., Andrzejczak, D., Wojtas, A., Golembiowska, K., and Zawilska, J. B. (2018). Methcathinone and 3-fluoromethcathinone stimulate spontaneous horizontal locomotor activity in mice and elevate extracellular dopamine and serotonin levels in the mouse striatum. *Neurotox. Res.* 35, 594–605. doi: 10.1007/s12640-018-9973-4

Wood, K. M., and Hashemi, P. (2013). Fast-scan cyclic voltammetry analysis of dynamic serotonin reponses to acute escitalopram. *ACS Chem. Neurosci.* 4, 715–720. doi: 10.1021/cn4000378

Wood, K. M., Zeqja, A., Nijhout, H. F., Reed, M. C., Best, J., and Hashemi, P. (2014). Voltammetric and mathematical evidence for dual transport mediation of serotonin clearance in vivo. *J. Neurochem.* 130, 351–359. doi: 10.1111/jnc.12733

Yang, H., Sampson, M. M., Senturk, D., and Andrews, A. M. (2015). Sex- and SERT-mediated differences in stimulated serotonin revealed by fast microdialysis. *ACS Chem. Neurosci.* 6, 1487–1501. doi: 10.1021/acschemneuro.5b00132

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Saylor, Hersey, West, Buchanan, Berger, Nijhout, Reed, Best and Hashemi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

CORRECTION
published: 09 July 2019
doi: 10.3389/fnins.2019.00726

Corrigendum: *In vivo* Hippocampal Serotonin Dynamics in Male and Female Mice: Determining Effects of Acute Escitalopram Using Fast Scan Cyclic Voltammetry

Rachel A. Saylor[1†], Melinda Hersey[1,2†], Alyssa West[1], Anna Marie Buchanan[1,2], Shane N. Berger[1], H. Frederik Nijhout[3], Michael C. Reed[4], Janet Best[5] and Parastoo Hashemi[1]*

[1] Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, United States, [2] Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, United States, [3] Department of Biology, Duke University, Durham, NC, United States, [4] Department of Mathematics, Duke University, Durham, NC, United States, [5] Department of Mathematics, The Ohio State University, Columbus, OH, United States

OPEN ACCESS

Approved by:
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

***Correspondence:**
Parastoo Hashemi
hashemi@mailbox.sc.edu

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: 27 June 2019
Accepted: 28 June 2019
Published: 09 July 2019

Citation:
Saylor RA, Hersey M, West A, Buchanan AM, Berger SN, Nijhout HF, Reed MC, Best J and Hashemi P (2019) Corrigendum: In vivo Hippocampal Serotonin Dynamics in Male and Female Mice: Determining Effects of Acute Escitalopram Using Fast Scan Cyclic Voltammetry. Front. Neurosci. 13:726. doi: 10.3389/fnins.2019.00726

Keywords: serotonin, hippocampus, SSRI, FSCV, FSCAV, sex, depression

A Corrigendum on

***In vivo* Hippocampal Serotonin Dynamics in Male and Female Mice: Determining Effects of Acute Escitalopram Using Fast Scan Cyclic Voltammetry**

by Saylor RA, Hersey M, West A, Buchanan AM, Berger SN, Nijhout HF, Reed MC, Best J and Hashemi P (2019) Front. Neurosci. 13:362. doi: 10.3389/fnins.2019.00362

In the original article, there was a mistake in the legend for Figure 5 as published. The legend for Figure 6 was accidently used instead. The correct legend appears below.

Figure 5 | The male (blue, $n = 5$) and female (red, $n = 5$) basal serotonin levels were collected for 30 min. A saline injection (marked by a yellow bar) preceded another 30 min of basal serotonin data was collected. At the gray bar, escitalopram (ESCIT, 10 mg kg^{-1}) was administered and 60 min of basal serotonin data was collection. SEM is shown as a lighter bar behind.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Copyright © 2019 Saylor, Hersey, West, Buchanan, Berger, Nijhout, Reed, Best and Hashemi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 24 April 2019
doi: 10.3389/fnins.2019.00245

Serotonin Deficiency Increases Context-Dependent Fear Learning Through Modulation of Hippocampal Activity

Jonas Waider[1], Sandy Popp[1], Boris Mlinar[2], Alberto Montalbano[2], Francesco Bonfiglio[2], Benjamin Aboagye[1], Elisabeth Thuy[1], Raphael Kern[1], Christopher Thiel[1], Naozumi Araragi[3,4], Evgeniy Svirin[1,5], Angelika G. Schmitt-Böhrer[6], Renato Corradetti[2], Christopher A. Lowry[7] and Klaus-Peter Lesch[1,5,8]*

[1] Division of Molecular Psychiatry, Center of Mental Health, University of Würzburg, Würzburg, Germany, [2] Department of Neuroscience, Psychology, Drug Research, and Child Health, University of Florence, Florence, Italy, [3] Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany, [4] Charité – Universitätsmedizin Berlin, Berlin, Germany, [5] Laboratory of Psychiatric Neurobiology, Institute of Molecular Medicine, I.M. Sechenov First Moscow State Medical University, Moscow, Russia, [6] Department of Psychiatry, Psychosomatics, and Psychotherapy, Center of Mental Health, University of Würzburg, Würzburg, Germany, [7] Department of Integrative Physiology and Center for Neuroscience, University of Colorado Boulder, Boulder, CO, United States, [8] Department of Translational Psychiatry, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands

OPEN ACCESS

Edited by:
Guillaume Lucas, INSERM U1215 Neurocentre Magendie, France

Reviewed by:
Luc Maroteaux, INSERM U839 Institut du Fer à Moulin, France
Bettina Bert, Freie Universität Berlin, Germany
Stefano Comai, Vita-Salute San Raffaele University, Italy

***Correspondence:**
Jonas Waider
Waider_J@ukw.de

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *29 September 2018*
Accepted: *01 March 2019*
Published: *24 April 2019*

Citation:
Waider J, Popp S, Mlinar B, Montalbano A, Bonfiglio F, Aboagye B, Thuy E, Kern R, Thiel C, Araragi N, Svirin E, Schmitt-Böhrer AG, Corradetti R, Lowry CA and Lesch K-P (2019) Serotonin Deficiency Increases Context-Dependent Fear Learning Through Modulation of Hippocampal Activity. Front. Neurosci. 13:245. doi: 10.3389/fnins.2019.00245

Brain serotonin (5-hydroxytryptamine, 5-HT) system dysfunction is implicated in exaggerated fear responses triggering various anxiety-, stress-, and trauma-related disorders. However, the underlying mechanisms are not well understood. Here, we investigated the impact of constitutively inactivated 5-HT synthesis on context-dependent fear learning and extinction using tryptophan hydroxylase 2 (*Tph2*) knockout mice. Fear conditioning and context-dependent fear memory extinction paradigms were combined with c-Fos imaging and electrophysiological recordings in the dorsal hippocampus (dHip). *Tph2* mutant mice, completely devoid of 5-HT synthesis in brain, displayed accelerated fear memory formation and increased locomotor responses to foot shock. Furthermore, recall of context-dependent fear memory was increased. The behavioral responses were associated with increased c-Fos expression in the dHip and resistance to foot shock-induced impairment of hippocampal long-term potentiation (LTP). In conclusion, increased context-dependent fear memory resulting from brain 5-HT deficiency involves dysfunction of the hippocampal circuitry controlling contextual representation of fear-related behavioral responses.

Keywords: tryptophan hydroxylase 2, knockout, fear learning, extinction, long-term potentiation, hippocampus, immediate-early gene, serotonin deficiency

INTRODUCTION

Anxiety disorders are common and result in substantial economic costs to individuals and society (Bereza et al., 2009). The current global prevalence of anxiety disorders is approximately 7.3%, ranging from 5.4 to 10.4% (Baxter et al., 2013). Many individuals meet diagnostic criteria for multiple anxiety disorders and comorbidity with other psychiatric disorders (Kessler et al., 2005;

Miyazaki et al., 2011). A hallmark of anxiety disorders is dysfunctional acquisition and extinction of conditioned fear memories (Grillon, 2002; Milad et al., 2008).

Evidence suggests that serotonin plays an important role in control of anxiety and fear responses (Lesch et al., 1996; Lowry et al., 2005; Maier and Watkins, 2005; Maier et al., 2006; Baratta et al., 2016; Bocchio et al., 2016). Mice with a targeted inactivation of *Tph2* have provided insights into the role of 5-HT in the modulation of anxiety-like behaviors. Previous studies of lifelong deficiency of brain 5-HT synthesis are consistent with the hypothesis that the brain serotonergic system plays an important role in control of anxiety-like behaviors (Mosienko et al., 2015), fear learning, and behavioral responses to stress (Gutknecht et al., 2015), effects that might be due to alterations in GABAergic transmission (Jorgensen et al., 2013; Waider et al., 2013). Furthermore, mice with defects in 5-HT system development leading to reduction of 5-HT neurons showed differential anxiety-like behaviors and fear memory (Hendricks et al., 2003; Dai et al., 2008; Schaefer et al., 2009; Kiyasova et al., 2011; Song et al., 2011; Brooks et al., 2014). Indeed, the 5-HT system is thought to play an essential role in the regulation of fear memory in rodents (Graeff and Zangrossi, 2010; Bocchio et al., 2016). Studies in animals demonstrate a direct anatomical connection between the main sources of serotonin in the brain, the brainstem dorsal and median raphe nuclei as well as forebrain limbic structures, such as the medial prefrontal cortex, hippocampus, and amygdala, that control anxiety and fear responses (Maier et al., 2006; Hale and Lowry, 2011; Fernandez et al., 2016; Muzerelle et al., 2016). Of particular interest to contextual fear conditioning is the dorsal hippocampus (dHip; Bauer, 2015), which receives serotonergic projections primarily from the median raphe nucleus (Azmitia and Whitaker-Azmitia, 1995; McQuade and Sharp, 1997; Lowry, 2002).

Consistent with this hypothesis, acute administration of selective 5-HT reuptake inhibitors (SSRIs) 60 min before testing results in a decrease in contextual fear expression (Hashimoto et al., 1996; Li et al., 2001; Gravius et al., 2006), while it increases conditioned fear expression in auditory fear conditioning setting (Burghardt et al., 2007). Furthermore, peripheral administration of SSRIs decreases neuronal activity, immediate-early gene expression, and plasticity in the hippocampus (Staubli and Otaky, 1994; Igelstrom and Heyward, 2012; Ravinder et al., 2013). In addition to these effects of serotonergic signaling on fear expression, other studies provide support for a role for multiple 5-HT receptor types in the dHip in conditioned fear memory consolidation (Schmidt et al., 2017).

We previously showed that *Tph2* mutant ($Tph2^{-/-}$) mice display enhanced acquisition of conditioned fear and escape-oriented behavior in response to aversive foot shock, in association with altered basolateral amygdala function (Waider et al., 2017). Here, we investigated the impact of a lifelong abscence of brain 5-HT synthesis on the contextual domain of fear learning, using fear conditioning combined with an extinction paradigm, functional immunohistochemistry, and electrophysiological recordings within the hippocampal formation.

MATERIALS AND METHODS

Animals

Adult male $Tph2^{+/+}$, $Tph2^{+/-}$, and $Tph2^{-/-}$ mice on a mixed Sv129/C57BL/6N genetic background (Gutknecht et al., 2012), 2–5 months of age, were housed individually in a controlled environment (12 h/12 h light/dark cycle, light phase 7 am–7 pm, 21 ± 0.5°C room temperature, 50 ± 5% humidity) with food and water *ad libitum*. Mice were allowed to acclimate for 1 week before being subjected to fear conditioning experiments. Tests were performed during the light phase between 10:00 and 15:00. All experiments were performed in accordance with the European Parliament and Council Directive (2010/63/EU) and were approved by local authorities (55.2-2531.01-57/12) and (IT: 938/2017-PR).

Fear Conditioning and Contextual Fear Extinction Training

In order to assess context-dependent fear memory and extinction, $Tph2^{-/-}$, $Tph2^{+/-}$, and $Tph2^{+/+}$ mice (n = 8–9/genotype) were exposed to a fear conditioning protocol as previously described (Waider et al., 2017) and subsequently subjected to a context-dependent extinction protocol (EXT; **Figure 1**). Briefly, on day 0, mice were placed by a blinded operator in randomized order into the fear conditioning test box (TSE Systems, Homburg, Germany), which was comprised of a transparent Perspex arena (23 cm × 23 cm × 35 cm) on a stainless steel foot shock grid (floor bars 4 mm diameter, distance rod center to rod center 8.9 mm) that was connected to a shocker-scrambler unit for delivering foot shocks of defined duration and intensity (Raab et al., 2018). The arena was placed inside in a square-shaped base frame (outer size: 31 cm × 31 cm) with integrated animal detection sensors (XY and Z axes featuring 16 sensors mounted 14 mm apart). All sensors were scanned with a sampling rate of up to 100 Hz to monitor the animal's position and movement at high spatial and temporal resolution. The test box was operated in a sound-attenuating housing (52 cm × 52 cm × 65 cm) featuring a loudspeaker and two lamps in the ceiling for software-controlled application of acoustic stimuli and continuous house-light illumination (set to 100 lux in all testing phases), respectively.

Mice were allowed to freely explore for 2 min the test box before receiving three pairings of a 20 s tone cue (80 dB, 4 kHz), co-terminating with a 2 s foot shock (FS, 0.6 mA) at an inter-trial interval (ITI) of 1 min. Mice were removed from the chamber 2 min after the last tone-shock pairing. Starting 24 h after conditioning, mice were tested for recall and extinction of context-dependent fear on two consecutive days (day 1 and 2). Each extinction session consisted of re-exposing the animals to the conditioning context for 800 s without negative reinforcement.

Freezing was defined as no light-beam interruption for at least 2 s and expressed as percentage of time relative to total session duration (Rivero et al., 2015; Raab et al., 2018). Additionally, other indices of locomotor activity and exploratory behavior (i.e., distance moved, mean and maximum velocity, activity vs.

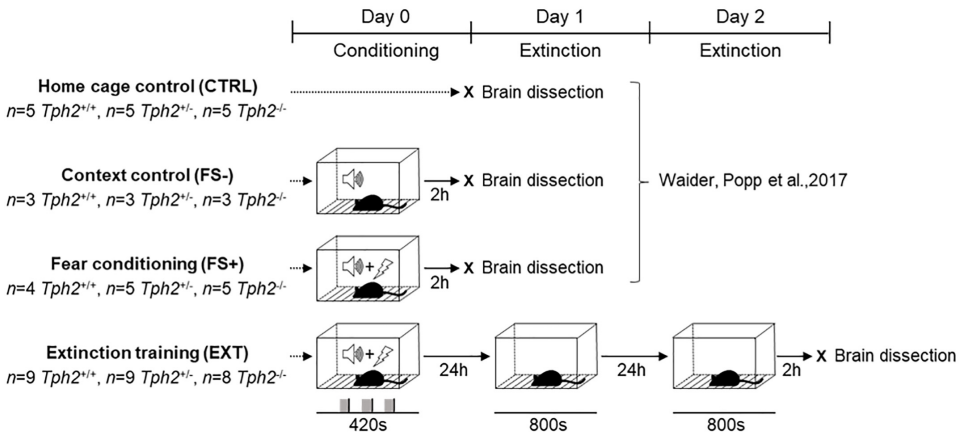


FIGURE 1 | Experimental design for c-Fos immunoreactivity after fear conditioning and context-dependent extinction. *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice were exposed to a fear conditioning (FC) protocol and subsequently subjected to a context-dependent extinction protocol. On day 0 mice were placed in the conditioning chamber receiving three pairings of a 20 s tone cue co-terminating with a 2 s foot shock. On day 1 and again on day 2, mice were tested for recall and extinction of context-dependent fear memory using an extinction session consisting of re-exposing the animals to the conditioning context for 800 s without negative reinforcement. Two hours after extinction on day 2, brains were dissected for hippocampal c-Fos expression analysis (EXT) and compared to controls that were left undisturbed in the home cage (CTRL), controls that were exposed to the FC context and tone cue without foot shocks (FS–), and mice that underwent FC with tone-signaled foot shocks (FS+) with brain dissection 2 h after the test (Waider et al., 2017).

inactivity, rearing) were measured continuously by the light-beam detection system. Inactivity was defined as the percentage of time the animal's speed fell below a threshold of 2 cm/s. Using the same fear conditioning system (TSE FCS 303410 series), Misane et al. (2005) have shown that there is a close linear correlation between operator-scored freezing (using a time-sampling procedure in which the animal was instantly scored as either freezing or active every 10 s) and computer-derived inactivity (activity threshold 1 cm/s) both in context- and tone-dependent memory tests. Moreover, Toth et al. (2012) have shown that there is no difference between hand-scored freezing, TSE-determined freezing (defined as no light-beam interruption for at least 3 s), and Noldus Ethovision-determined inactivity.

To determine acquisition of the conditioned fear response, freezing was measured continuously throughout the training session. The maximum movement velocity was taken as an indicator of the animal's unconditioned reactivity to foot shock (Waider et al., 2017). During extinction training, freezing was continuously recorded in 20 s time-bins and, except for the first 20 s, averaged into blocks of 1 min to assess recall and extinction of contextual fear memory.

C-Fos and Parvalbumin Immunostaining in the Dorsal Hippocampus

Two hours after extinction on day 2, the brains of the mice that underwent context-dependent extinction training (EXT) were prepared for immunostaining of parvalbumin (PV) and c-Fos as previously described (Waider et al., 2017). The brain sections were analyzed and compared with the brains of mice that were either left undisturbed in the home cage (CTRL), exposed to the fear conditioning context and tone without foot shocks (FS-), or underwent fear conditioning with tone-signaled foot shocks (FS+) as previously described (Waider et al., 2017; **Figure 1**). In brief, serially cut 30 μm-thick cryostat sections were used for immunofluorescent stainings. Primary antibodies used were mouse anti-PV (1:200; Swant, Marly, Switzerland) and rabbit anti-c-Fos (1:400; Santa Cruz Biotechnology, Dallas, TX, United States). Sections were incubated in 1:400 diluted secondary antibodies, goat anti-mouse 488 and goat anti-rabbit 555 (Invitrogen, Carlsbad, CA, United States). Pictures were acquired with a motorized inverted system epifluorescence microscope IX81 (Olympus, Tokyo, Japan). Pictures were taken with 20× objective in x-y directions. Images were then processed using CellSense (Olympus, Tokyo, Japan), and corrected for contrast and brightness using ImageJ v2.0.0 (Schindelin et al., 2012). Three to five sections from −1.06 mm bregma to −2.06 mm bregma of the dHip, spaced 180 μm apart, were delineated with contours according to a stereotactic atlas of the mouse brain (Franklin and Paxinos, 1997). Immunoreactive (ir) cells were counted as c-Fos-ir only when the nucleus, according to DAPI (4′, 6-diamidino-2-phenylindole) counterstaining, showed complete fluorescent signal. When the PV signal superimposed or surrounded the cell nucleus, it was counted as c-Fos/PV-ir. The sum of counted cells of all sections per mouse were divided by the total contour area to calculate the cell densities per region of interest.

Electrophysiology

To investigate the impact of 5-HT deficiency on hippocampal plasticity under basal conditions and after contextual fear conditioning with unsignaled foot shocks, an independent cohort of mice was either left undisturbed (naïve controls) or subjected to a foot shock procedure according to Dai et al. (2008). Mice were placed in the box and allowed to freely explore for 2 min before receiving five foot shocks (0.5 mA, 2 s) with ITI of 2 min. Two minutes after the last foot shock, mice were

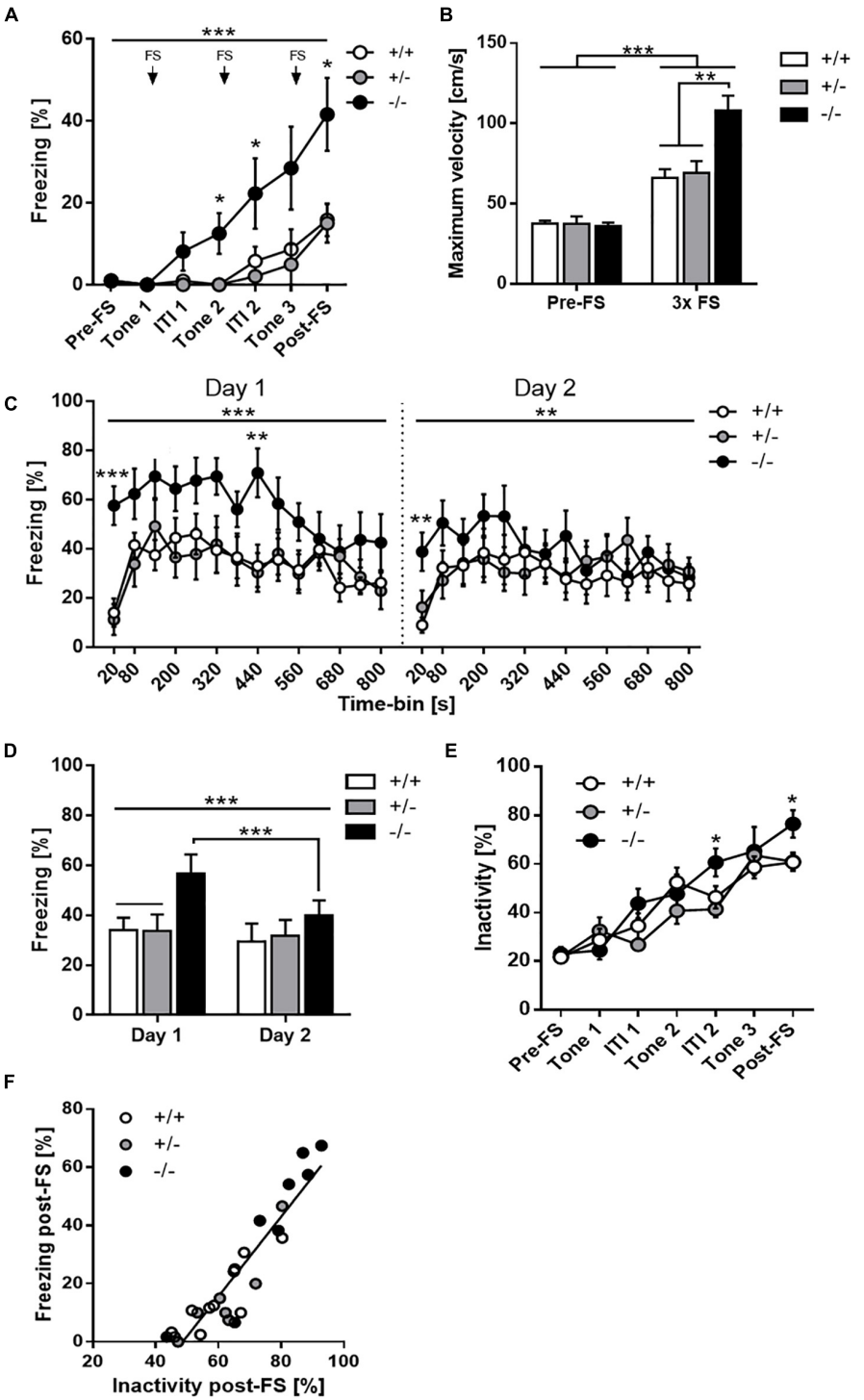


FIGURE 2 | Increased context-dependent fear memory recall but effective contextual extinction learning due to 5-HT deficiency. **(A)** Time-course analysis of the conditioned fear response of *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice during acquisition training showing relative freezing levels before the first pairing of a tone cue with a foot shock (pre-FS), during tone presentations and inter-trial intervals (ITI), and after the last foot shock (post-FS). **(B)** Unconditioned foot shock reactivity, measured as maximum movement velocity before the first pairing of a tone cue with a foot shock (pre-FS) and following the foot shock (3 × FS). **(C)** Time-course analysis of the freezing response during contextual fear memory recall and extinction training on day 1 and 2 after conditioning. **(D)** Average freezing scores in the two extinction sessions. **(E)** Time-course analysis of the inactivity level during acquisition training showing relative inactivity levels. **(F)** Positive relationship between freezing scores obtained after the last foot shock (post-FS) in the conditioning session and inactivity scores. Data are shown as means + or ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

removed from the chamber and slices were prepared. Field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the dHip were recorded in transversal slices as previously described (Morini et al., 2011; Mlinar et al., 2015). LTP was induced by theta burst stimulation (TBS) comprised of a single train of 5 bursts of 5 stimuli (100 Hz intra-burst frequency, 5 Hz burst frequency). Stimulation intensity for baseline measurement and LTP induction was set to evoke fEPSP corresponding to 35–40% of the maximal response. Typically, more than one slice was used per mouse and the results of all determinations per genotype and treatment are shown and analyzed in order to account for the overall variability of LTP responses in the different genotypes. Mean values from replicates (2–4) in the same animal were used for genotype x treatment statistical analysis reported in results.

Statistical Analyses

Data were analyzed using IBM SPSS Statistics 21 (IBM Corp., Armonk, NY, United States) or GraphPad Prism version 6.00 (GraphPad Software, San Diego, CA, United States). Behavioral data were analyzed by two- or three-way mixed analysis of variance (ANOVA) with genotype as the between-subjects factor and with time and day as within-subjects factors. When appropriate, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity.

An ANCOVA was performed in addition to the regular ANOVA to adjust for the effect of post-shock freezing (=covariate) on subsequent freezing during extinction sessions. Immunohistochemical and electrophysiological data were analyzed by two-way ANOVA with group and genotype as between-subjects factors. Unless otherwise indicated, Bonferroni *post hoc* tests were performed to evaluate significant main effects or simple effects following a significant interaction. Correlations between behavioral parameters and c-Fos expression were calculated using the Pearson correlation coefficient.

Results are presented as mean ± SEM unless stated otherwise. The significance level was set at $p < 0.05$ and $p < 0.1$ was highlighted as approaching statistical significance.

RESULTS

Increased Acquisition of Fear Conditioning in *Tph2*$^{-/-}$ Mice

Two-way mixed ANOVA for freezing during fear acquisition training (**Figure 2A**) revealed a significant main effect of time [$F_{(2.8,64.4)} = 17.54$, $p < 0.001$] and genotype [$F_{(2,23)} = 7.70$, $p = 0.003$], as well as a time × genotype interaction [$F_{(5.6,64.4)} = 2.73$, $p = 0.023$]. *Post hoc* tests showed that freezing levels were very low before the first tone-shock pairing and gradually increased thereafter over the course of training. However, *Tph2*$^{-/-}$ mice acquired the conditioned fear response more rapidly than *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice, as evidenced by significantly elevated freezing levels *Tph2*$^{-/-}$ mice from the second tone presentation onward. Furthermore, the reactivity to foot shock, as measured by the maximum movement velocity (Vmax), was significantly increased in *Tph2*$^{-/-}$ compared to *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice [genotype effect: $F_{(2,23)} = 9.63$, $p < 0.001$; **Figure 2B**].

Moreover, comparison of baseline (pre-FS) Vmax and foot shock-induced Vmax revealed a significant increase in all three genotypes (*Tph2*$^{+/+}$: $p < 0.01$, *Tph2*$^{+/-}$: $p < 0.001$ and *Tph2*$^{-/-}$: $p < 0.0001$), thereby demonstrating that *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$ mice showed a clear response to FS, which was further exaggerated in *Tph2*$^{-/-}$ mice [phase x genotype interaction: $F_{(2,23)} = 8.223$, $p = 0.002$].

Besides freezing, we analyzed inactivity using an activity threshold of 2 cm/s. Baseline (pre-FS) inactivity (immobility/resting: ∼20–25%) did not significantly differ between genotypes ($p = 0.861$; **Figure 2E**). Inactivity increased linearly across tone-FS trials in all three genotypes [main effect of phase: $F_{(4.2,96.2)} = 55.73$, $p < 0.0001$] and reached maximum levels after three tone-FS pairings (∼61% in *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$, ∼76% in *Tph2*$^{-/-}$ mice). Similar to freezing, *Tph2*$^{-/-}$ mice were significantly less active than *Tph2*$^{+/-}$ and *Tph*$^{+/+}$ mice after the 2nd and 3rd tone-FS pairing [phase x genotype interaction: $F_{(8.4,96.2)} = 2.27$, $p = 0.026$]. Moreover, there was a close linear correlation between post-shock freezing and post-shock inactivity ($r = 0.926$, $p < 0.0001$) with similar steepness of regression slopes in *Tph2*$^{+/+}$, *Tph2*$^{+/-}$ and *Tph2*$^{-/-}$ mice (**Figure 2F**).

Increased Recall but Effective Extinction of Contextual Fear Memory in *Tph2*$^{-/-}$ Mice

Analysis of freezing during extinction training on day 1 and 2 after fear conditioning revealed a significant time-bin x genotype [$F_{(9.5,149.5)} = 2.02$, $p = 0.041$, **Figure 2C**] and day × genotype [$F_{(2,299)} = 5.29$, $p = 0.013$, **Figure 2D**] interaction, but no genotype main effect [$F_{(2,23)} = 2.09$, $p = 0.147$]. *Post hoc* tests showed that, within sessions, *Tph2*$^{-/-}$ mice promptly froze upon placement into the conditioned context, while freezing onset was slightly delayed in *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice. Moreover, freezing levels of *Tph2*$^{-/-}$ mice remained elevated during the first minutes of testing but were indistinguishable from the other genotypes at the end of the session due to a steeper decline of the freezing response in *Tph2*$^{-/-}$ compared to *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice (**Figure 2C**). Accordingly, *Tph2*$^{-/-}$ mice showed a significantly stronger decrease of freezing than *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice across the two extinction sessions (**Figure 2D**).

Since *Tph2*$^{-/-}$ mice displayed significantly enhanced acquisition of conditioned fear and post-shock freezing levels in the conditioning session, we performed an analysis of covariance to control for differences in post-shock freezing levels. Three-way mixed ANCOVA confirmed a significant effect of post-shock freezing [$F_{(1,22)} = 12.56$, $p = 0.002$] as well as a time-bin x genotype [$F_{(9.9,138.6)} = 2.46$, $p = 0.011$] and day x genotype [$F_{(2,286)} = 2.73$, $p = 0.087$] interaction on the freezing scores obtained during extinction training. *Post hoc* tests again showed that *Tph2*$^{-/-}$ mice froze significantly more than *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice during the first 20 s of context re-exposure. However, freezing levels were indistinguishable among genotypes thereafter. Furthermore, *Tph2*$^{-/-}$ mice

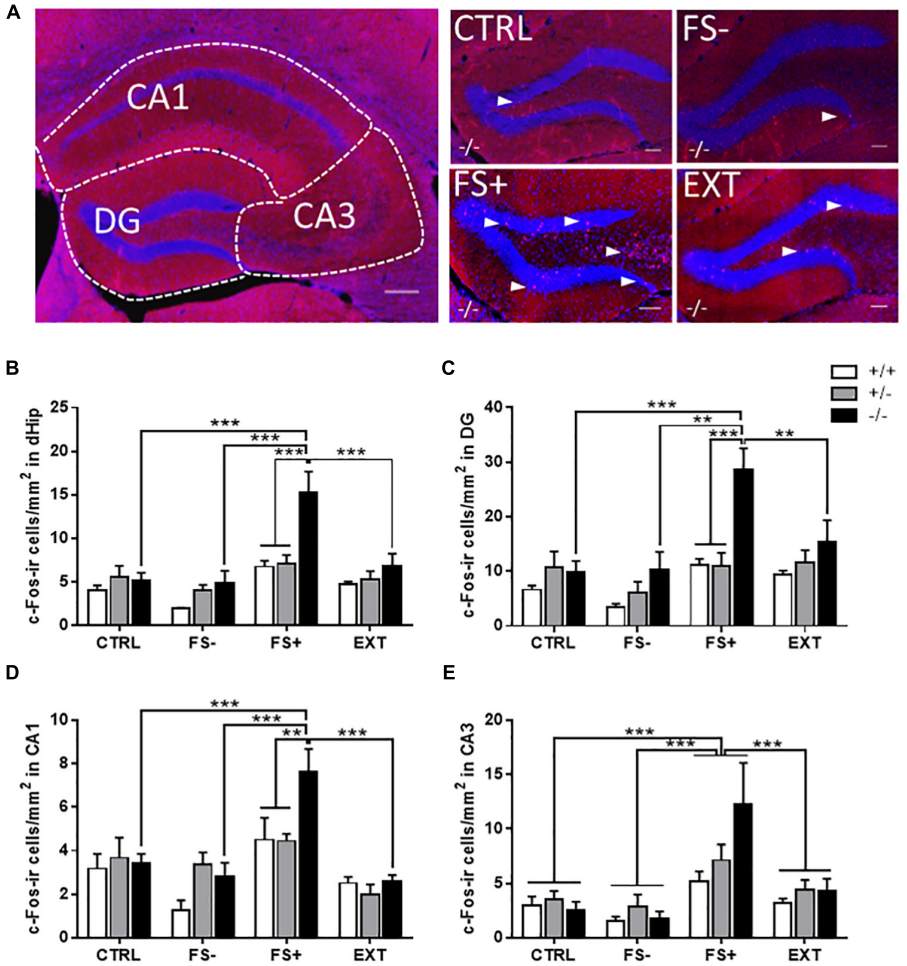


FIGURE 3 | Increased c-Fos activity in the dorsal hippocampus (dHip) of *Tph2*$^{-/-}$ mice following fear conditioning. (**A**, left panel) Overview of the dHip indicating the subregions dentate gyrus (DG), cornu ammonis area 1 (CA1), and cornu ammonis area 3 (CA3) and (**A**, right panel) higher magnification images depicting the DG of representative *Tph2*$^{-/-}$ mice for the different groups: home cage controls (CTRL), context controls subjected to the conditioning procedure without foot shocks (FS–), fear-conditioned mice receiving three tone-signaled foot shocks (FS+), fear-conditioned mice that underwent two extinction training sessions (EXT). Quantification of c-Fos-ir cell densities in **(B)** the total dHip, **(C)** DG, **(D)** CA1, and **(E)** CA3 of *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice of the four different groups. Arrowheads in **(A)** indicate c-Fos-ir cells (red), DAPI-stained cell nuclei (blue). Scale bars in **(A)**: 200 μm (left panel) and 100 μm (right panel). Data are shown as means + SEM. $^{**}p < 0.01$; $^{***}p < 0.001$.

were able to extinguish the conditioned fear more efficiently than *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice, as evidenced by a stronger decline of the freezing response both within and across the two extinction sessions.

Taken together, these results indicate that 5-HT deficiency due to *Tph2* inactivation augmented context-dependent fear memory recall and facilitated both intra- and intersession extinction learning.

Increased c-Fos Activation Due to Fear Conditioning in *Tph2*$^{-/-}$ Mice

Because of the strong effect of 5-HT deficiency on post-shock freezing, an indicator of short-term memory for contextual fear (Fanselow, 1980), we analyzed c-Fos activation in the dHip and its subregions, dentate gyrus (DG), cornu ammonis area 1 (CA1), and 3 (CA3) of *Tph2*$^{-/-}$, *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ mice that were either left undisturbed in the home cage (CTRL), exposed to the fear conditioning procedure without foot shocks (FS−) or with foot shocks (FS+), or subjected to fear conditioning and subsequent extinction training (EXT) (**Figure 3**).

An increased density of c-Fos-ir cells was found in the dHip, especially in the granule cell layer and hilus of the DG (**Figure 3A**) of FS+ *Tph2*$^{-/-}$ mice. Quantification confirmed a significant genotype x group interaction for the density of c-Fos-ir cells in the total dHip [$F_{(6,51)} = 3.05$, $p = 0.0126$; **Figure 3B**], in the DG [$F_{(6,51)} = 2.31$, $p = 0.0477$; **Figure 3C**], and in the CA1 area [$F_{(6,51)} = 2.59$, $p = 0.0288$; **Figure 3D**]. *Post hoc* analyses showed that the number of c-Fos-ir cells was increased in the total dHip, DG and CA1 of *Tph2*$^{-/-}$ after fear conditioning relative to respective *Tph2*$^{+/-}$ and *Tph2*$^{+/+}$ FS+ controls as well as to CTRL, FS−, and EXT *Tph2*$^{-/-}$ mice ($p < 0.01$). A similar activation

pattern was observed in the CA3 region, although only a group main effect was detected [$F_{(3,51)}$ = 9.59, $p < 0.0001$; **Figure 3E**], with a significantly increased density of c-Fos-ir cells in FS+ animals compared to CTRL, FS−, and EXT mice (all $p < 0.001$).

Correlation analysis of the conditioned fear response (post-shock freezing) with the c-Fos-ir cell density in FS− and FS+ animals detected a strong positive relationship specifically in *Tph2*$^{-/-}$ mice ($r \geq 0.7501$, $p \leq 0.0321$; **Table 1**). A similar but less exclusive correlation pattern was observed for the density of c-Fos-ir cells with the unconditional response to FS (shock reactivity). Taken together, these data indicate that fear conditioning in *Tph2*$^{-/-}$ mice increased hippocampal activity, while context-dependent extinction training of fear memory normalized hippocampal c-Fos expression within the dHip of *Tph2*$^{-/-}$ mice.

Foot Shock Reduces C-Fos Activation of PV-ir Cells in 5-HT-Deficient Mice

Since PV-ir neurons in the hippocampus were previously shown to be involved in contextual memory (Donato et al., 2013), we analyzed c-Fos-ir neurons in the dHip by double-immunofluorescent staining with PV, a marker of a subset of inhibitory GABAergic interneurons (Gulyas et al., 1999). Because c-Fos-ir densities did not differ between CTRL and FS− animals but were highly increased in fear-conditioned FS+ *Tph2*$^{-/-}$ mice, we focused our further analyses on the FS+ group compared to the CTRL group (**Figure 4**). PV-ir cells were predominantly found in the CA3 and CA1 regions of the dHip, specifically in the stratum lacunosum, pyramidal layer, and stratum oriens with a high density of c-Fos/PV double-ir neurons in the CA1 region (**Figures 4B,E**). In the total dHip, ANOVA detected an almost significant genotype x group interaction for the density of c-Fos/PV double-ir neurons [$F_{(2,24)}$ = 3.14, p = 0.06; **Figure 4C**]. PV-ir neurons in FS+ *Tph2*$^{-/-}$ mice showed reduced c-Fos-ir cells relative to CTRL *Tph2*$^{-/-}$ mice (p = 0.053), while FS+ *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$ mice displayed no alterations compared to respective CTRL mice. Within the FS+ group, *Tph2*$^{+/-}$ mice showed the highest c-Fos/PV-ir density compared to *Tph2*$^{+/+}$ (p = 0.05) and *Tph2*$^{-/-}$ (p = 0.009) mice.

In CA1, a similar but significant genotype x group interaction was detected for the density of c-Fos/PV double-ir neurons [$F_{(2,24)}$ = 4.77, p = 0.018; **Figure 4E**]. *Post hoc* tests revealed that CTRL *Tph2*$^{-/-}$ mice showed increased c-Fos/PV double-ir cell densities relative to CTRL *Tph2*$^{+/+}$ (p = 0.047). However, FS+ *Tph2*$^{-/-}$ mice showed reduced c-Fos/PV double-ir cell densities relative to CTRL *Tph2*$^{-/-}$ as well as FS+ *Tph2*$^{+/-}$ ($p < 0.05$) and FS+ *Tph2*$^{+/+}$ ($p < 0.1$) mice. Neither differences among genotypes nor among CTRL and FS+ groups were found for the density of PV-ir neurons in dHip (**Figure 4D**) and CA1 (**Figure 4F**). Altogether these data indicate increased recruitment of PV neurons in the dorsal CA1 region of CTRL *Tph2*$^{-/-}$ mice, which is absent after fear conditioning.

LTP Impairment by Inescapable Foot Shock Is Absent in *Tph2*-Deficient Mice

In the CA1 region, plasticity is modulated by endogenous 5-HT (Mlinar et al., 2015). Here, we investigated the impact of 5-HT deficiency on TBS-induced LTP of fEPSP in the hippocampal CA1 region of *Tph2*-deficient mice (**Figure 5C**). Similar LTP of fEPSP responses were found across genotypes in naïve mice (**Figure 5A**, upper panels), indicating that basic mechanisms underlying LTP are preserved in the life-long absence of 5-HT. Since the 5-HT system has been implicated in foot shock-induced impairment of LTP (Dai et al., 2008), we compared LTP in slices obtained from animals exposed to repeated foot shock stress (**Figure 5A**, lower panels, **B,D**). Two-way ANOVA revealed a significant genotype x group interaction [$F_{(2,30)}$ = 9.37 p = 0.0484; **Figure 5D**]. *Post hoc* analysis confirmed decreased LTP in *Tph2*$^{+/+}$ (p = 0.0011) and *Tph2*$^{+/-}$ (p = 0.002) mice after foot shock relative to naïve controls, an effect that was absent in *Tph2*$^{-/-}$ mice (**Figures 5A,B,D**) indicating that presence of 5-HT during foot shock is required for the stress-induced impairment of LTP.

DISCUSSION

We previously showed that lifelong absence in brain 5-HT synthesis following constitutive *Tph2* inactivation in mice enhanced acquisition of conditioned fear and promoted unconditioned escape responses to electric foot shock (Waider et al., 2017). Here, we were able to replicate and extend these findings, which indicate that

TABLE 1 | Pearson's correlation of shock reactivity and post-shock freezing with c-Fos density in the dorsal hippocampus and its subregions of FS− and FS+ animals.

		Shock reactivity				Post-shock freezing			
		Total	**+/+**	**+/−**	**−/−**	**Total**	**+/+**	**+/−**	**−/−**
dHip	*r*	0.6636	0.7071	0.5517	0.7493	0.6193	0.2035	0.2821	0.8590
	p	0.0006	0.0756	0.1563	0.0324	0.0016	0.6616	0.4985	0.0063
DG	*r*	0.6592	0.7065	0.3972	0.8409	0.6083	0.2151	0.1633	0.9279
	p	0.0006	0.0759	0.3298	0.0089	0.0021	0.6432	0.6992	0.0009
CA1	*r*	0.6453	0.4586	0.8015	0.7217	0.5401	−0.0100	0.6363	0.7501
	p	0.0009	0.3006	0.0168	0.0433	0.0078	0.9830	0.0898	0.0321
CA3	*r*	0.5561	0.7337	0.4562	0.5655	0.5950	0.3135	0.2460	0.7541
	p	0.0059	0.0605	0.2558	0.1441	0.0027	0.4936	0.5570	0.0307

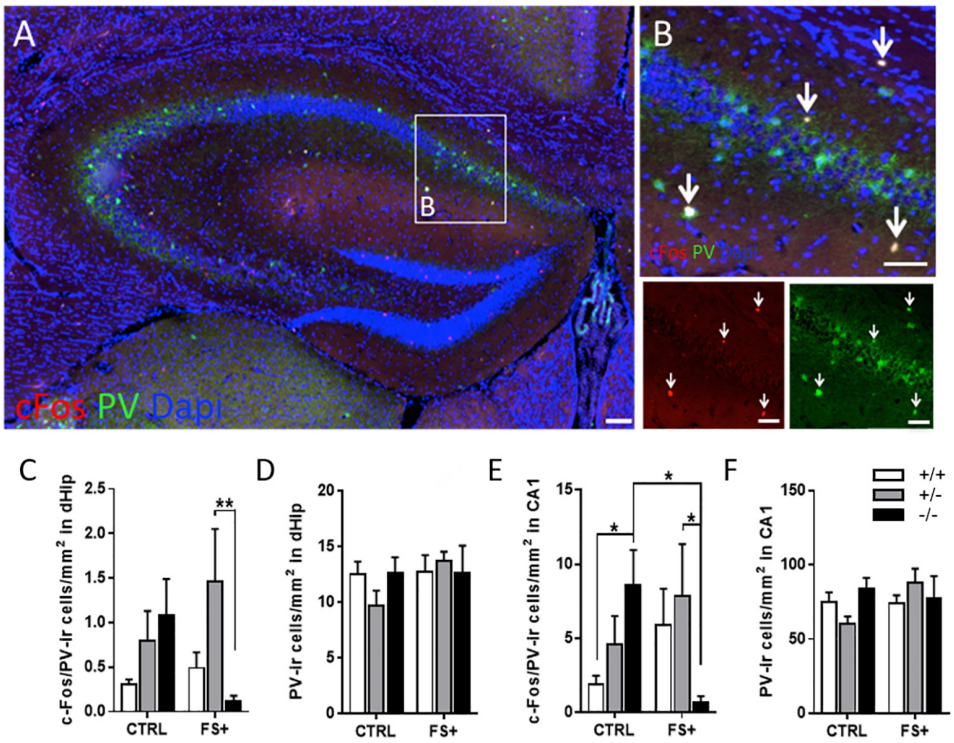


FIGURE 4 | Lifelong brain 5-HT synthesis deficiency prevents activation of parvalbumin-ir neurons in fear conditioning. **(A)** Anti-parvalbumin (PV) (green) and c-Fos (red) immunofluorescent staining with DAPI in the dorsal hippocampus (dHip) of a fear conditioned *Tph2*$^{+/-}$ mouse. **(B)** Magnified with magnified area of cornu ammonis area 1 (CA1). Density of c-Fos/PV-ir cells of home cage control (CTRL) and fear-conditioned (FS+) *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice in **(C)** dHip and **(E)** CA1. PV-ir cell density of CTRL and FS+ *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice in **(D)** dHip and **(F)** CA1. Data are shown as means + SEM. *$p < 0.05$; **$p < 0.01$. Two-way ANOVA followed by Fisher's LSD *post hoc* test. Scale bar: 100 μm.

constitutive *Tph2* inactivation results in faster acquisition of conditioned fear and increased escape-like behavior in response to foot shock, as well as enhanced contextual representation of the fear memory, but functional extinction of the context. By combining fear conditioning with immediate-early gene expression, our data emphasize that lifelong 5-HT deficiency renders the dHip hyperexcitable in fear conditioning.

The dHip encodes the contextual component of fear learning during early acquisition phases (Fanselow and Dong, 2010; Maren et al., 2013). Furthermore, direct glutamatergic projections from the BLA to the dHip have been shown to be both necessary and sufficient for repeated long-term foot shock-mediated memory impairments (Rei et al., 2015), while neurons in the BLA become activated during hippocampal theta network activity or optogenetic stimulation of CA1 pyramidal neurons (Bienvenu et al., 2012; Bazelot et al., 2015).

Because compensation through a 5-HT-dependent mechanism are absent in *Tph2*$^{-/-}$ mice (Gutknecht et al., 2012; Waider et al., 2017), resilience to foot shock-mediated memory impairments may be derived directly from absence of 5-HT signaling in the dHip. Indeed, inescapable foot shock and novel aversive contexts were reported to impair contextual memory and the induction of LTP in the hippocampal CA1 region (Foy et al., 1987; Sacchetti et al., 2002; Dai et al., 2008).

Moreover, it has long been suggested that 5-HT counteracts the consolidation of stressful memories, presumably mediated by 5-HT1A receptors in the dHip, which then may lead to tolerance with chronic aversive events (Graeff et al., 1996). In contrast to *Lmx1b* cKO mice, which lack raphe 5-HT neurons (Dai et al., 2008), 5-HT neurons in *Tph2*$^{-/-}$ mice are unable to synthesize 5-HT, but are functionally preserved (Gutknecht et al., 2012; Montalbano et al., 2015).

Here, we chose a consolidated foot shock protocol (Dai et al., 2008) to investigate whether the absence of 5-HT influences hippocampal LTP formation to allow direct comparison of our data with those obtained using a different animal model of 5-HT system impairment. Although the protocol applied for the LTP and the behavioral experiments differed and, therefore, quantitative correlation of plasticity impairment with the behavioral effects was not possible, our results demonstrate that 5-HT is required for foot shock-induced impairment of LTP. Indeed, 5-HT has been shown to shift hippocampal activity along the longitudinal axis toward the ventral part (Mlinar and Corradetti, 2018). Thus, an absence of this mechanism in *Tph2*$^{-/-}$ mice may result in an overactivation of the hippocampal circuitry, manifested as enhanced c-Fos immunostaining and overrepresentation of the contextual

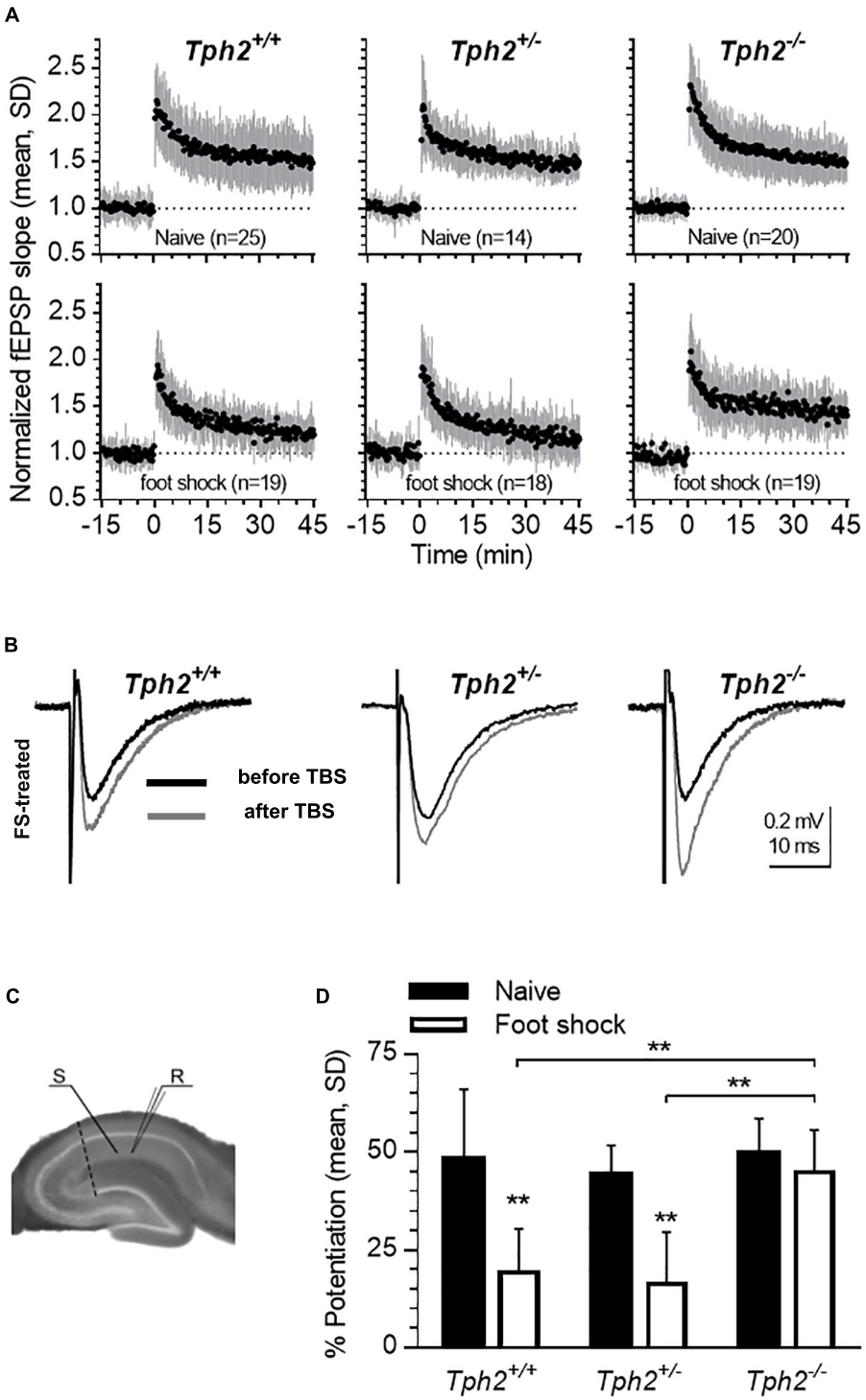


FIGURE 5 | Lack of 5-HT impedes foot shock-induced reduction in hippocampal long-term potentiation (LTP). **(A)** Time-course of LTP in slices obtained from naïve and foot shock-treated mice (*Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice; naïve: n = 8,6,5; foot shock: n = 5,6,6). Mean ± SD of fEPSP slope values normalized to the baseline mean from 5 to 0 min before theta burst stimulation (TBS). Numbers (n) in graphs indicate slices tested, including replicates per mouse. **(B)** Representative traces of recordings foot shock-treated *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice before and after TBS. **(C)** Positioning of stimulation electrode (S) on Schaffer collaterals and recording electrode (R) in CA1. The dashed line indicates a cut made between cornu ammonis area 1 (CA1) and cornu ammonis area 3 (CA3) to prevent recurrent propagation of action potentials. **(D)** LTP is expressed as the percent change measured 40–45 min after TBS in respect to baseline between naïve and foot shock-treated *Tph2*$^{+/+}$, *Tph2*$^{+/-}$, and *Tph2*$^{-/-}$ mice. $^{**}p < 0.01$. Two-way ANOVA, followed by Sidak's *post hoc* test.

component in fear learning. In line with this, a mouse model lacking hippocampal serotonergic input showed alterations in contextual fear memory with no differences in LTP formation under baseline conditions (Fernandez et al., 2017).

However, recall of context-dependent aversive memory is exaggerated in *Tph2*$^{-/-}$ mice, supporting the view that 5-HT mediates inhibition of context-dependent aversive memories through inhibition of LTP, while under non-aversive conditions, increased endogenous 5-HT release facilitated LTP induction in the CA1 region, and may underlie the *in vivo* positive effects of augmented 5-HT tone on cognitive performance (Mlinar et al., 2015).

In this respect, it may be of interest in a follow up study to analyze the response of the ventral hippocampus, which is involved in emotional fear processing (Fanselow and Dong, 2010), and is modulated differentially by 5-HT along its longitudinal axis (Mlinar and Corradetti, 2018).

Formation of context-dependent memories requires changes in the expression of calcium-binding proteins of GABAergic interneurons, including hippocampal PV-ir cells (Bienvenu et al., 2012; Donato et al., 2013, 2015). In line with previous studies, 5-HT has been shown to modulate PV-specific neurons in the hippocampus (Gulyas et al., 1999). Our results hint toward an involvement of CA1 PV-ir neurons in context-dependent fear conditioning, which is prevented in *Tph2*$^{-/-}$ mice, although activity of the hippocampus is generally increased. Especially in *Tph2*$^{+/-}$ mice, fear conditioning recruited the highest number of PV-ir neurons. Thus, it seems that 5-HT-dependent activation of PV neurons may protect the dHip from overactivation. Furthermore, the increased hippocampal activity in *Tph2*$^{-/-}$ mice due to fear conditioning may prevent an increased response of the BLA, as observed in *Tph2*$^{+/-}$ mice, to inhibit flight or panic responses (Waider et al., 2017). Thus, further studies are required to investigate the differential role of 5-HT on PV neuron-dependent theta synchronization in Hip and BLA (Amilhon et al., 2015).

Increased active coping in novel, aversive, and inescapable situations is mediated through an amygdala-ventrolateral periaqueductal gray (vlPAG) circuit, mediated through the dorsolateral PAG (Hale et al., 2012; Tovote et al., 2016). 5-HT neurons of the vlPAG inhibit panic-like responses mediated by dorsolateral PAG neurons (Pobbe et al., 2011; Spannuth et al., 2011). Indeed, *Tph2*$^{-/-}$ mice show increased post-shock freezing and increased flight responses, although the BLA did not respond to foot shock with an altered activity compared to *Tph2*$^{+/+}$ mice (Waider et al., 2017). Thus, the increased hippocampal response in mice completely devoid of 5-HT-mediated regulatory mechanisms may influence the PAG in its role to translate inputs from the amygdala into an appropriate behavioral response, resulting in increased freezing and flight behaviors.

In conclusion, exaggerated context-dependent fear memory and shock reactivity resulting from brain 5-HT deficiency likely involves dysfunction of the raphe-hippocampal innervation controlling fear-related behavioral responses and is presumably due to the failure of 5-HT receptor-mediated inhibition of hippocampal circuitries. Furthermore, our data indicate largely effective extinction learning, during repetitive context exposure without negative reinforcement and without 5-HT functioning. Thus, context-dependent extinction training may represent a strategy to adapt behavioral therapy for patients suffering from 5-HT system dysfunction associated with anxiety-, stress-, and trauma-related disorders.

AUTHOR CONTRIBUTIONS

JW, RC, ASB, and KPL designed and supervised the study. JW, SP, BM, AM, FB, BA, ET, RK, CT, NA, ES, and ASB performed and analyzed the experiments. JW, SP, BM, RC, CL, and KPL interpreted the results and wrote the manuscript.

FUNDING

This study was supported by the German Research Foundation (DFG: SFB TRR58-A05 to KPL and WA 3446/2-1 to JW), the European Union's Seventh Framework Program (FP7/2007–2013) under Grant No. 602805 (Aggressotype), the Horizon 2020 Research and Innovation Program under Grant No. 728018 (Eat2beNICE), the 5-100 Russian Academic Excellence Project (to KPL) and the University of Florence (ex60% to RC).

ACKNOWLEDGMENTS

We thank P. Gaspar for helpful discussion as well as N. Schraud for technical assistance. We are grateful to Zachary D. Barger for proofreading the manuscript.

REFERENCES

Amilhon, B., Huh, C. Y., Manseau, F., Ducharme, G., Nichol, H., Adamantidis, A., et al. (2015). Parvalbumin interneurons of hippocampus tune population activity at theta frequency. *Neuron* 86, 1277–1289. doi: 10.1016/j.neuron.2015.05.027

Azmitia, E., and Whitaker-Azmitia, P. (1995). "Anatomy, cell biology, and plasticity of the serotonergic system. Neuropsychopharmacological implications for the actions of psychotropic drugs," in *Psychopharmacology: the Fourth Generation of Progress*, eds F. E. Bloom and D. J. Kupfer (New York, NY: Raven Press), 443–449.

Baratta, M. V., Kodandaramaiah, S. B., Monahan, P. E., Yao, J., Weber, M. D., Lin, P. A., et al. (2016). Stress enables reinforcement-elicited serotonergic consolidation of fear memory. *Biol. Psychiatry* 79, 814–822. doi: 10.1016/j.biopsych.2015.06.025

Bauer, E. P. (2015). Serotonin in fear conditioning processes. *Behav. Brain Res.* 277, 68–77. doi: 10.1016/j.bbr.2014.07.028

Baxter, A. J., Scott, K. M., Vos, T., and Whiteford, H. A. (2013). Global prevalence of anxiety disorders: a systematic review and meta-regression. *Psychol. Med.* 43, 897–910. doi: 10.1017/S003329171200147X

Bazelot, M., Bocchio, M., Kasugai, Y., Fischer, D., Dodson, P. D., Ferraguti, F., et al. (2015). Hippocampal theta input to the amygdala shapes feedforward inhibition to gate heterosynaptic plasticity. *Neuron* 87, 1290–1303. doi: 10.1016/j.neuron.2015.08.024

Bereza, B. G., Machado, M., and Einarson, T. R. (2009). Systematic review and quality assessment of economic evaluations and quality-of-life studies related to

generalized anxiety disorder. *Clin. Ther.* 31, 1279–1308. doi: 10.1016/j.clinthera.2009.06.004

Bienvenu, T. C., Busti, D., Magill, P. J., Ferraguti, F., and Capogna, M. (2012). Cell-type-specific recruitment of amygdala interneurons to hippocampal theta rhythm and noxious stimuli in vivo. *Neuron* 74, 1059–1074. doi: 10.1016/j.neuron.2012.04.022

Bocchio, M., McHugh, S. B., Bannerman, D. M., Sharp, T., and Capogna, M. (2016). Serotonin, amygdala and fear: assembling the puzzle. *Front. Neural Circuits* 10:24. doi: 10.3389/fncir.2016.00024

Brooks, L. R., Enix, C. L., Rich, S. C., Magno, J. A., Lowry, C. A., and Tsai, P. S. (2014). Fibroblast growth factor deficiencies impact anxiety-like behavior and the serotonergic system. *Behav. Brain Res.* 264, 74–81. doi: 10.1016/j.bbr.2014.01.053

Burghardt, N. S., Bush, D. E. A., McEwen, B. S., and LeDoux, J. E. (2007). Acute selective serotonin reuptake inhibitors increase conditioned fear expression: blockade with a 5-HT(2C) receptor antagonist. *Biol. Psychiatry* 62, 1111–1118. doi: 10.1016/j.biopsych.2006.11.023

Dai, J. X., Han, H. L., Tian, M., Cao, J., Xiu, J. B., Song, N. N., et al. (2008). Enhanced contextual fear memory in central serotonin-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11981–11986. doi: 10.1073/pnas.0801329105

Donato, F., Chowdhury, A., Lahr, M., and Caroni, P. (2015). Early- and late-born parvalbumin basket cell subpopulations exhibiting distinct regulation and roles in learning. *Neuron* 85, 770–786. doi: 10.1016/j.neuron.2015.01.011

Donato, F., Rompani, S. B., and Caroni, P. (2013). Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504, 272–276. doi: 10.1038/nature12866

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *Pavlov. J. Biol. Sci.* 15, 177–182.

Fanselow, M. S., and Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65, 7–19. doi: 10.1016/j.neuron.2009.11.031

Fernandez, S. P., Cauli, B., Cabezas, C., Muzerelle, A., Poncer, J. C., and Gaspar, P. (2016). Multiscale single-cell analysis reveals unique phenotypes of raphe 5-HT neurons projecting to the forebrain. *Brain Struct. Funct.* 221, 4007–4025. doi: 10.1007/s00429-015-1142-4

Fernandez, S. P., Muzerelle, A., Scotto-Lomassese, S., Barik, J., Gruart, A., Delgado-Garcia, J. M., et al. (2017). Constitutive and acquired serotonin deficiency alters memory and hippocampal synaptic plasticity. *Neuropsychopharmacology* 42, 512–523. doi: 10.1038/npp.2016.134

Foy, M. R., Stanton, M. E., Levine, S., and Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav. Neural Biol.* 48, 138–149. doi: 10.1016/S0163-1047(87)90664-9

Franklin, K. B. J., and Paxinos, G. (1997). *The Mouse Brain in Stereotaxic Coordinates*. San Diego: Academic Press.

Graeff, F. G., Guimarães, F. S., De Andrade, T. G. C. S., and Deakin, J. F. (1996). Role of 5-HT in stress, anxiety, and depression. *Pharmacol. Biochem. Behav.* 54, 129–141. doi: 10.10160091-3057(95)02135-3

Graeff, F. G., and Zangrossi, H. Jr. (2010). The dual role of serotonin in defense and the mode of action of antidepressants on generalized anxiety and panic disorders. *Cent. Nerv. Syst. Agents Med. Chem.* 10, 207–217. doi: 10.2174/187152491100603020 7

Gravius, A., Barberi, C., Schafer, D., Schmidt, W. J., and Danysz, W. (2006). The role of group I metabotropic glutamate receptors in acquisition and expression of contextual and auditory fear conditioning in rats - a comparison. *Neuropharmacology* 51, 1146–1155. doi: 10.1016/j.neuropharm.2006.07.008

Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biol. Psychiatry* 52, 958–975. doi: 10.1016/S0006-3223(02)01665-7

Gulyas, A. I., Acsady, L., and Freund, T. F. (1999). Structural basis of the cholinergic and serotonergic modulation of GABAergic neurons in the hippocampus. *Neurochem. Int.* 34, 359–372. doi: 10.1016/S0197-0186(99)00041-8

Gutknecht, L., Araragi, N., Merker, S., Waider, J., Sommerlandt, F. M., Mlinar, B., et al. (2012). Impacts of brain serotonin deficiency following Tph2 inactivation on development and raphe neuron serotonergic specification. *PLoS One* 7:e43157. doi: 10.1371/journal.pone.0043157

Gutknecht, L., Popp, S., Waider, J., Sommerlandt, F. M., Goppner, C., Post, A., et al. (2015). Interaction of brain 5-HT synthesis deficiency, chronic stress and sex differentially impact emotional behavior in Tph2 knockout mice. *Psychopharmacology* 232, 2429–2441. doi: 10.1007/s00213-015-3879-0

Hale, M. W., and Lowry, C. A. (2011). Functional topography of midbrain and pontine serotonergic systems: implications for synaptic regulation of serotonergic circuits. *Psychopharmacology* 213, 243–264. doi: 10.1007/s00213-010-2089-z

Hale, M. W., Shekhar, A., and Lowry, C. A. (2012). Stress-related serotonergic systems: implications for symptomatology of anxiety and affective disorders. *Cell. Mol. Neurobiol.* 32, 695–708. doi: 10.1007/s10571-012-9827-1

Hashimoto, S., Inoue, T., and Koyama, T. (1996). Serotonin reuptake inhibitors reduce conditioned fear stress-induced freezing behavior in rats. *Psychopharmacology* 123, 182–186. doi: 10.1007/BF02246175

Hendricks, T. J., Fyodorov, D. V., Wegman, L. J., Lelutiu, N. B., Pehek, E. A., Yamamoto, B., et al. (2003). Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. *Neuron* 37, 233–247. doi: 10.1016/S0896-6273(02)01167-4

Igelstrom, K. M., and Heyward, P. M. (2012). Inhibition of hippocampal excitability by citalopram. *Epilepsia* 53, 2034–2042. doi: 10.1111/j.1528-1167.2012.03660.x

Jorgensen, C. V., Jacobsen, J. P., Caron, M. G., Klein, A. B., Knudsen, G. M., and Mikkelsen, J. D. (2013). Cerebral 5-HT2A receptor binding, but not mGluR2, is increased in tryptophan hydroxylase 2 decrease-of-function mice. *Neurosci. Lett.* 555, 118–122. doi: 10.1016/j.neulet.2013.08.073

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., and Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 617–627. doi: 10.1001/archpsyc.62.6.617

Kiyasova, V., Fernandez, S. P., Laine, J., Stankovski, L., Muzerelle, A., Doly, S., et al. (2011). A genetically defined morphologically and functionally unique subset of 5-HT neurons in the mouse raphe nuclei. *J. Neurosci.* 31, 2756–2768. doi: 10.1523/JNEUROSCI.4080-10.2011

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Li, X. B., Inoue, T., Hashimoto, S., and Koyama, T. (2001). Effect of chronic administration of flesinoxan and fluvoxamine on freezing behavior induced by conditioned fear. *Eur. J. Pharmacol.* 425, 43–50. doi: 10.1016/S0014-2999(01)01159-1

Lowry, C. A. (2002). Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. *J. Neuroendocrinol.* 14, 911–923. doi: 10.1046/j.1365-2826.2002.00861.x

Lowry, C. A., Johnson, P. L., Hay-Schmidt, A., Mikkelsen, J., and Shekhar, A. (2005). Modulation of anxiety circuits by serotonergic systems. *Stress* 8, 233–246. doi: 10.1080/10253890500492787

Maier, S. F., Amat, J., Baratta, M. V., Paul, E., and Watkins, L. R. (2006). Behavioral control, the medial prefrontal cortex, and resilience. *Dialogues Clin. Neurosci.* 8, 397–406.

Maier, S. F., and Watkins, L. R. (2005). Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neurosci. Biobehav. Rev.* 29, 829–841. doi: 10.1016/j.neubiorev.2005.03.021

Maren, S., Phan, K. L., and Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat. Rev. Neurosci.* 14, 417–428. doi: 10.1038/nrn3492

McQuade, R., and Sharp, T. (1997). Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J. Neurochem.* 69, 791–796. doi: 10.1046/j.1471-4159.1997.69020791.x

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., and Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. *J. Psychiatr. Res.* 42, 515–520. doi: 10.1016/j.jpsychires.2008.01.017

Misane, I., Tovote, P., Meyer, M., Spiess, J., Ogren, S. O., and Stiedl, O. (2005). Time-dependent involvement of the dorsal hippocampus in trace fear conditioning in mice. *Hippocampus* 15, 418–426. doi: 10.1002/hipo.20067

Miyazaki, M., Yoshino, A., and Nomura, S. (2011). Relationships between anxiety severity, diagnosis of multiple anxiety disorders, and comorbid major

depressive disorder. *Asian J. Psychiatry* 4, 293–296. doi: 10.1016/j.ajp.2011.10.003

Mlinar, B., and Corradetti, R. (2018). Differential modulation of CA1 impulse flow by endogenous serotonin along the hippocampal longitudinal axis. *Hippocampus* 28, 217–225. doi: 10.1002/hipo.22825

Mlinar, B., Stocca, G., and Corradetti, R. (2015). Endogenous serotonin facilitates hippocampal long-term potentiation at CA3/CA1 synapses. *J. Neural Transm.* 122, 177–185. doi: 10.1007/s00702-014-1246-7

Montalbano, A., Waider, J., Barbieri, M., Baytas, O., Lesch, K.-P., Corradetti, R., et al. (2015). Cellular resilience: 5-HT neurons in Tph2$^{-/-}$ mice retain normal firing behavior despite the lack of brain 5-HT. *Eur. Neuropsychopharmacol.* 25, 2022–2035. doi: 10.1016/j.euroneuro.2015.08.021

Morini, R., Mlinar, B., Baccini, G., and Corradetti, R. (2011). Enhanced hippocampal long-term potentiation following repeated MDMA treatment in Dark-Agouti rats. *Eur. Neuropsychopharmacol.* 21, 80–91. doi: 10.1016/j.euroneuro.2010.07.007

Mosienko, V., Beis, D., Pasqualetti, M., Waider, J., Matthes, S., Qadri, F., et al. (2015). Life without brain serotonin: reevaluation of serotonin function with mice deficient in brain serotonin synthesis. *Behav. Brain Res.* 277, 78–88. doi: 10.1016/j.bbr.2014.06.005

Muzerelle, A., Scotto-Lomassese, S., Bernard, J. F., Soiza-Reilly, M., and Gaspar, P. (2016). Conditional anterograde tracing reveals distinct targeting of individual serotonin cell groups (B5-B9) to the forebrain and brainstem. *Brain Struct. Funct.* 221, 535–561. doi: 10.1007/s00429-014-0924-4

Pobbe, R. L., Zangrossi, H. Jr., Blanchard, D. C., and Blanchard, R. J. (2011). Involvement of dorsal raphe nucleus and dorsal periaqueductal gray 5-HT receptors in the modulation of mouse defensive behaviors. *Eur. Neuropsychopharmacol.* 21, 306–315. doi: 10.1016/j.euroneuro.2010.05.004

Raab, A., Popp, S., Lesch, K. P., Lohse, M. J., Fischer, M., Deckert, J., et al. (2018). Increased fear learning, spatial learning as well as neophobia in Rgs2(-/-) mice. *Genes Brain Behav.* 17:e12420. doi: 10.1111/gbb.12420

Ravinder, S., Burghardt, N. S., Brodsky, R., Bauer, E. P., and Chattarji, S. (2013). A role for the extended amygdala in the fear-enhancing effects of acute selective serotonin reuptake inhibitor treatment. *Transl. Psychiatry* 3:e209. doi: 10.1038/tp.2012.137

Rei, D., Mason, X., Seo, J., Graff, J., Rudenko, A., Wang, J., et al. (2015). Basolateral amygdala bidirectionally modulates stress-induced hippocampal learning and memory deficits through a p25/Cdk5-dependent pathway. *Proc. Natl. Acad. Sci. U.S.A.* 112, 7291–7296. doi: 10.1073/pnas.1415845112

Rivero, O., Selten, M. M., Sich, S., Popp, S., Bacmeister, L., Amendola, E., et al. (2015). Cadherin-13, a risk gene for ADHD and comorbid disorders, impacts GABAergic function in hippocampus and cognition. *Transl. Psychiatry* 5:e655. doi: 10.1038/tp.2015.152

Sacchetti, B., Lorenzini, C. A., Baldi, E., Bucherelli, C., Roberto, M., Tassoni, G., et al. (2002). Time-dependent inhibition of hippocampal LTP in vitro following contextual fear conditioning in the rat. *Eur. J. Neurosci.* 15, 143–150. doi: 10.1046/j.0953-816x.2001.01844.x

Schaefer, T. L., Vorhees, C. V., and Williams, M. T. (2009). Mouse plasmacytoma-expressed transcript 1 knock out induced 5-HT disruption results in a lack of cognitive deficits and an anxiety phenotype complicated by hypoactivity and defensiveness. *Neuroscience* 164, 1431–1443. doi: 10.1016/j.neuroscience.2009.09.059

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682. doi: 10.1038/nmeth.2019

Schmidt, S. D., Furini, C. R. G., Zinn, C. G., Cavalcante, L. E., Ferreira, F. F., Behling, J. A. K., et al. (2017). Modulation of the consolidation and reconsolidation of fear memory by three different serotonin receptors in hippocampus. *Neurobiol. Learn. Mem.* 142(Pt A), 48–54. doi: 10.1016/j.nlm.2016.12.017

Song, N. N., Xiu, J. B., Huang, Y., Chen, J. Y., Zhang, L., Gutknecht, L., et al. (2011). Adult raphe-specific deletion of LMX1B leads to central serotonin deficiency. *PLoS One* 6:e15998. doi: 10.1371/journal.pone.0015998

Spannuth, B. M., Hale, M. W., Evans, A. K., Lukkes, J. L., Campeau, S., and Lowry, C. A. (2011). Investigation of a central nucleus of the amygdala/dorsal raphe nucleus serotonergic circuit implicated in fear-potentiated startle. *Neuroscience* 179, 104–119. doi: 10.1016/j.neuroscience.2011.01.042

Staubli, U., and Otaky, N. (1994). Serotonin controls the magnitude of LTP induced by theta bursts via an action on NMDA-receptor-mediated responses. *Brain Res.* 643, 10–16. doi: 10.1016/0006-8993(94)90003-5

Toth, I., Dietz, M., Peterlik, D., Huber, S. E., Fendt, M., Neumann, I. D., et al. (2012). Pharmacological interference with metabotropic glutamate receptor subtype 7 but not subtype 5 differentially affects within- and between-session extinction of Pavlovian conditioned fear. *Neuropharmacology* 62, 1619–1626. doi: 10.1016/j.neuropharm.2011.10.021

Tovote, P., Esposito, M. S., Botta, P., Chaudun, F., Fadok, J. P., Markovic, M., et al. (2016). Midbrain circuits for defensive behaviour. *Nature* 534, 206–212. doi: 10.1038/nature17996

Waider, J., Popp, S., Lange, M. D., Kern, R., Kolter, J. F., Kobler, J., et al. (2017). Genetically driven brain serotonin deficiency facilitates panic-like escape behavior in mice. *Transl. Psychiatry* 7:e1246. doi: 10.1038/tp.2017.209

Waider, J., Proft, F., Langlhofer, G., Asan, E., Lesch, K. P., and Gutknecht, L. (2013). GABA concentration and GABAergic neuron populations in limbic areas are differentially altered by brain serotonin deficiency in Tph2 knockout mice. *Histochem. Cell Biol.* 139, 267–281. doi: 10.1007/s00418-012-1029-x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Waider, Popp, Mlinar, Montalbano, Bonfiglio, Aboagye, Thuy, Kern, Thiel, Araragi, Svirin, Schmitt-Böhrer, Corradetti, Lowry and Lesch. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Neuroscience

REVIEW
published: 30 April 2019
doi: 10.3389/fnins.2019.00404

Overcoming Resistance to Selective Serotonin Reuptake Inhibitors: Targeting Serotonin, Serotonin-1A Receptors and Adult Neuroplasticity

*Faranak Vahid-Ansari, Min Zhang, Amin Zahrai and Paul R. Albert**

Brain and Mind Research Institute, Ottawa Hospital Research Institute (Neuroscience), University of Ottawa, Ottawa, ON, Canada

Major depressive disorder (MDD) is the most prevalent mental illness contributing to global disease burden. Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are the first-line treatment for MDD, but are only fully effective in 30% of patients and require weeks before improvement may be seen. About 30% of SSRI-resistant patients may respond to augmentation or switching to another antidepressant, often selected by trial and error. Hence a better understanding of the causes of SSRI resistance is needed to provide models for optimizing treatment. Since SSRIs enhance 5-HT, in this review we discuss new findings on the circuitry, development and function of the 5-HT system in modulating behavior, and on how 5-HT neuronal activity is regulated. We focus on the 5-HT1A autoreceptor, which controls 5-HT activity, and the 5-HT1A heteroreceptor that mediates 5-HT actions. A series of mice models now implicate increased levels of 5-HT1A autoreceptors in SSRI resistance, and the requirement of hippocampal 5-HT1A heteroreceptor for neurogenic and behavioral response to SSRIs. We also present clinical data that show promise for identifying biomarkers of 5-HT activity, 5-HT1A regulation and regional changes in brain activity in MDD patients that may provide biomarkers for tailored interventions to overcome or bypass resistance to SSRI treatment. We identify a series of potential strategies including inhibiting 5-HT auto-inhibition, stimulating 5-HT1A heteroreceptors, other monoamine systems, or cortical stimulation to overcome SSRI resistance.

Keywords: serotonin, antidepressant, autoreceptor, knockout, imaging, brain stimulation, noradrenalin, brain-derived growth factor

OPEN ACCESS

Edited by:
Nasser Haddjeri, Institut National de la Santé et de la Recherche Médicale (INSERM), France

Reviewed by:
Luc Maroteaux, INSERM U839 Institut du Fer à Moulin, France
Guillaume Lucas, INSERM U1215 Neurocentre Magendie, France
Benjamin Adam Samuels, Rutgers, The State University of New Jersey, United States

***Correspondence:**
Paul R. Albert
palbert@uottawa.ca

Specialty section:
This article was submitted to Neuropharmacology, a section of the journal Frontiers in Neuroscience

Received: *16 November 2018*
Accepted: *09 April 2019*
Published: *30 April 2019*

Citation:
Vahid-Ansari F, Zhang M, Zahrai A and Albert PR (2019) Overcoming Resistance to Selective Serotonin Reuptake Inhibitors: Targeting Serotonin, Serotonin-1A Receptors and Adult Neuroplasticity. Front. Neurosci. 13:404. doi: 10.3389/fnins.2019.00404

INTRODUCTION

Major depressive disorder (MDD) has a high incidence and low remission rate with the current therapeutic strategies. Major depression is the largest contributor to global disability by years lived with disability, and anxiety disorders rank sixth (World Health Organization [WHO], 2017). The annual prevalence of depression is 4.4% overall, 3.6% in men and 5.1% in women (Baxter et al., 2014; World Health Organization [WHO], 2017). Major depression is diagnosed by persistent symptoms such as sadness, irritability, anhedonia or changes in appetite or sleep patterns that could result in suicidal thoughts and attempts (Kessler and Bromet, 2013). Genetic or biomarkers for major depression remain elusive, and current genome-wide association studies indicate that individual genetic polymorphisms contribute only a small increase in risk for depression.

Brain imaging studies are beginning to reveal changes in functional connectivity associated with major depression that may predict treatment response (Drysdale et al., 2017; Dunlop et al., 2017). However, at present diagnosis of depression is made by psychiatric interviews, and treatment is not always effective.

Selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for major depression but are only effective for remission in 30% of patients (Rush et al., 2009). Furthermore, a latency of 2–3 weeks is required for response, and even longer to ascertain remission. Thus, a better understanding of how SSRIs mediate their actions could be useful to identify biomarkers or predictors of SSRI response and to enhance treatment efficacy.

SSRIs enhance the function of the serotonin (5-hydroxytryptamine, 5-HT) system and 5-HT has long been implicated as a mediator of antidepressant actions (Cowen, 2008). As a neuro-glial modulator, 5-HT functions throughout the body to regulate a diversity of homeostatic systems. In the brain, 5-HT is implicated in regulation of pain, sleep, appetite, stress, mood, and emotion (Jacobs and Azmitia, 1992). In this review, we discuss the actions of 5-HT in the nervous system and on behavior and how 5-HT activity is regulated, focusing on the 5-HT1A receptor, which both controls 5-HT activity and mediates 5-HT actions (Albert, 2012; Garcia-Garcia et al., 2014). We address how SSRI actions are mediated, mechanisms that promote resistance to chronic SSRI treatment, and how SSRI resistance may be predicted and overcome. In addition, while most animal studies were using males, we have noted studies that include females. Taken together, there is strong evidence from rodent models that increased 5-HT1A autoreceptor function contributes to depression and SSRI resistance, while activation of hippocampal 5-HT1A heteroreceptors is required for SSRI action. Several potential targets to bypass these mechanisms of SSRI resistance are highlighted including reducing 5-HT auto-inhibition, activating the 5-HT system pharmacologically or through brain stimulation, activating 5-HT1A heteroreceptors, or bypassing the 5-HT system by activating other monoamine systems (**Figure 1**).

In this review we focus mechanisms of resistance to SSRIs involving altered activity of 5-HT neurons via 5-HT1A autoreceptors and how these changes can be overcome by targeting 5-HT1A receptors, non-5-HT mechanisms or activity-induced neuroplasticity. Several alternative strategies proposed to bypass the auto-inhibition of 5-HT neurons by targeting other 5-HT receptor subtypes are not discussed here, but have been reviewed recently. These include use of 5-HT4 agonists (Samuels et al., 2016), 5-HT2C or 5-HT7 antagonists (Ramaker and Dulawa, 2017), or 5-HT1B (Nautiyal and Hen, 2017) or 5-HT2B ligands (Quentin et al., 2018) as novel antidepressant strategies that may overcome SSRI resistance (Artigas, 2013).

THE SEROTONIN SYSTEM

Serotonin: Circuitry and Development

The cells responsible for brain 5-HT synthesis uniquely express tryptophan hydroxylase-2 (TPH2), the rate-limiting enzyme for 5-HT synthesis (Walther et al., 2003; Lesch et al., 2012) and are localized in the mesencephalon, pons and medulla oblongata. The majority of 5-HT cells are located in the raphe nuclei in midbrain, in the dorsal, median and caudal raphe sub-regions, which are interconnected [e.g., between dorsal and caudal raphe (Bang et al., 2012)]. The anatomy of the raphe 5-HT projections were characterized initially using histochemical fluorescence (Ungerstedt, 1971), and then using labeled with [^{3}H]5-HT, and verified using unilateral injection of the selective serotonin neurotoxin 5,7-dihydroxytryptamine (Azmitia and Segal, 1978; Jacobs and Azmitia, 1992). The rostral dorsal (DR) and median (MR) raphe nuclei project 5-HT fibers via the median forebrain bundle to forebrain areas, while the caudal raphe nuclei innervate cerebellar and spinal targets (Gaspar and Lillesaar, 2012; Maddaloni et al., 2017). Thus, virtually the entire central nervous system receives 5-HT input (Jacobs and Azmitia, 1992). Serotonin neurons within the DR are functionally heterogeneous (Calizo et al., 2011; Okaty et al., 2015; Fernandez et al., 2016) and show diverse projections. Fiber tracing has revealed that individual 5-HT neurons are highly branched and send input to multiple forebrain structures (Gagnon and Parent, 2014). Retrograde tracing using retrobeads indicates that DR subregions may preferentially project to different targets (Waselus et al., 2006) and global mapping of 5-HT projections using viral retrograde tracing has revealed two main projection subtypes: from ventral DR to anterior cortical regions, and from dorsal DR to subcortical regions (Ren et al., 2018). Interestingly, these 5-HT pathways have opposing effects on anxiety and depression phenotypes. In particular, the 5-HT pathway projecting to the amygdala was activated by reward and punishment and promoted anxiety phenotypes, while the frontal cortex projection was activated by reward but inhibited by punishment and promoted an anti-depressed phenotype (Ren et al., 2018). Targeting 5-HT release in the frontal cortex could thus produce a specific and more robust antidepressant effect, avoiding the anxiogenic effects often seen with acute SSRI treatment and thought to involve activation of the amygdala (Arrant et al., 2013). Thus, globally targeting 5-HT using SSRI treatment is likely to activate antagonistic pathways that could contribute to adverse acute effects or to resistance to SSRI treatment.

The raphe nuclei in turn receive inputs from the numerous regions to which the 5-HT neurons project (Pollak Dorocic et al., 2014; Weissbourd et al., 2014; Ren et al., 2018). The majority of projections to the DR are from the hypothalamus, amygdala, medulla and cortex, with the central amygdala sending projections mainly to GABAergic DR interneurons, while the cortical projections are mainly to 5-HT neurons. In contrast, the MR receives sparse input from amygdala, prefrontal cortex (PFC) or other cortical areas, but stronger from hypothalamus and midbrain (Pollak Dorocic et al., 2014). Importantly, DR neurons projecting to anterior cortex receive strongest innervation for cortical regions, while DR cell targeting amygdala receive more innervation from the amygdala (Ren et al., 2018), suggesting a role for feedback regulation of 5-HT projections by the target neurons.

The development of the 5-HT system was addressed in the early 1980s, immunohistochemical studies showed that raphe 5-HT cells are generated from embryonic day (e) e11-15 of gestation in rats and the initial axonal sprouting of

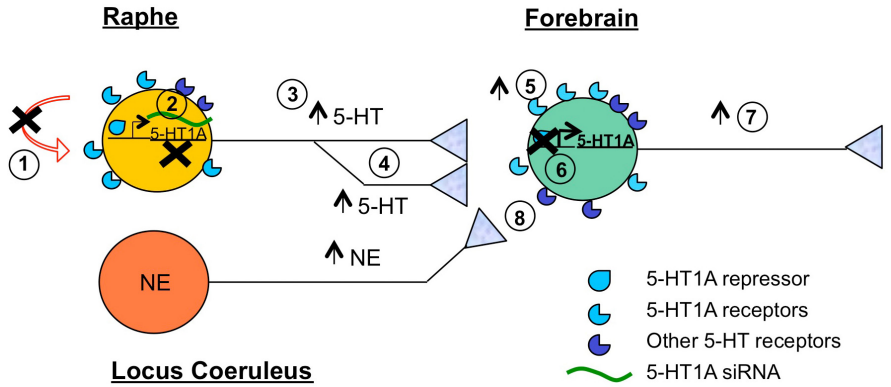


FIGURE 1 | Summary of target sites to overcome SSRI resistance. Shown is a 5-HT neuron (yellow) that is refractory to SSRI treatment. Brain regions highlighted include raphe containing 5-HT neurons, locus coeruleus containing norepinephrine (NE) neurons (orange), and forebrain (green), which includes frontal cortex, hippocampus, amygdala, brain regions implicated in antidepressant response. Several potential sites of intervention to augment SSRI response or bypass SSRI treatment are shown. These include: (1) Blocking (X) auto-inhibition (via 5-HT1A autoreceptor or signaling blockers); (2) Reducing 5-HT1A autoreceptor expression (via desensitization, HTR1A gene repression or 5-HT-targeted 5-HT1A siRNA); (3) Increasing (arrow) 5-HT neuronal activity (via cortical stimulation); (4) Increasing 5-HT neuroplasticity (e.g., synaptogenesis); (5) Increasing 5-HT1A heteroreceptor activity (via biased 5-HT1A agonists); (6) Increasing 5-HT1A heteroreceptor expression (via inhibiting 5-HT1A-selective repressor activity); (7) Increasing cortical activity (via ketamine, cortical stimulation); (8) Bypassing 5-HT (via activation of other monoamines such as NE using transporter blockers or agonists).

5-HT-containing neurons occurs at e12 (Wallace and Lauder, 1983). By e17, most of the forebrain areas and the frontal part of the neocortex become innervated by 5-HT fibers. The cortical plate is innervated by 5-HT by e18, which forms a deep bundle of fibers sprouting laterally/dorsally within the cortical areas (Jacobs and Azmitia, 1992). The 5-HT innervation terminates in occipital cortex suggesting that 5-HT fibers encircle the brain in a rostro-caudal direction (Wallace and Lauder, 1983). By e21, increases in 5-HT axonal density and terminal formation in subcortical regions are detectable and the latter continues postnatally, resembling the adult brain by post-natal day (p) p3. These early studies were further verified in mice by tracing of GFP-labeled 5-HT neurons through postnatal development (Maddaloni et al., 2017). By p6 increased 5-HT fiber density and terminals are seen in the thalamus, hypothalamus, and cerebellum. At p10-14, 5-HT fibers reach mature levels in all cortex layers and by p21 all terminal fields are fully innervated by 5-HT (Dori et al., 1996; Dinopoulos et al., 1997). Starting from p28, the fibers differentiate to attain an adult morphology (Maddaloni et al., 2017). Using knockout approaches, it has been shown that development of 5-HT projections is dependent on several axonal guidance, planar cell polarity factors (e.g., SLIT1/2, Frizzled3, and Vangl2) (Kiyasova and Gaspar, 2011) and cellular adhesion molecules (e.g., protocadherins) (Katori et al., 2009). In addition to a developmental role, 5-HT is also important for maintenance of 5-HT circuitry in adulthood. Using conditional TPH2GFP knockin mice, 5-HT depletion was found to increase GFP-labeled 5-HT fiber density in the hippocampus, while reducing it in other brain regions, such as the thalamic paraventricular nucleus (Pratelli et al., 2017), implicating 5-HT in development but also maintenance of 5-HT circuitry.

The timing of 5-HT development corresponds with a critical period that has been identified for the development of anxiety and depression phenotypes (Albert et al., 2014; Garcia-Garcia et al., 2014). For example, transient knockout or inhibition of 5-HT1A receptors during the early postnatal-adolescent period results in a persistent anxiety/depression phenotype that is not rescued by gene re-activation in adulthood (Gross et al., 2002; Lo Iacono and Gross, 2008; Donaldson et al., 2014; Garcia-Garcia et al., 2017b). More recently it has been shown that early life manipulation of the 5-HT system, including early life SSRI treatment, alters 5-HT innervation and neural circuitry in adult and impacts adult behavior (Gaspar et al., 2003; Gingrich et al., 2017; Teissier et al., 2017). In this regard, disruption of 5-HT projections by conditional deletion of protocaderin-alphaC2 results in a mild depression-like phenotype in mice, implicating forebrain 5-HT innervation in behavioral phenotypes (Katori et al., 2009; Chen et al., 2017), and as potential target for new antidepressant treatments (**Figure 1**). The lack of effect on behavior of adulthood 5-HT1A gene rescue in the forebrain of 5-HT1A knockout mice (Gross et al., 2002) suggests that developmental alterations in 5-HT innervation may confer behavioral phenotypes in adulthood that are more resistant to SSRI treatment, although this remains to be tested in these models.

Serotonin Dynamics: Synthesis and Reuptake

The differentiation of neuronal progenitors to express serotonergic markers like TPH2 is primarily driven by the transcription factor Pet-1, which is expressed only in 5-HT neurons and directly activates the TPH2 gene (Liu et al., 2010; Jacobsen et al., 2011). The activity of TPH is quite low at birth and reaches 60% of adult levels by p2 in rats (Deguchi and Barchas, 1972). Similarly, 5-HT levels reach 75% of adult

values by p2 and maximize by p3 (Bennett and Giarman, 1965). The level of extracellular 5-HT in the brain is tightly controlled by the neuronal uptake system at the presynaptic nerve endings to maintain internal homeostasis. Serotonin transporter proteins (SERT) are expressed at release sites and efficiently transport released 5-HT back into the cell via a high affinity Na^+/Cl^- dependent active transport system (Amara and Kuhar, 1993). These high affinity transporters are the targets of many antidepressant compounds. Imipramine and related tricyclic compounds were shown to have antidepressant activity (Lehmann et al., 1958) and then found to inhibit both SERT and the norepinephrine transporter (NET) resulting in a longer half-life of the neurotransmitter in the synaptic cleft (Pletscher, 1991). These and other observations led to the hypothesis that the reduced activity of monoamine systems, like NE and/or 5-HT are associated with depression (Schildkraut, 1965; Coppen, 1967).

5-HT Synaptic Contacts and Neuroplasticity

Several modes of 5-HT neurotransmission have been described. Classically, 5-HT neurons arising from the midbrain raphe nuclei are thought to project throughout the brain form direct synapses with target neurons. Interestingly, 5-HT is also released non-synaptically from varicosities (Descarries et al., 1975), a diffusion-based neurotransmission termed "volume transmission" (Fuxe et al., 2007). The 5-HT in an extra-synaptic space preferentially modulates the activity of excitatory/inhibitory synapses, in contrast to its neurotransmitter functions at dendrites or cell bodies (De-Miguel and Trueta, 2005; Kiss, 2008). To finely modulate the activity of excitatory and inhibitory neurons, 5-HT projections form close contacts with these cells, or synaptic triads, as detected by electron microscopy in rodent (Ciranna, 2006). Belmer et al. (2016, 2017) have mapped changes in 5-HTergic axonal density and the formation of triadic connectivity within different corticolimbic regions. They detected 5-HTT+ varicosities in close proximity to presynaptic excitatory and inhibitory nerve terminals. Asymmetrical synapses/excitatory triads were identified in cortical and hippocampal areas, while symmetrical synapses/inhibitory triads were mainly located in subcortical areas. These results suggest that 5-HT projections may preferentially target excitatory vs. inhibitory neurotransmission, depending on the region. The balance between 5-HT regulation of glutamate vs. GABA neurons in the prefrontal cortex is postulated to account for behavioral phenotypes observed upon reduction or activation of 5-HT neurotransmission in transgenic mouse models. Our model postulates that the behavioral phenotype shifts as 5-HT activity increases from none (anxious/aggressive) to low (anxious/depressed) to high (anxious, not depressed) in part due to a dose-dependent shift in 5-HT targeting from glutamate to GABA neurons in the PFC (Albert et al., 2014). The role of 5-HT triads in behavior remains unclear, and the presence of 5-HT triads in human subjects remains to be addressed.

There is evidence that environmental stress can modify the activity of the 5-HT system in a region-specific manner. For example, acute exposure of rats to swim stress increases 5-HT in the striatum but decreases it in the lateral septum and amygdala (Waselus et al., 2006), with no changes in the cortex and hippocampus (Kirby et al., 1995). Stress-induced regional activation targets different 5-HT neuron populations in the DR, but the mechanisms that trigger this specificity remain unclear. In addition, there is evidence that stress-induced neuroplastic changes may remove or alter 5-HT innervation to modify 5-HT action, which can be reversed by deep brain stimulation (Veerakumar et al., 2014). A salient loss of 5-HT fiber density was found in the orbitofrontal cortex (OFC) in depressed subjects, suggesting that region-specific modifications of 5-HT innervation contribute to the pathology of depression (Rajkowska et al., 2017). Harnessing 5-HT neuroplasticity using deep brain stimulation may provide a new treatment strategy in depressed patients that may be resistant to SSRI treatment due to deficient 5-HT innervation in some forebrain areas (**Figure 1**). Taken together, alterations in the activity of the 5-HT system, including in 5-HT synthesis, 5-HT innervation, or 5-HT degradation differentially impact the activity of the different brain areas and provide potential targets for antidepressant therapy (**Figure 1**).

In addition to differences in 5-HT projections, different subregions of the raphe nuclei have been associated with depression and anxiety. The caudal DR and MR share similar origins and many projections compared to the rostral DR (Commons, 2016). Increasing evidence is suggesting different functional roles of the rostral DR and caudal DR/MR in anxiety and depression phenotypes. Activation of caudal DR by inescapable shock stress or by CRH injection is associated with depression-like behavior providing stronger support for a pro-depression effect of caudal DR (Hammack et al., 2002). In the post-natal FLX model, reduced activity of the rostral DR was associated with behavioral despair, while hyper-activity of the MR led to anxiety-like behavior (Teissier et al., 2015). A recent paper shows that knockout of CACNA1C L-type calcium channel subunit gene in 5-HT neurons results in behavioral despair associated with increased activity of caudal DR and inhibition of the rostral DR, via 5-HT1A receptor activation (Ehlinger and Commons, 2019). In post-mortem raphe tissue from depressed suicide compared to control brains, TPH2 RNA is increased in mid-caudal DR (Bach-Mizrachi et al., 2008) and 5-HT1A receptors in higher in mid-rostral DR than in caudal DR (Stockmeier et al., 1998; Boldrini et al., 2008), which may suggest reduced 5-HT1A-mediated auto-inhibition of the caudal DR vs. rostral DR. Thus, region-specific activation of the caudal DR/MR and 5-HT1A-induced inhibition of rostral DR appear to associate with depression and possibly anxiety phenotypes.

5-HT1A RECEPTORS

5-HT1A Autoreceptor and Heteroreceptor Function

Additional important targets of antidepressant treatment are the 5-HT receptors, particularly the 5-HT1A receptor (**Figure 1**). A large family of at least 14 distinct receptor subtypes mediates the actions of 5-HT on brain function (Hoyer et al., 2002).

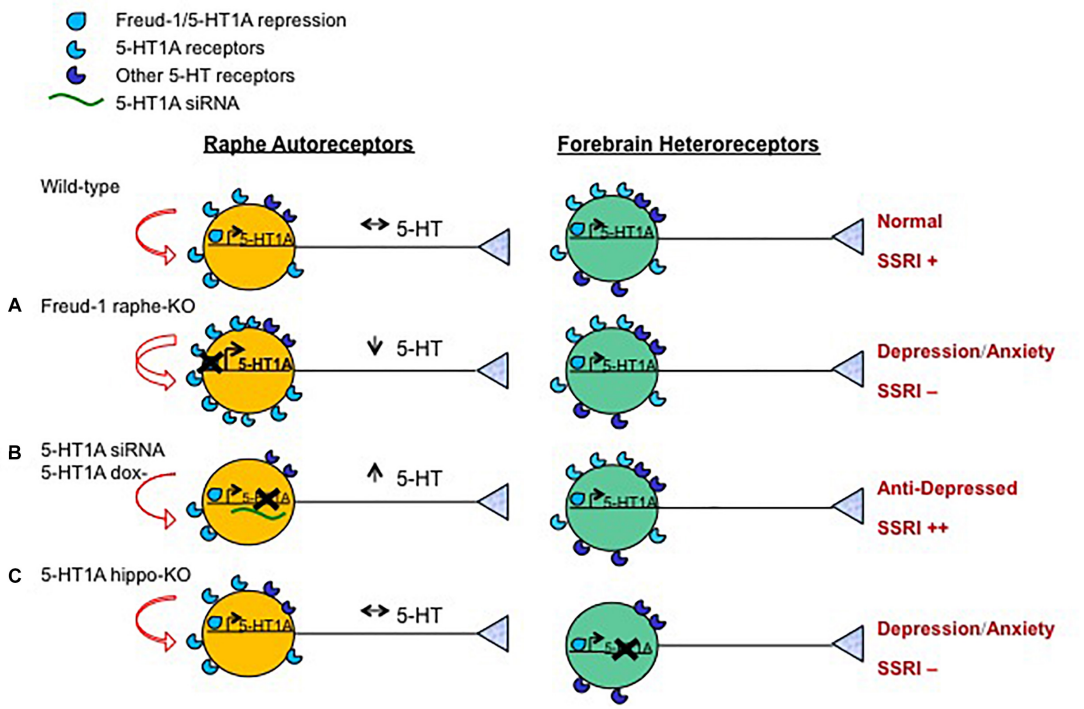


FIGURE 2 | Opposite roles of 5-HT1A autoreceptor vs. heteroreceptors in behavior and SSRI response. Shown are the effects on the serotonin system and behavior in mouse or rat knockout/knockdown (X) genetic models compared to wild-type animals. Models included were generated with: **(A)** Over-expression of 5-HT1A autoreceptors (using knockout of 5-HT1A repressor Freud-1/CC2D1A in adult 5-HT neurons); **(B)** knock-down of 5-HT1A autoreceptors (using raphe-targeted 5-HT1A siRNA (Bortolozzi et al., 2012) or inducible genetic knock-down); or **(C)** loss (using gene knockout) of 5-HT1A heteroreceptors in hippocampal granule cells. The effect of these knockouts on 5-HT1A transcription (right angle arrow) and receptor levels, 5-HT auto-inhibition (curved red arrows), 5-HT neuronal activity (black arrows), depression- or anxiety-like behavior and response to chronic SSRI treatment (++, + or −) are shown. Increasing the expression of 5-HT1A autoreceptors reduces 5-HT activity leading to anxiety and/or depression-like behavior resistant to SSRI treatment (Vahid-Ansari et al., 2017). Knockdown of adult 5-HT1A autoreceptors induces a stress-resilient state and enhances SSRI responsiveness (++) (Richardson-Jones et al., 2010), but can also lead to an anxiogenic response to subchronic SSRI with extensive knockdown (Turcotte-Cardin et al., 2019). The loss of hippocampal granule cell 5-HT1A heteroreceptors leads to depression and anxiety phenotypes and prevents SSRI response (Samuels et al., 2015).

Much attention has focused on the 5-HT1A receptor subtype, among the most abundant and widely expressed 5-HT receptors in the brain (Barnes and Sharp, 1999; Beliveau et al., 2017). 5-HT1A receptors have a dual function: as somatodendritic autoreceptors located on 5-HT neurons in the raphe nuclei; and as postsynaptic heteroreceptors, which exist on target non-5-HT neurons in 5-HT projecting areas (**Figure 2**) (Riad et al., 2001; Albert, 2012; Garcia-Garcia et al., 2014). Serotonin released in the raphe activates 5-HT1A autoreceptors to negatively regulate the firing of the serotonin system (Blier et al., 1998; Albert and Francois, 2010; Garcia-Garcia et al., 2014) (**Figure 2**), although with greater inhibition in DR compared to MR (Beck et al., 2004). This 5-HT1A-mediated auto-inhibition is often not observed *in vitro*, unless physiological levels of tryptophan are added to support 5-HT synthesis (Liu et al., 2005). Release of serotonin at target neurons activates 5-HT1A heteroreceptors that are most abundantly expressed in the hippocampus, septum, amygdala, and PFC (Albert et al., 1990), where it mediates 5-HT actions on fear, anxiety, stress, and cognitive function (Albert et al., 2014; Garcia-Garcia et al., 2014). In the forebrain, 5-HT1A heteroreceptors are expressed on two antagonistic neuronal populations, to modulate the activity of excitatory glutamatergic pyramidal neurons and inhibitory GABAergic interneurons, in parallel (Celada et al., 2013; Albert et al., 2014). Thus, 5-HT1A receptors both negatively regulate global 5-HT activity and mediate 5-HT responses in target neurons.

The roles of 5-HT1A receptors in brain function have been tested pharmacologically by the administration of 5-HT1A receptor-selective compounds (Fletcher et al., 1996). However, these compounds do not discriminate between 5-HT1A auto- or heteroreceptors, but target all 5-HT1A receptors. They include the agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8OH-DPAT), which is selective for 5-HT1A receptors, but can activate 5-HT7 receptors with 10-fold lower affinity. The antagonist WAY100635 is highly specific in blocking 5-HT1A receptors, but activates dopamine-D4 receptors with 10-fold lower potency (Chemel et al., 2006). Systemic administration of 8-OH-DPAT produces acute hyperphagia, hypothermia, and an anxiolytic effect in rodents. The behavioral and physiological effects of 8OH-DPAT are blocked by pretreatment with the 5-HT1A antagonist, WAY 100635. In clinical trials, 5-HT1A partial agonists, such as buspirone, are currently used as

anxiolytics (Lucki, 1996). Buspirone and other 5-HT1A receptor partial agonists and antagonists are also reported to enhance the therapeutic effects of antidepressants (Blier and Ward, 2003), as seen in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (Trivedi et al., 2006a) (**Figure 1**). These anxiolytic compounds appear to trigger distinct signaling pathways from full agonists like 5-HT or 8OH-DPAT (Albert et al., 1999; Pauwels and Colpaert, 2003), which may contribute to their preferential actions on anxiety and to augment SSRI actions, but this remains to be clarified.

5-HT1A Receptor Signaling

The 5-HT1A receptor signals via inhibitory G proteins (Gi/Go) to reduce neuronal excitability and inhibit firing rate. The 5-HT1A auto- and hetero-receptors have the same intronless coding sequence and share canonical Gi/Go signaling pathways including: inhibition of adenylyl cyclase to reduce PKA activity; opening of G-protein inward rectifying potassium (GIRK) channels to hyperpolarize membrane potential; and inhibition of voltage-gated calcium channels (Ca^{2+}) to reduce calcium influx and calcium-calmodulin kinase activity (Raymond et al., 1999; Albert and Vahid-Ansari, 2018). Nevertheless, they appear to signal differently to G proteins and to tyrosine kinase effectors such as ERK1/2 and Akt-GSK3 (Polter and Li, 2010; Albert and Vahid-Ansari, 2018). For example, 5-HT1A autoreceptors couple to inhibition of ERK1/2 activation in raphe RN46A cells (Kushwaha and Albert, 2005), while the hippocampal 5-HT1A heteroreceptor activates ERK1/2 via a PKC-mediated pathway (Adayev et al., 1999; Mogha et al., 2012). The latter pathway is implicated in 5-HT-induced hippocampal synaptogenesis in development (Mogha et al., 2012), while the former may participate in long-term 5-HT1A-induced auto-inhibition of raphe function. Recent transgenic models have implicated hippocampal 5-HT1A-Gi2 signaling and Akt-GSK3β in fluoxetine-induced anti depressant actions in mice (Talbot et al., 2010; Polter et al., 2012). Importantly, global 5-HT1A knockout (Santarelli et al., 2003) or specific deletion of the gene in hippocampal dentate gyrus granule cells (Samuels et al., 2015) prevents fluoxetine-induced hippocampal neurogenesis and anti depressant and anti-anxiety actions, implicating hippocampal 5-HT1A receptors in fluoxetine action (**Figure 2**). Taken together, 5-HT1A autoreceptors and heteroreceptors signal to diverse, sometimes opposing pathways to mediate the acute and sustained effects of the serotonin system on anxiety and depression (Albert and Vahid-Ansari, 2018). Identifying biased 5-HT1A ligands that target autoreceptor vs. heteroreceptor signaling may provide useful tools to separately target these receptors that have opposite effects on depression and SSRI response (**Figure 2**) (Assie et al., 2010; Becker et al., 2016).

SEROTONIN PROJECTIONS AND BEHAVIOR

Optogenetic and Chemogenetic Studies

The 5-HT system plays important roles in physiological and behavioral function. Despite the large body of studies implicating functional perturbations in this system in a variety of behaviors, the role of specific 5-HT projections from different parts of the raphe remains unclear. Recently, optogenetic studies directly activating 5-HT neurons have revealed novel and sometimes opposing roles for DR stimulation in behavior. For example, Liu and coworkers showed that the acute activation of 5-HT cells in DR results in reinforcement (Liu et al., 2014), while others found a role for this pathway in patience for delayed reward, with no role in reinforcement (Fonseca et al., 2015). Similarly, in one study acute photostimulation of DR 5-HT cells reversibly decreased mechanosensory responses in behaving mice (Dugue et al., 2014), while another showed that phasic optogenetic activation of DR 5-HT neurons produces a transient inhibition in locomotion, but also a persistent increase after chronic stimulation (Correia et al., 2017). Optogenetic stimulation of 5-HT neurons in the MR produced an anxiogenic effect in the elevated plus maze while no behavior changes were found when stimulating the DR (Ohmura et al., 2014). However, stimulating DR projections to the dorsal bed nucleus of the stria terminalis (BNST) to activate inhibitory 5-HT1A receptors was anxiolytic in the elevated plus maze test, and inhibition of those 5-HT axons was instead anxiogenic (Garcia-Garcia et al., 2017a). Oppositely, optogenetic activation at higher power of a DR projection to the ventral BNST activates (via excitatory 5-HT2C receptors) an anxiogenic CRH neurons that silence anxiolytic BNST output neurons (Marcinkiewcz et al., 2016). This latter circuit appears mediate an acute SSRI-induced anxiogenic response. It is likely that 5-HT input to different neuronal populations (e.g., glutamatergic vs. GABAergic) via different receptors (e.g., 5-HT1A vs. 5-HT2) can have opposing effects on behavior (Albert et al., 2014). Thus, targeting the 5-HT system using SSRIs may trigger antagonist neural circuits in some individuals that minimize their efficacy as anti-anxiety or antidepressant treatments, or even lead to adverse effects like anxiogenesis. In these cases, augmentation of SSRI with 5-HT1A partial agonists may enhance the antidepressant response by modulating receptor-specific stimulation by 5-HT.

With regard to the role of different subsets of 5-HT neurons in anxiety- or depression-like behavior, a recent report in mice shows that activating 5-HT neurons of the DR with different projections can have opposite effects on anxiety (Ren et al., 2018). Chemogenetic activation of DR 5-HT neurons projecting to the amygdala, BNST and paraventricular hypothalamus promoted anxiety-like behavior. These 5-HT neurons in the DR express SERT but not vGLUT3 and conditional knockout of TPH2 in this pathway revealed a prodepressant effect of this pathway. In contrast, a distinct population of 5-HT neurons projecting to the anterior cortical areas including the OFC, improved coping behavior in the face of challenge, and had an anti-anxiety effect. The role of 5-HT in these actions was verified by conditional knockout of TPH2 in this pathway. Interestingly, in human depressed subjects a deficiency in 5-HT axon length in OFC Layer VI has been reported (Rajkowska et al., 2017). In mice, the OFC-projecting 5-HT cells co-express SERT and VGLUT3 and are located in the ventral DR (Ren et al., 2018). In agreement, specific inhibition of the DR in mice had prodepressant actions, while MR inhibition had the opposite effect (Teissier et al., 2015).

Chemogenetic stimulation of the raphe induced antidepressant and anti-anxiety effects, which were blunted in a depression model (Teissier et al., 2015). Similarly, optogenetic stimulation of the MR also induced anxiety-like behavior (Ohmura et al., 2014). These studies lead to the hypothesis that the heterogeneity of 5-HT neuron projections in the DR and MR is associated with distinct behavioral outcomes, and their activity can be modified by changes in activity associated with depression (Teissier et al., 2015). New clinical approaches involving stimulation of discrete brain regions implicated in depression may activate the appropriate 5-HT circuits to induce stable remission, particularly in the presence of concurrent SSRI treatment (**Figure 1**). In this regard, a recent prospective study has used deep transcranial magnetic stimulation (dTMS) in SSRI-resistant patients and show enhanced response with SSRI compared to dTMS alone (Tendler et al., 2018).

Serotonin and Neuroplasticity

The adult brain can adapt to environmental and internal stimuli with structural and functional changes known as plasticity and the 5-HT system appears to enhance neuroplasticity of target brain areas (Azmitia, 1999; Batsikadze et al., 2013; Castren and Hen, 2013; Kraus et al., 2017). For example, neuroplasticity following monocular deprivation in adult rats is restored by chronic SSRI treatment (Maya Vetencourt et al., 2008). In human depressed post-mortem brain compared to controls, a lower density of SERT+ 5-HT axons is seen in orbitofrontal cortex (Rajkowska et al., 2017). Conversely, excessive 5-HT during development is associated with dystrophic 5-HT projections in human autism (Daubert and Condron, 2010; Azmitia et al., 2011a,b) and defects in cortical pyramidal and interneuron migration, as seen in 5-HTT–/– mice (Riccio et al., 2009; Riccio et al., 2011). In rodents and humans, chronic SSRI treatment increases the expression in hippocampus and cortex of neurotrophins like brain-derived neurotrophic factor (BDNF), in part via activation of the transcription factor, CREB (D'Sa and Duman, 2002) to mediate behavioral improvement (Jin et al., 2017). Reductions in hippocampal BDNF are seen in human depression (Chen et al., 2001) and associated with the BDNF-Val66Met and 5-HTTLPR risk alleles and reduced 5-HT1A receptor function (Chen et al., 2006; Homberg et al., 2014). Yet, the effect of these polymorphisms on response to SSRI treatment in human depression remains controversial (Domschke et al., 2010).

The 5-HT system also has a modulatory effect on long-term synaptic plasticity of glutamatergic neurotransmission underlying LTP and/or LTD in learning and memory, with no changes in glutamate level (Fernandez et al., 2017). In the amygdala, 5-HT release and activation of 5-HT1A heteroreceptors induced a reduction in excitatory synaptic transmission followed by a 5-HT4 receptor-mediated potentiation (Huang and Kandel, 2007). Conditioned fear stress increases 5-HT levels in the amygdala and mediates LTP via 5-HT1A receptor activation (Yokoyama et al., 2005; Johansen et al., 2011). 5-HT1A receptor activation reduces EPSPs in several brain regions by activities such as down-regulation of NR2B receptors in cortical pyramidal neurons (Yuen et al., 2005) or reductions of AMPA currents and surface expression of GluR2/3 receptors (Schiapparelli et al., 2005). Thus, 5-HT1A heteroreceptors can alter plasticity through a variety of mechanisms including regulation of glutamate receptors, synapse formation, alterations in 5-HT projections. The ability to target these post-synaptic signaling mechanisms could enhance response to SSRI treatment (**Figure 1**).

In addition, 5-HT neurons themselves undergo neuroplasticity in response to chronic antidepressant treatment. In particular, chronic deep brain stimulation of the prefrontal cortex increases social interaction in chronic social defeat mice. Cortical stimulation was associated with recovery of 5-HT neuron firing activity, reduction of 5-HT neuron dendritic length and branching, increased glutamatergic synapses in the DR, and recovery of 5-HT synaptic density and/or size in the PFC, hippocampus and amygdala (Veerakumar et al., 2014). Thus chronic brain stimulation can induce 5-HT plasticity both in the raphe and in its projections to target regions.

5-HT1A-Mediated Neurogenesis and Neuroplasticity

In addition to regulating neuroplasticity, 5-HT1A receptors have been implicated in adult hippocampal neurogenesis (Santarelli et al., 2003; Banasr et al., 2004; Klempin et al., 2010). Direct activation of 5-HT1A receptors using 8OH-DPAT increases progenitor cell proliferation, which is blocked by 5-HT1A antagonist WAY100635 (Banasr et al., 2004; Klempin et al., 2010) or an inhibitor of ERK1/2 signaling (Cai et al., 2019). The effects of chronic SSRI treatment on hippocampal neurogenesis and anxiety- or depression-like behavior are blocked using 5-HT1A antagonists (Klempin et al., 2010) or by knockout of 5-HT1A receptors, globally or specifically on granule cells of the hippocampus (Santarelli et al., 2003; Samuels et al., 2015) (**Figure 2**). These findings suggest that the behavioral effects of chronic SSRI treatment are mediated by stimulation of hippocampal neurogenesis, which requires 5-HT1A heteroreceptors. On the other hand, mice with over-expression of 5-HT1A heteroreceptors show increased adult neurogenesis (in females but not males) (Noto et al., 2016), suggesting that 5-HT1A receptor levels can drive increase in hippocampal neurogenesis. However, knockout models of loss of 5-HT1A receptors or of 5-HT did not alter basal neurogenesis (Santarelli et al., 2003; Diaz et al., 2013). In addition, the role of 5-HT1A receptors in adult hippocampal neurogenesis in humans has not been addressed. The extent of hippocampal neurogenesis in adult humans, though supported by solid evidence, remains difficult to assess (Kempermann et al., 2018). Directly targeting 5-HT1A-induced neurogenesis could bypass resistance to SSRIs associated with reduced activity of 5-HT neurons (e.g., due to increase 5-HT1A autoreceptors, **Figure 2**).

Thus, the therapeutic action of antidepressants is dependent on a balanced activity of 5-HT1A auto vs. heteroreceptors (Samuels et al., 2015) (**Figure 2**). Activation of 5-HT1A autoreceptors reduces 5-HT activity and response to SSRI treatment. Activation of 5-HT1A heteroreceptors stimulates hippocampal neurogenesis and regulates dendritic maturation

in the hippocampus and frontal cortex associated with the antidepressant response (Samuels et al., 2015). Consistent with roles in neuroplasticity and neurogenesis, levels of 5-HT1A auto- and heteroreceptors appear to correlate oppositely with cortical gray matter thickness. Multimodal imaging studies show that in MDD patients, an increase in raphe 5-HT1A binding potential is correlated with reduced cortical thickness values and fewer 5-HT tracts projecting to the cortex, while increased terminal 5-HT1A receptors correlate with increased gray matter volume in several cortical and hippocampal regions (Kraus et al., 2012; Zanderigo et al., 2018). Therefore, higher activity of 5-HT1A autoreceptors residing on serotoninergic raphe cells puts the brakes on 5-HT neurotransmission in target areas and affects their synaptic plasticity.

SEROTONIN, ANTIDEPRESSANTS, AND DEPRESSION

Clinical Studies

Several lines of evidence have implicated reduced 5-HT as a key risk factor for major depression. The current first-line therapy for major depression targets the 5-HT system (Trivedi et al., 2006b). In particular, selective 5-HT reuptake inhibitors (SSRIs), such as fluoxetine or citalopram, specifically block SERT to selectively increase 5-HT neurotransmission. This selectivity for 5-HT results in less severe adverse effects compared to tricyclic antidepressants like imipramine that also target noradrenalin reuptake (Cipriani et al., 2016, 2018) and can block alpha1-adrenergic receptors, inducing orthostatic hypotension in some patients (Glassman, 1984). Chronic treatment with SSRI was shown to be effective in major depression, anxiety, and several other mood disorders, implicating serotonin (Charney et al., 1990). Acute tryptophan depletion to acutely reduce 5-HT, also supports a role for decreased 5-HT in depression, or at least in the relapse of recovered depressed patients (Leyton et al., 1997; Delgado et al., 1999; Young and Leyton, 2002; Jans et al., 2007). 5-HT and its metabolites are reduced in the cerebrospinal fluid of depressed patients and especially of depressed suicide victims (Asberg and Traskman, 1981; Asberg, 1997; Mann and Malone, 1997; Moberg et al., 2011). Reductions cortical 5-HT receptor levels are also seen in PET imaging studies of living depressed patients and in post-mortem studies (Yatham et al., 2000; Mintun et al., 2004; Berney et al., 2008; Savitz and Drevets, 2009; Hesselgrave and Parsey, 2013; An et al., 2016). Several studies have found that normal women have significantly higher 5-HT1A receptor (Parsey et al., 2002; Jovanovic et al., 2008) and lower 5-HTT binding potentials (Jovanovic et al., 2008; Underwood et al., 2018) than men, in raphe and several cortical regions. By contrast in male but not female MDD, 5-HT1A autoreceptors were increased compared to controls (Kaufman et al., 2015), while females showed reduced PFC 5-HT1A receptors (Szewczyk et al., 2009). In post-mortem studies, decreases in cortical 5-HT1A receptor RNA and binding site levels are observed (Lopez-Figueroa et al., 2004; Stockmeier et al., 2009; Szewczyk et al., 2009), but in some areas increases are seen (Anisman et al., 2008). Oppositely, increased levels of 5-HT1A autoreceptor binding have been reported in the DR, particularly in the rostral DR (Stockmeier et al., 1998; Boldrini et al., 2008). Similarly in PET imaging studies, reductions in cortical 5-HT1A heteroreceptors and increases in raphe 5-HT1A autoreceptors have been found in depressed subjects compared to controls (Savitz et al., 2009; Hesselgrave and Parsey, 2013; Kautzky et al., 2017; Milak et al., 2018). These results suggest a reduced activity of the 5-HT system driven by increased 5-HT1A autoreceptors and/or reduced 5-HT1A heteroreceptors may predispose to MDD. In agreement, functional variants affecting gene expression in the 5-HT system, such as the 5-HTTLPR and 5-HT1A rs6295 polymorphisms have been implicated in disease susceptibility and response to antidepressants (Serretti et al., 2007; Le Francois et al., 2008; Newman-Tancredi and Albert, 2012; Pettitt, 2015). However, these markers alone are not robust enough to predict response to SSRI treatment (Kato et al., 2015), which remains insufficient. The STAR*D study showed that only one-third of patients given the SSRI citalopram as first-line treatment achieved remission and that about 10–15% more responded to combination therapy (Trivedi et al., 2006b). Therefore, in many patients, targeting the serotonin system or other monoamine systems is insufficient for benefit. Patients who fail two or three types of treatments are classified as treatment-resistant (Rush et al., 2009). Identifying animal models that can be used to address mechanisms of treatment resistance and how to overcome it remains a major challenge (O'Leary et al., 2014; Willner and Belzung, 2015).

Rodent Models of SSRI Resistance

Several transgenic and knockout mouse models indicate the role of dis-inhibition of the 5-HT system in response to SSRI treatment (**Figure 2**). A common theme is that 5-HT1A autoreceptor-mediated inhibition of 5-HT neurons prevents behavioral responses to SSRI treatment (Artigas et al., 1996; Blier and Ward, 2003). First, the time course of 5-HT1A autoreceptor desensitization follows the latency for clinical response to SSRI and other antidepressants, suggesting that reduced 5-HT auto-inhibition is required for SSRI response (Blier and Ward, 2003). Second, pharmacological inhibition [e.g., using pindolol (Blier and Ward, 2003)] or inducible repression of 5-HT1A autoreceptors in adult 5-HT neurons was shown to enhance and accelerate response to SSRI treatment (Richardson-Jones et al., 2010). Similarly, acute down-regulation of 5-HT1A autoreceptors using 5-HT1A-siRNA targeted to 5-HT neurons induces an acute antidepressant response in rats (Bortolozzi et al., 2012). However, mice with more extensive knockdown (in 90% of 5-HT neurons) of 5-HT1A autoreceptors results in an anxiogenic response to sub-chronic (9 d) SSRI treatment in both sexes, which may be due to hyper-activation of the 5-HT system (Turcotte-Cardin et al., 2019). Third, targeted gene deletion of a repressor of the 5-HT1A receptor gene (Freud-1) in adult 5-HT neurons to up-regulate 5-HT1A autoreceptors, reduces 5-HT neuronal activity and results in a fluoxetine-resistant anxiety and depression phenotype in both male and female mice (Vahid-Ansari et al., 2017). Taken together, these different rodent models implicate 5-HT1A autoreceptors in SSRI resistance (**Figure 2**).

In contrast to 5-HT1A autoreceptors, specific deletion of hippocampal granule cell 5-HT1A heteroreceptors prevents SSRI/CC2D1A-induced hippocampal neurogenesis and antidepressant actions (Samuels et al., 2015) (**Figure 2**). Similarly, mice with global knockout of 5-HT1A receptors are also resistant to SSRI treatment (Santarelli et al., 2003). However, chronic desipramine, which targets 5-HT and noradrenalin transporters, reversed the behavioral phenotype (Santarelli et al., 2003). Thus, while 5-HT1A autoreceptors inhibit SSRI response, 5-HT1A heteroreceptors in the hippocampus are required for SSRI actions. Antidepressants that target a different system such as desipramine, can still mediate the behavioral response. This provides empirical support for the concept that patients that do not respond to SSRIs should be switched to a different class of antidepressant (Blier, 2014). However, patients do not entirely lack 5-HT1A receptors and may still respond to augmentation with drugs that inhibit or desensitize 5-HT1A autoreceptors, such as buspirone or pindolol (Artigas et al., 2018). Despite promising results showing that pindolol augmentation could accelerate and enhance SSRI response patients (Artigas et al., 1994; Blier and Bergeron, 1995; Portella et al., 2009), not all studies have shown benefit, especially in SSRI-resistant cohorts (McAskill et al., 1998; Berman et al., 1999; Perez et al., 1999). This could reflect inadequate dosing (Martinez et al., 2000), non-selective effects of pindolol on both 5-HT1A auto- and heteroreceptors (Newman-Tancredi et al., 2001), or antagonism of beta-adrenergic receptors. Partial block of postsynaptic 5-HT1A receptors by pindolol may prevent pindolol's benefits due to block of 5-HT1A autoreceptors, emphasizing the need for compounds with higher selectivity to block presynaptic or activate post-synaptic 5-HT1A receptors (Garcia-Garcia et al., 2014; Vidal et al., 2018).

Given the sex differences in depression prevalence and 5-HT1A receptor levels in humans, a few studies have addressed sex differences in transgenic models of 5-HT1A receptor regulation. For example, knockout of the HTR1A repressor Deaf1 results in increased 5-HT1A autoreceptor expression but greater functional uncoupling in females, and a sex- and test-dependent anxiety phenotype (Luckhart et al., 2016). Similarly knockout in adult 5-HT neurons of MeCP2, an enhancer Deaf1 repressor activity, also increased 5-HT1A receptor expression, resulting in a similar sex- and test-dependent anxiety and depression phenotypes (Philippe et al., 2018). In contrast, knockout in 5-HT neurons of the stronger repressor Freud-1 resulted in a consistent anxiety and depression phenotype in all tests (Vahid-Ansari et al., 2017). The extent of up-regulation of 5-HT1A autoreceptors in the latter model may overwhelm endogenous sex-dependent differences to result in SSRI-resistant anxiety and depression. Similarly, over-expression of 5-HT1A heteroreceptors mainly in cortical regions reduced depression-like behavior in males but not females, yet both responded equally to SSRI treatment (Gunther et al., 2011).

Another mouse model of SSRI resistance is the BDNF Val66Met mouse, in which the human BDNF gene and polymorphism was knocked-in to the mouse locus, which phenocopies humans with this polymorphism (Chen et al., 2006). The BDNF (Met) mice display an anxiety-like phenotype that is resistant to chronic FLX (Chen et al., 2006), ketamine (Liu et al., 2012) or voluntary exercise (Ieraci et al., 2016). However, the heterozygous BDNF (+/Met) mice that fail to respond to FLX responded to chronic desipramine (Yu et al., 2012). Clinically, antidepressants targeting multiple neurotransmitter systems, such as imipramine that targets 5-HT and noradrenalin, appear more effective than SSRIs, but may also have lower acceptability and compliance rates (Cipriani et al., 2016, 2018). An alternative strategy to directly reverse BDNF deficiency is intranasal administration of a viral brain-permeant BDNF construct, which reversed the depression phenotype in mice following chronic mild stress (Ma et al., 2016).

Taken together, these animal model studies implicate alterations in 5-HT and BDNF in resistance to SSRI treatment. Patients with polymorphisms (5-HTTLPR, 5-HT1A rs6295, BDNF Val66Met) that alter the function of key genes in these systems may be more resistant to SSRI treatment, but responsive to treatments that target other systems (noradrenalin) or that can augment 5-HT activity to permit SSRI action (**Figure 1**). Still, there is no reliable marker to predict which treatment option would be best.

HOW TO IMPROVE TREATMENT FOR MAJOR DEPRESSION?

Markers for SSRI Response

Although SSRIs remain the first-line treatment for MDD, due to the low remission rate (30% of patients remit) and long latency, many patients must be switched to other antidepressants, or given augmentation to try to enhance SSRI response. In the STAR*D study, patients not responding to SSRI were switched to another antidepressant or augmented with add-on treatments, leading to around 50–60% remission rate and 40% treatment resistant patients failing to respond to two chronically used common antidepressants (Rush et al., 2009). Citalopram non-responders switched to another SSRI (27% response) or a different class of antidepressant (25–6% response) had similar response rates (**Figure 1**). Thus, there was no clear indication of which treatment would be best for a given patient who fails to respond or remit on SSRI treatment.

Resistance to SSRIs appears to be associated with genetic polymorphisms in 5-HT-related genes, like the 5-HTT (SLC6A4) and HTR1A genes. For example, the HTR1A rs6295 polymorphism has been associated with depression, suicide and SSRI resistance (Le Francois et al., 2008). Other gene polymorphisms including BDNF (rs10501087 and Val66Met rs6265), 5HT2A receptor (rs7997012) and CREB1 (rs7569963) have been shown to interact with each other in predicting SSRI resistance (Kautzky et al., 2015). Among those, BDNF rs6265 has been the focus of many studies with the most robust association with SSRI response (Niitsu et al., 2013). More recently, progress has been made in using functional MRI (fMRI) of resting state functional connectivity to identify depression subtypes and subjects that are resistant to SSRI or cognitive-behavioral therapy (Dunlop et al., 2017), and responsive to targeted repetitive transcranial

magnetic stimulation (rTMS) (Drysdale et al., 2017). In a small cohort of treatment-resistant patients, deep brain stimulation (DBS) targeting a hub of the depression circuitry [subgenual cingulate gyrus (Cg25)] improved negative mood and depressive illness (Kennedy et al., 2011). However, stimulation of the subcallosal cingulate white matter was ineffective (Holtzheimer et al., 2017), suggesting that further study is required.

Recently, altered left-right asymmetry in electro-encephalogram (EEG) has been suggested to predict response to the SSRI escitalopram, with elevated alpha and reduced delta power in the right hemisphere predicting non-response (Baskaran et al., 2018). Interestingly, the HTR1A rs6295 risk polymorphism is also associated with EEG asymmetry with greater right frontal activity (Bismark et al., 2010), and has also been associated with resistance to SSRI treatment. The EEG approach has also been applied to detect increased 5-HT1A autoreceptor activity in response to buspirone in a small cohort of depressed compared to normal controls (McAllister-Williams et al., 2014), and might provide a non-invasive marker of resistance to SSRI treatment due to increase in 5-HT1A autoreceptor function.

Ketamine and Serotonin

In clinical research studies, there has been a large interest in the effects of single sub-anesthetic dose of infused ketamine (0.5 mg/kg, in 40 min) in treatment resistant depression patients (Zarate et al., 2006, 2012). However, after single infusion the antidepressant response was not sustained. Further studies showed that repeated twice-weekly ketamine treatment maintains the antidepressant response for up to 2 weeks (Singh et al., 2016). In control male volunteers, ketamine-induced interneuron inhibition is associated with increased synaptic glutamate in the anterior cingulate cortex (Stone et al., 2012). Therefore, region-dependent effects of ketamine may be associated with the efficacy of ketamine to treat TRD.

The exact mechanisms of ketamine action remain unclear, with the recent finding that ketamine metabolites without NMDA blocking activity exert antidepressant actions (Zanos et al., 2016). Low dose ketamine is thought to block NMDA receptors preferentially on GABAergic interneurons to enhance AMPA receptor signaling to release to mTOR to increase synaptogenesis in prefrontal cortical pyramidal neurons (Zanos and Gould, 2018). Ketamine metabolites appear to directly activate AMPA signaling to trigger this pathway to induce the rapid (1 h) antidepressant actions of ketamine. Recent studies in rodents implicate the 5-HT system in ketamine action (Nishitani et al., 2014; du Jardin et al., 2016; Fukumoto et al., 2016; Pham et al., 2017). In particular, ketamine increases 5-HT levels in prefrontal cortex (Pham et al., 2017), and mediates antidepressant-like activity in the forced swimming test assayed 24 h post-treatment via activation of PFC 5-HT1A heteroreceptors (Fukumoto et al., 2016, 2018). This sustained antidepressant-like activity requires activation of raphe AMPA receptors that recruits the prefrontal cortex neural circuit (Pham et al., 2017; Pham and Gardier, 2019). Furthermore, AMPA receptor-dependent 5-HT release and activation of PFC 5-HT1A receptors mediates the antidepressant actions of an mGlu2/3 receptor antagonist in the forced swim test (24 h), via 5-HT1A signaling to Akt-mTOR (Fukumoto et al., 2016, 2018). Given the role of 5-HT in the sustained actions of ketamine and mGluR2/3 antagonists, it would be interesting to test their efficacy in SSRI-resistant mouse models.

CONCLUSION

A multi-factorial disease like depression cannot always be managed using a one-dimensional strategy, such as targeting the 5-HT system. While SSRIs may be effective in some patients initially, epigenetic and environmental factors can prevent or gradually erode the response to treatment (Albert and Lemonde, 2004). This review illustrates how region-specific activation of 5-HT mechanisms including synthesis, 5-HT neuronal activity, 5-HT neuroplasticity, and 5-HT-induced hippocampal neurogenesis could augment SSRI response (**Figure 1**). This activation could be mediated by targeted brain stimulation to regions with abnormal brain activity in imaging (Drysdale et al., 2017; Dunlop et al., 2017) or EEG analysis (Baskaran et al., 2018). Furthermore, strategies that overcome 5-HT1A-mediated autoinhibition of 5-HT activity may also overcome resistance to SSRIs, and could include augmentation with 5-HT1A partial agonists or switching to drugs that target additional or other systems such as noradrenalin, dopamine or glutamate (**Figure 1**). However, there remain no clear biomarkers that can predict resistance to antidepressants, or whether augmentation or switching antidepressants is best. By testing responses in SSRI resistant models that mimic or phenocopy human genetic polymorphisms such as 5-HT1A autoreceptor over-expression or BDNF Val66Met genotype, progress is being made to understand the mechanistic underpinnings of SSRI resistance. In addition to providing potential markers, such as risk polymorphisms or imaging changes, a mechanistic understanding is providing novel ways of targeting the 5-HT system, such as SSRI-conjugated siRNAs to target 5-HT neurons (Bortolozzi et al., 2012). Ultimately, non-invasive methods to identify treatment-resistance may lead to novel strategies that combine brain stimulation with neurotransmitter modulation to accelerate and enhance antidepressant response (Tendler et al., 2018).

AUTHOR CONTRIBUTIONS

FV-A and PA conceived the content. FV-A, AZ, and MZ wrote the first draft. PA revised the final draft. All authors approved the final draft.

FUNDING

Supported by CIHR grants MOP115098 and MOP123426 to PA.

REFERENCES

Adayev, T., El-Sherif, Y., Barua, M., Penington, N. J., and Banerjee, P. (1999). Agonist stimulation of the serotonin1A receptor causes suppression of anoxia-induced apoptosis via mitogen-activated protein kinase in neuronal HN2-5 cells. *J. Neurochem.* 72, 1489–1496.

Albert, P. R. (2012). Transcriptional regulation of the 5-HT1A receptor: implications for mental illness. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2402–2415. doi: 10.1098/rstb.2011.0376

Albert, P. R., and Francois, B. L. (2010). Modifying 5-HT1A receptor gene expression as a new target for antidepressant therapy. *Front. Neurosci.* 4:35. doi: 10.3389/fnins.2010.00035

Albert, P. R., and Lemonde, S. (2004). 5-HT1A receptors, gene repression, and depression: guilt by association. *Neuroscientist* 10, 575–593.

Albert, P. R., Neve, K. A., Bunzow, J. R., and Civelli, O. (1990). Coupling of a cloned rat dopamine-D2 receptor to inhibition of adenylyl cyclase and prolactin secretion. *J. Biol. Chem.* 265, 2098–2104.

Albert, P. R., Sajedi, N., Lemonde, S., and Ghahremani, M. H. (1999). Constitutive G(i2)-dependent activation of adenylyl cyclase type II by the 5-HT1A receptor. Inhibition by anxiolytic partial agonists. *J. Biol. Chem.* 274, 35469–35474.

Albert, P. R., and Vahid-Ansari, F. (2018). The 5-HT1A receptor: signaling to behavior. *Biochimie* (in press). doi: 10.1016/j.biochi.2018.10.015

Albert, P. R., Vahid-Ansari, F., and Luckhart, C. (2014). Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT1A receptor expression. *Front. Behav. Neurosci.* 8:199. doi: 10.3389/fnbeh.2014.00199

Amara, S. G., and Kuhar, M. J. (1993). Neurotransmitter transporters: recent progress. *Annu. Rev. Neurosci.* 16, 73–93.

An, Y., Chen, C., Inoue, T., Nakagawa, S., Kitaichi, Y., Wang, C., et al. (2016). Mirtazapine exerts an anxiolytic-like effect through activation of the median raphe nucleus-dorsal hippocampal 5-HT pathway in contextual fear conditioning in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 70, 17–23. doi: 10.1016/j.pnpbp.2016.04.014

Anisman, H., Du, L., Palkovits, M., Faludi, G., Kovacs, G. G., Szontagh-Kishazi, P., et al. (2008). Serotonin receptor subtype and p11 mRNA expression in stress-relevant brain regions of suicide and control subjects. *J. Psychiatry Neurosci.* 33, 131–141.

Arrant, A. E., Coburn, E., Jacobsen, J., and Kuhn, C. M. (2013). Lower anxiogenic effects of serotonin agonists are associated with lower activation of amygdala and lateral orbital cortex in adolescent male rats. *Neuropharmacology* 73, 359–367. doi: 10.1016/j.neuropharm.2013.05.030

Artigas, F. (2013). Serotonin receptors involved in antidepressant effects. *Pharmacol. Ther.* 137, 119–131. doi: 10.1016/j.pharmthera.2012.09.006

Artigas, F., Bortolozzi, A., and Celada, P. (2018). Can we increase speed and efficacy of antidepressant treatments? Part I: general aspects and monoamine-based strategies. *Eur. Neuropsychopharmacol.* 28, 445–456. doi: 10.1016/j.euroneuro.2017.10.032

Artigas, F., Perez, V., and Alvarez, E. (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch. Gen. Psychiatry* 51, 248–251.

Artigas, F., Romero, L., de Montigny, C., and Blier, P. (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci.* 19, 378–383.

Asberg, M. (1997). Neurotransmitters and suicidal behavior. The evidence from cerebrospinal fluid studies. *Ann. N. Y. Acad. Sci.* 836, 158–181.

Asberg, M., and Traskman, L. (1981). Studies of CSF 5-HIAA in depression and suicidal behaviour. *Adv. Exp. Med. Biol.* 133, 739–752.

Assie, M. B., Bardin, L., Auclair, A. L., Carilla-Durand, E., Depoortere, R., Koek, W., et al. (2010). F15599, a highly selective post-synaptic 5-HT(1A) receptor agonist: in-vivo profile in behavioural models of antidepressant and serotonergic activity. *Int. J. Neuropsychopharmacol.* 13, 1285–1298. doi: 10.1017/S1461145709991222

Azmitia, E. C. (1999). Serotonin neurons, neuroplasticity, and homeostasis of neural tissue. *Neuropsychopharmacology* 21, 33S–45S.

Azmitia, E. C., and Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* 179, 641–667.

Azmitia, E. C., Singh, J. S., Hou, X. P., and Wegiel, J. (2011a). Dystrophic serotonin axons in postmortem brains from young autism patients. *Anat. Rec.* 294, 1653–1662. doi: 10.1002/ar.21243

Azmitia, E. C., Singh, J. S., and Whitaker-Azmitia, P. M. (2011b). Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology* 60, 1347–1354. doi: 10.1016/j.neuropharm.2011.02.002

Bach-Mizrachi, H., Underwood, M. D., Tin, A., Ellis, S. P., Mann, J. J., and Arango, V. (2008). Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. *Mol. Psychiatry* 13, 813–820. doi: 10.1038/sj.mp.4002143

Banasr, M., Hery, M., Printemps, R., and Daszuta, A. (2004). Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology* 29, 450–460.

Bang, S. J., Jensen, P., Dymecki, S. M., and Commons, K. G. (2012). Projections and interconnections of genetically defined serotonin neurons in mice. *Eur. J. Neurosci.* 35, 85–96. doi: 10.1111/j.1460-9568.2011.07936.x

Barnes, N. M., and Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38, 1083–1152.

Baskaran, A., Farzan, F., Milev, R., Brenner, C. A., Alturi, S., Pat McAndrews, M., et al. (2018). The comparative effectiveness of electroencephalographic indices in predicting response to escitalopram therapy in depression: a pilot study. *J. Affect. Disord.* 227, 542–549. doi: 10.1016/j.jad.2017.10.028

Batsikadze, G., Paulus, W., Kuo, M. F., and Nitsche, M. A. (2013). Effect of serotonin on paired associative stimulation-induced plasticity in the human motor cortex. *Neuropsychopharmacology* 38, 2260–2267. doi: 10.1038/npp.2013.127

Baxter, A. J., Scott, K. M., Ferrari, A. J., Norman, R. E., Vos, T., and Whiteford, H. A. (2014). Challenging the myth of an "epidemic" of common mental disorders: trends in the global prevalence of anxiety and depression between 1990 and 2010. *Depress. Anxiety* 31, 506–516. doi: 10.1002/da.22230

Beck, S. G., Pan, Y. Z., Akanwa, A. C., and Kirby, L. G. (2004). Median and dorsal raphe neurons are not electrophysiologically identical. *J. Neurophysiol.* 91, 994–1005.

Becker, G., Bolbos, R., Costes, N., Redoute, J., Newman-Tancredi, A., and Zimmer, L. (2016). Selective serotonin 5-HT1A receptor biased agonists elicit distinct brain activation patterns: a pharmacoMRI study. *Sci. Rep.* 6:26633.

Beliveau, V., Ganz, M., Feng, L., Ozenne, B., Hojgaard, L., Fisher, P. M., et al. (2017). A high-resolution in vivo atlas of the human brain's serotonin system. *J. Neurosci.* 37, 120–128. doi: 10.1523/JNEUROSCI.2830-16.2016

Belmer, A., Klenowski, P. M., Patkar, O. L., and Bartlett, S. E. (2017). Mapping the connectivity of serotonin transporter immunoreactive axons to excitatory and inhibitory neurochemical synapses in the mouse limbic brain. *Brain Struct. Funct.* 222, 1297–1314. doi: 10.1007/s00429-016-1278-x

Belmer, A., Patkar, O. L., Pitman, K. M., and Bartlett, S. E. (2016). Serotonergic neuroplasticity in alcohol addiction. *Brain Plast.* 1, 177–206.

Bennett, D. S., and Giarman, N. J. (1965). Schedule of appearance of 5-hydroxy-tryptamine (serotonin) and associated enzymes in the developing rat brain. *J. Neurochem.* 12, 911–918.

Berman, R. M., Anand, A., Cappiello, A., Miller, H. L., Hu, X. S., Oren, D. A., et al. (1999). The use of pindolol with fluoxetine in the treatment of major depression: final results from a double-blind, placebo-controlled trial. *Biol. Psychiatry* 45, 1170–1177.

Berney, A., Nishikawa, M., Benkelfat, C., Debonnel, G., Gobbi, G., and Diksic, M. (2008). An index of 5-HT synthesis changes during early antidepressant treatment: alpha-[(11)C]methyl-l-tryptophan PET study. *Neurochem. Int.* 52, 701–708.

Bismark, A. W., Moreno, F. A., Stewart, J. L., Towers, D. N., Coan, J. A., Oas, J., et al. (2010). Polymorphisms of the HTR1a allele are linked to frontal brain electrical asymmetry. *Biol. Psychol.* 83, 153–158. doi: 10.1016/j.biopsycho.2009.12.002

Blier, P. (2014). Rational site-directed pharmacotherapy for major depressive disorder. *Int. J. Neuropsychopharmacol.* 17, 997–1008. doi: 10.1017/S1461145713000400

Blier, P., and Bergeron, R. (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J. Clin. Psychopharmacol.* 15, 217–222.

Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., and de Montigny, C. (1998). Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann. N. Y. Acad. Sci.* 861, 204–216.

Blier, P., and Ward, N. M. (2003). Is there a role for 5-HT(1A) agonists in the treatment of depression? *Biol. Psychiatry* 53, 193–203.

Boldrini, M., Underwood, M. D., Mann, J. J., and Arango, V. (2008). Serotonin-1A autoreceptor binding in the dorsal raphe nucleus of depressed suicides. *J. Psychiatr. Res.* 42, 433–442.

Bortolozzi, A., Castane, A., Semakova, J., Santana, N., Alvarado, G., Cortes, R., et al. (2012). Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. *Mol. Psychiatry* 17, 612–623. doi: 10.1038/mp.2011.92

Cai, C. Y., Wu, H. Y., Luo, C. X., Zhu, D. Y., Zhang, Y., Zhou, Q. G., et al. (2019). Extracellular regulated protein kinaseis critical for the role of 5-HT1a receptor in modulating nNOS expression and anxiety-related behaviors. *Behav. Brain Res.* 357–358, 88–97. doi: 10.1016/j.bbr.2017.12.017

Calizo, L. H., Akanwa, A., Ma, X., Pan, Y. Z., Lemos, J. C., Craige, C., et al. (2011). Raphe serotonin neurons are not homogenous: electrophysiological, morphological and neurochemical evidence. *Neuropharmacology* 61, 524–543.

Castren, E., and Hen, R. (2013). Neuronal plasticity and antidepressant actions. *Trends Neurosci.* 36, 259–267. doi: 10.1016/j.tins.2012.12.010

Celada, P., Puig, M. V., and Artigas, F. (2013). Serotonin modulation of cortical neurons and networks. *Front. Integr. Neurosci.* 7:25. doi: 10.3389/fnint.2013.00025

Charney, D. S., Krystal, J. H., Delgado, P. L., and Heninger, G. R. (1990). Serotonin-specific drugs for anxiety and depressive disorders. *Annu. Rev. Med.* 41, 437–446.

Chemel, B. R., Roth, B. L., Armbruster, B., Watts, V. J., and Nichols, D. E. (2006). WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology* 188, 244–251.

Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F., and Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry* 50, 260–265.

Chen, W. V., Nwakeze, C. L., Denny, C. A., O'Keeffe, S., Rieger, M. A., and Mountoufaris, G. (2017). Pcdhalphac2 is required for axonal tiling and assembly of serotonergic circuitries in mice. *Science* 356, 406–411. doi: 10.1126/science.aal3231

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C. J., et al. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314, 140–143.

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., et al. (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet* 391, 1357–1366. doi: 10.1016/S0140-6736(17)32802-7

Cipriani, A., Zhou, X., Del Giovane, C., Hetrick, S. E., Qin, B., Whittington, C., et al. (2016). Comparative efficacy and tolerability of antidepressants for major depressive disorder in children and adolescents: a network meta-analysis. *Lancet* 388, 881–890. doi: 10.1016/S0140-6736(16)30385-3

Ciranna, L. (2006). Serotonin as a modulator of glutamate- and GABA-mediated neurotransmission: implications in physiological functions and in pathology. *Curr. Neuropharmacol.* 4, 101–114.

Commons, K. G. (2016). Ascending serotonin neuron diversity under two umbrellas. *Brain Struct. Funct.* 221, 3347–3360. doi: 10.1007/s00429-015-1176-7

Coppen, A. (1967). The biochemistry of affective disorders. *Br. J. Psychiatry* 113, 1237–1264.

Correia, P. A., Lottem, E., Banerjee, D., Machado, A. S., Carey, M. R., and Mainen, Z. F. (2017). Transient inhibition and long-term facilitation of locomotion by phasic optogenetic activation of serotonin neurons. *eLife* 6:e20975. doi: 10.7554/eLife.20975

Cowen, P. J. (2008). Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends Pharmacol. Sci.* 29, 433–436. doi: 10.1016/j.tips.2008.05.004

Daubert, E. A., and Condron, B. G. (2010). Serotonin: a regulator of neuronal morphology and circuitry. *Trends Neurosci.* 33, 424–434. doi: 10.1016/j.tins.2010.05.005

Deguchi, T., and Barchas, J. (1972). Regional distribution and developmental change of tryptophan hydroxylase activity in rat brain. *J. Neurochem.* 19, 927–929.

Delgado, P. L., Miller, H. L., Salomon, R. M., Licinio, J., Krystal, J. H., Moreno, F. A., et al. (1999). Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol. Psychiatry* 46, 212–220.

De-Miguel, F. F., and Trueta, C. (2005). Synaptic and extrasynaptic secretion of serotonin. *Cell. Mol. Neurobiol.* 25, 297–312.

Descarries, L., Beaudet, A., and Watkins, K. C. (1975). Serotonin nerve terminals in adult rat neocortex. *Brain Res.* 100, 563–588.

Diaz, S. L., Narboux-Neme, N., Trowbridge, S., Scotto-Lomassese, S., Kleine Borgmann, F. B., Jessberger, S., et al. (2013). Paradoxical increase in survival of newborn neurons in the dentate gyrus of mice with constitutive depletion of serotonin. *Eur. J. Neurosci.* 38, 2650–2658. doi: 10.1111/ejn.12297

Dinopoulos, A., Dori, I., and Parnavelas, J. G. (1997). The serotonin innervation of the basal forebrain shows a transient phase during development. *Brain Res. Dev. Brain Res.* 99, 38–52.

Domschke, K., Lawford, B., Laje, G., Berger, K., Young, R., Morris, P., et al. (2010). Brain-derived neurotrophic factor (BDNF) gene: no major impact on antidepressant treatment response. *Int. J. Neuropsychopharmacol.* 13, 93–101. doi: 10.1017/S1461145709000030

Donaldson, Z. R., Piel, D. A., Santos, T. L., Richardson-Jones, J., Leonardo, E. D., Beck, S. G., et al. (2014). Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior. *Neuropsychopharmacology* 39, 291–302. doi: 10.1038/npp.2013.185

Dori, I., Dinopoulos, A., Blue, M. E., and Parnavelas, J. G. (1996). Regional differences in the ontogeny of the serotonergic projection to the cerebral cortex. *Exp. Neurol.* 138, 1–14.

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., et al. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat. Med.* 23, 28–38. doi: 10.1038/nm.4246

D'Sa, C., and Duman, R. S. (2002). Antidepressants and neuroplasticity. *Bipolar Disord.* 4, 183–194.

du Jardin, K. G., Muller, H. K., Elfving, B., Dale, E., Wegener, G., and Sanchez, C. (2016). Potential involvement of serotonergic signaling in ketamine's antidepressant actions: a critical review. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 71, 27–38. doi: 10.1016/j.pnpbp.2016.05.007

Dugue, G. P., Lorincz, M. L., Lottem, E., Audero, E., Matias, S., Correia, P. A., et al. (2014). Optogenetic recruitment of dorsal raphe serotonergic neurons acutely decreases mechanosensory responsivity in behaving mice. *PLoS One* 9:e105941. doi: 10.1371/journal.pone.0105941

Dunlop, B. W., Rajendra, J. K., Craighead, W. E., Kelley, M. E., McGrath, C. L., Choi, K. S., et al. (2017). Functional connectivity of the subcallosal cingulate cortex and differential outcomes to treatment with cognitive-behavioral therapy or antidepressant medication for major depressive disorder. *Am. J. Psychiatry* 174, 533–545. doi: 10.1176/appi.ajp.2016.16050518

Ehlinger, D. G., and Commons, K. G. (2019). Cav1.2 L-type calcium channels regulate stress coping behavior via serotonin neurons. *Neuropharmacology* 144, 282–290. doi: 10.1016/j.neuropharm.2018.08.033

Fernandez, S. P., Cauli, B., Cabezas, C., Muzerelle, A., Poncer, J. C., and Gaspar, P. (2016). Multiscale single-cell analysis reveals unique phenotypes of raphe 5-HT neurons projecting to the forebrain. *Brain Struct. Funct.* 221, 4007–4025.

Fernandez, S. P., Muzerelle, A., Scotto-Lomassese, S., Barik, J., Gruart, A., Delgado-Garcia, J. M., et al. (2017). Constitutive and acquired serotonin deficiency alters memory and hippocampal synaptic plasticity. *Neuropsychopharmacology* 42, 512–523. doi: 10.1038/npp.2016.134

Fletcher, A., Forster, E. A., Bill, D. J., Brown, G., Cliffe, I. A., Hartley, J. E., et al. (1996). Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT1A receptor antagonist. *Behav. Brain Res.* 73, 337–353.

Fonseca, M. S., Murakami, M., and Mainen, Z. F. (2015). Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. *Curr. Biol.* 25, 306–315. doi: 10.1016/j.cub.2014.12.002

Fukumoto, K., Iijima, M., and Chaki, S. (2016). The antidepressant effects of an mGlu2/3 receptor antagonist and ketamine require AMPA receptor stimulation in the mPFC and subsequent activation of the 5-HT neurons in the DRN. *Neuropsychopharmacology* 41, 1046–1056. doi: 10.1038/npp.2015.233

Fukumoto, K., Iijima, M., Funakoshi, T., and Chaki, S. (2018). Role of 5-HT1A receptor stimulation in the medial prefrontal cortex in the sustained antidepressant effects of ketamine. *Int. J. Neuropsychopharmacol.* 21, 371–381. doi: 10.1093/ijnp/pyx116

Fuxe, K., Dahlstrom, A., Hoistad, M., Marcellino, D., Jansson, A., and Rivera, A. (2007). From the Golgi-Cajal mapping to the transmitter-based characterization of the neuronal networks leading to two modes of brain communication: wiring and volume transmission. *Brain Res. Rev.* 55, 17–54.

Gagnon, D., and Parent, M. (2014). Distribution of VGLUT3 in highly collateralized axons from the rat dorsal raphe nucleus as revealed by single-neuron reconstructions. *PLoS One* 9:e87709. doi: 10.1371/journal.pone.0087709

Garcia-Garcia, A. L., Canetta, S., Stujenske, J. M., Burghardt, N. S., Ansorge, M. S., Dranovsky, A., et al. (2017a). Serotonin inputs to the dorsal BNST modulate anxiety in a 5-HT1A receptor-dependent manner. *Mol. Psychiatry* 23, 1990–1997. doi: 10.1038/mp.2017.165

Garcia-Garcia, A. L., Meng, Q., Canetta, S., Gardier, A. M., Guiard, B. P., Kellendonk, C., et al. (2017b). Serotonin signaling through prefrontal cortex 5-HT1A receptors during adolescence can determine baseline mood-related behaviors. *Cell Rep.* 18, 1144–1156. doi: 10.1016/j.celrep.2017.01.021

Garcia-Garcia, A. L., Newman-Tancredi, A., and Leonardo, E. D. (2014). 5-HT(1A) receptors in mood and anxiety: recent insights into autoreceptor versus heteroreceptor function. *Psychopharmacology* 231, 623–636. doi: 10.1007/s00213-013-3389-x

Gaspar, P., Cases, O., and Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* 4, 1002–1012.

Gaspar, P., and Lillesaar, C. (2012). Probing the diversity of serotonin neurons. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2382–2394. doi: 10.1098/rstb.2011.0378

Gingrich, J. A., Malm, H., Ansorge, M. S., Brown, A., Sourander, A., Suri, D., et al. (2017). New insights into how serotonin selective reuptake inhibitors shape the developing brain. *Birth Defects Res.* 109, 924–932. doi: 10.1002/bdr2.1085

Glassman, A. H. (1984). Cardiovascular effects of tricyclic antidepressants. *Annu. Rev. Med.* 35, 503–511.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., et al. (2002). Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 416, 396–400.

Gunther, L., Rothe, J., Rex, A., Voigt, J. P., Millan, M. J., Fink, H., et al. (2011). 5-HT(1A)-receptor over-expressing mice: genotype and sex dependent responses to antidepressants in the forced swim-test. *Neuropharmacology* 61, 433–441. doi: 10.1016/j.neuropharm.2011.03.004

Hammack, S. E., Richey, K. J., Schmid, M. J., LoPresti, M. L., Watkins, L. R., and Maier, S. F. (2002). The role of corticotropin-releasing hormone in the dorsal raphe nucleus in mediating the behavioral consequences of uncontrollable stress. *J. Neurosci.* 22, 1020–1026.

Hesselgrave, N., and Parsey, R. V. (2013). Imaging the serotonin 1A receptor using [11C]WAY100635 in healthy controls and major depression. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368:20120004. doi: 10.1098/rstb.2012.0004

Holtzheimer, P. E., Husain, M. M., Lisanby, S. H., Taylor, S. F., Whitworth, L. A., McClintock, S., et al. (2017). Subcallosal cingulate deep brain stimulation for treatment-resistant depression: a multisite, randomised, sham-controlled trial. *Lancet Psychiatry* 4, 839–849. doi: 10.1016/S2215-0366(17)30371-1

Homberg, J. R., Molteni, R., Calabrese, F., and Riva, M. A. (2014). The serotonin-BDNF duo: developmental implications for the vulnerability to psychopathology. *Neurosci. Biobehav. Rev.* 43, 35–47. doi: 10.1016/j.neubiorev.2014.03.012

Hoyer, D., Hannon, J. P., and Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* 71, 533–554.

Huang, Y. Y., and Kandel, E. R. (2007). 5-Hydroxytryptamine induces a protein kinase A/mitogen-activated protein kinase-mediated and macromolecular synthesis-dependent late phase of long-term potentiation in the amygdala. *J. Neurosci.* 27, 3111–3119.

Ieraci, A., Madaio, A. I., Mallei, A., Lee, F. S., and Popoli, M. (2016). Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. *Neuropsychopharmacology* 41, 3070–3079. doi: 10.1038/npp.2016.120

Jacobs, B. L., and Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiol. Rev.* 72, 165–229.

Jacobsen, K. X., Czesak, M., Deria, M., Le Francois, B., and Albert, P. R. (2011). Region-specific regulation of 5-HT1A receptor expression by Pet-1-dependent mechanisms in vivo. *J. Neurochem.* 116, 1066–1076. doi: 10.1111/j.1471-4159.2010.07161.x

Jans, L. A., Riedel, W. J., Markus, C. R., and Blokland, A. (2007). Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. *Mol. Psychiatry* 12, 522–543.

Jin, H. J., Pei, L., Li, Y. N., Zheng, H., Yang, S., and Wan, Y. (2017). Alleviative effects of fluoxetine on depressive-like behaviors by epigenetic regulation of BDNF gene transcription in mouse model of post-stroke depression. *Sci. Rep.* 7:14926. doi: 10.1038/s41598-017-13929-5

Johansen, J. P., Cain, C. K., Ostroff, L. E., and LeDoux, J. E. (2011). Molecular mechanisms of fear learning and memory. *Cell* 147, 509–524. doi: 10.1016/j.cell.2011.10.009

Jovanovic, H., Lundberg, J., Karlsson, P., Cerin, A., Saijo, T., Varrone, A., et al. (2008). Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *Neuroimage* 39, 1408–1419.

Kato, M., Serretti, A., Nonen, S., Takekita, Y., Wakeno, M., Azuma, J., et al. (2015). Genetic variants in combination with early partial improvement as a clinical utility predictor of treatment outcome in major depressive disorder: the result of two pooled RCTs. *Transl. Psychiatry* 5:e513. doi: 10.1038/tp.2015.6

Katori, S., Hamada, S., Noguchi, Y., Fukuda, E., Yamamoto, T., Yamamoto, H., et al. (2009). Protocadherin-alpha family is required for serotonergic projections to appropriately innervate target brain areas. *J. Neurosci.* 29, 9137–9147. doi: 10.1523/JNEUROSCI.5478-08.2009

Kaufman, J., Sullivan, G. M., Yang, J., Ogden, R. T., Miller, J. M., Oquendo, M. A., et al. (2015). Quantification of the serotonin 1A receptor using PET: identification of a potential biomarker of major depression in males. *Neuropsychopharmacology* 40, 1692–1699. doi: 10.1038/npp.2015.15

Kautzky, A., Baldinger, P., Souery, D., Montgomery, S., Mendlewicz, J., Zohar, J., et al. (2015). The combined effect of genetic polymorphisms and clinical parameters on treatment outcome in treatment-resistant depression. *Eur. Neuropsychopharmacol.* 25, 441–453. doi: 10.1016/j.euroneuro.2015.01.001

Kautzky, A., James, G. M., Philippe, C., Baldinger-Melich, P., Kraus, C., and Kranz, G. S. (2017). The influence of the rs6295 gene polymorphism on serotonin-1A receptor distribution investigated with PET in patients with major depression applying machine learning. *Transl. Psychiatry* 7:e1150. doi: 10.1038/tp.2017.108

Kempermann, G., Gage, F. H., Aigner, L., Song, H., Curtis, M. A., Thuret, S., et al. (2018). Human adult neurogenesis: evidence and remaining questions. *Cell Stem Cell* 23, 25–30. doi: 10.1016/j.stem.2018.04.004

Kennedy, S. H., Giacobbe, P., Rizvi, S. J., Placenza, F. M., Nishikawa, Y., Mayberg, H. S., et al. (2011). Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. *Am. J. Psychiatry* 168, 502–510.

Kessler, R. C., and Bromet, E. J. (2013). The epidemiology of depression across cultures. *Annu. Rev. Public Health* 34, 119–138. doi: 10.1146/annurev-publhealth-031912-114409

Kirby, L. G., Allen, A. R., and Lucki, I. (1995). Regional differences in the effects of forced swimming on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Res.* 682, 189–196.

Kiss, J. P. (2008). Theory of active antidepressants: a nonsynaptic approach to the treatment of depression. *Neurochem. Int.* 52, 34–39.

Kiyasova, V., and Gaspar, P. (2011). Development of raphe serotonin neurons from specification to guidance. *Eur. J. Neurosci.* 34, 1553–1562. doi: 10.1111/j.1460-9568.2011.07910.x

Klempin, F., Babu, H., De Pietri Tonelli, D., Alarcon, E., Fabel, K., and Kempermann, G. (2010). Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front. Mol. Neurosci.* 3:14. doi: 10.3389/fnmol.2010.00014

Kraus, C., Castren, E., Kasper, S., and Lanzenberger, R. (2017). Serotonin and neuroplasticity - Links between molecular, functional and structural pathophysiology in depression. *Neurosci. Biobehav. Rev.* 77, 317–326. doi: 10.1016/j.neubiorev.2017.03.007

Kraus, C., Hahn, A., Savli, M., Kranz, G. S., Baldinger, P., Hoflich, A., et al. (2012). Serotonin-1A receptor binding is positively associated with gray matter

volume – a multimodal neuroimaging study combining PET and structural MRI. *Neuroimage* 63, 1091–1098. doi: 10.1016/j.neuroimage.2012.07.035

Kushwaha, N., and Albert, P. R. (2005). Coupling of 5-HT1A autoreceptors to inhibition of mitogen-activated protein kinase activation via Gbetagamma subunit signaling. *Eur. J. Neurosci.* 21, 721–732.

Le Francois, B., Czesak, M., Steubl, D., and Albert, P. R. (2008). Transcriptional regulation at a HTR1A polymorphism associated with mental illness. *Neuropharmacology* 55, 977–985. doi: 10.1016/j.neuropharm.2008.06.046

Lehmann, H. E., Cahn, C. H., and De Verteuil, R. L. (1958). The treatment of depressive conditions with imipramine (G 22355). *Can. Psychiatr. Assoc. J.* 3, 155–164.

Lesch, K. P., Araragi, N., Waider, J., van den Hove, D., and Gutknecht, L. (2012). Targeting brain serotonin synthesis: insights into neurodevelopmental disorders with long-term outcomes related to negative emotionality, aggression and antisocial behaviour. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2426–2443. doi: 10.1098/rstb.2012.0039

Leyton, M., Young, S. N., Blier, P., Ellenbogen, M. A., Palmour, R. M., Ghadirian, A. M., et al. (1997). The effect of tryptophan depletion on mood in medication-free, former patients with major affective disorder. *Neuropsychopharmacology* 16, 294–297.

Liu, C., Maejima, T., Wyler, S. C., Casadesus, G., Herlitze, S., and Deneris, E. S. (2010). Pet-1 is required across different stages of life to regulate serotonergic function. *Nat. Neurosci.* 13, 1190–1198. doi: 10.1038/nn.2623

Liu, R. J., Lambe, E. K., and Aghajanian, G. K. (2005). Somatodendritic autoreceptor regulation of serotonergic neurons: dependence on L-tryptophan and tryptophan hydroxylase-activating kinases. *Eur. J. Neurosci.* 21, 945–958.

Liu, R. J., Lee, F. S., Li, X. Y., Bambico, F., Duman, R. S., and Aghajanian, G. K. (2012). Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol. Psychiatry* 71, 996–1005. doi: 10.1016/j.biopsych.2011.09.030

Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., and Ma, M. (2014). Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron* 81, 1360–1374. doi: 10.1016/j.neuron.2014.02.010

Lo Iacono, L., and Gross, C. (2008). Alpha-Ca2+/calmodulin-dependent protein kinase II contributes to the developmental programming of anxiety in serotonin receptor 1A knock-out mice. *J. Neurosci.* 28, 6250–6257. doi: 10.1523/JNEUROSCI.5219-07.2008

Lopez-Figueroa, A. L., Norton, C. S., Lopez-Figueroa, M. O., Armellini-Dodel, D., Burke, S., Akil, H., et al. (2004). Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. *Biol. Psychiatry* 55, 225–233.

Luckhart, C., Philippe, T. J., Le Francois, B., Vahid-Ansari, F., Geddes, S. D., Beique, J. C., et al. (2016). Sex-dependent adaptive changes in serotonin-1A autoreceptor function and anxiety in Deaf1-deficient mice. *Mol. Brain* 9:77. doi: 10.1186/s13041-016-0254-y

Lucki, I. (1996). Serotonin receptor specificity in anxiety disorders. *J. Clin. Psychiatry* 57, 5–10.

Ma, X. C., Liu, P., Zhang, X. L., Jiang, W. H., Jia, M., Wang, C. X., et al. (2016). Intranasal delivery of recombinant AAV containing BDNF fused with HA2TAT: a potential promising therapy strategy for major depressive disorder. *Sci. Rep.* 6:22404. doi: 10.1038/srep22404

Maddaloni, G., Bertero, A., Pratelli, M., Barsotti, N., Boonstra, A., Giorgi, A., et al. (2017). Development of serotonergic fibers in the post-natal mouse brain. *Front. Cell. Neurosci.* 11:202. doi: 10.3389/fncel.2017.00202

Mann, J. J., and Malone, K. M. (1997). Cerebrospinal fluid amines and higher-lethality suicide attempts in depressed inpatients. *Biol. Psychiatry* 41, 162–171.

Marcinkiewcz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., et al. (2016). Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature* 537, 97–101. doi: 10.1038/nature19318

Martinez, D., Mawlawi, O., Hwang, D., Kent, J., Simpson, N., Parsey, R. V., et al. (2000). Positron emission tomography study of pindolol occupancy of 5-HT(1A) receptors in humans: preliminary analyses. *Nucl. Med. Biol.* 27, 523–527.

Maya Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., et al. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* 320, 385–388. doi: 10.1126/science.1150516

McAllister-Williams, R. H., Alhaj, H. A., Massey, A., Pankiv, J., and Reckermann, U. (2014). Somatodendritic 5-hydroxytryptamine1A (5-HT1A) autoreceptor function in major depression as assessed using the shift in electroencephalographic frequency spectrum with buspirone. *Psychol. Med.* 44, 767–777. doi: 10.1017/S0033291713001475

McAskill, R., Mir, S., and Taylor, D. (1998). Pindolol augmentation of antidepressant therapy. *Br. J. Psychiatry* 173, 203–208.

Milak, M. S., Pantazatos, S., Rashid, R., Zanderigo, F., DeLorenzo, C., and Hesselgrave, N. (2018). Higher 5-HT1A autoreceptor binding as an endophenotype for major depressive disorder identified in high risk offspring - A pilot study. *Psychiatry Res. Neuroimaging* 276, 15–23. doi: 10.1016/j.pscychresns.2018.04.002

Mintun, M. A., Sheline, Y. I., Moerlein, S. M., Vlassenko, A. G., Huang, Y., and Snyder, A. Z. (2004). Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. *Biol. Psychiatry* 55, 217–224.

Moberg, T., Nordstrom, P., Forslund, K., Kristiansson, M., Asberg, M., and Jokinen, J. (2011). CSF 5-HIAA and exposure to and expression of interpersonal violence in suicide attempters. *J. Affect. Disord.* 132, 173–178. doi: 10.1016/j.jad.2011.01.018

Mogha, A., Guariglia, S. R., Debata, P. R., Wen, G. Y., and Banerjee, P. (2012). Serotonin 1A receptor-mediated signaling through ERK and PKCalpha is essential for normal synaptogenesis in neonatal mouse hippocampus. *Transl. Psychiatry* 2:e66. doi: 10.1038/tp.2011.58

Nautiyal, K. M., and Hen, R. (2017). Serotonin receptors in depression: from A to B. *F1000Res.* 6:123. doi: 10.12688/f1000research.9736.1

Newman-Tancredi, A., and Albert, P. R. (2012). "Gene polymorphism at serotonin 5-HT1A receptors: moving towards personalized medicine for psychosis and mood deficits," in *Schizophrenia Research: Recent Advances*, ed. T. Sumiyoshi (New York, NY: Nova Publishers), 337–358.

Newman-Tancredi, A., Chaput, C., Touzard, M., and Millan, M. J. (2001). Pindolol antagonises G-protein activation at both pre- and postsynaptic serotonin 5-HT1A receptors: a [35S]GTPγS autoradiography study. *Naunyn Schmiedebergs Arch. Pharmacol.* 363, 391–398.

Niitsu, T., Fabbri, C., Bentini, F., and Serretti, A. (2013). Pharmacogenetics in major depression: a comprehensive meta-analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 45, 183–194. doi: 10.1016/j.pnpbp.2013.05.011

Nishitani, N., Nagayasu, K., Asaoka, N., Yamashiro, M., Shirakawa, H., Nakagawa, T., et al. (2014). Raphe AMPA receptors and nicotinic acetylcholine receptors mediate ketamine-induced serotonin release in the rat prefrontal cortex. *Int. J. Neuropsychopharmacol.* 17, 1321–1326. doi: 10.1017/S1461145714000649

Noto, B., Klempin, F., Alenina, N., Bader, M., Fink, H., and Sander, S. E. (2016). Increased adult neurogenesis in mice with a permanent overexpression of the postsynaptic 5-HT1A receptor. *Neurosci. Lett.* 633, 246–251. doi: 10.1016/j.neulet.2016.09.051

Ohmura, Y., Tanaka, K. F., Tsunematsu, T., Yamanaka, A., and Yoshioka, M. (2014). Optogenetic activation of serotonergic neurons enhances anxiety-like behaviour in mice. *Int. J. Neuropsychopharmacol.* 17, 1777–1783. doi: 10.1017/S1461145714000637

Okaty, B. W., Freret, M. E., Rood, B. D., Brust, R. D., Hennessy, M. L., deBairos, D., et al. (2015). Multi-scale molecular deconstruction of the serotonin neuron system. *Neuron* 88, 774–791. doi: 10.1016/j.neuron.2015.10.007

O'Leary, O. F., O'Brien, F. E., O'Connor, R. M., and Cryan, J. F. (2014). Drugs, genes and the blues: pharmacogenetics of the antidepressant response from mouse to man. *Pharmacol. Biochem. Behav.* 123, 55–76. doi: 10.1016/j.pbb.2013.10.015

Parsey, R. V., Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., et al. (2002). Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res.* 954, 173–182.

Pauwels, P. J., and Colpaert, F. C. (2003). Ca2+ responses in Chinese hamster ovary-K1 cells demonstrate an atypical pattern of ligand-induced 5-HT1A receptor activation. *J. Pharmacol. Exp. Ther.* 307, 608–614.

Perez, V., Soler, J., Puigdemont, D., Alvarez, E., and Artigas, F. (1999). A double-blind, randomized, placebo-controlled trial of pindolol augmentation in depressive patients resistant to serotonin reuptake inhibitors. Grup de Recerca en Trastorns Afectius. *Arch. Gen. Psychiatry* 56, 375–379.

Pettitt, A. (2015). Genetic variations in the serotonergic system mediate a combined, weakened response to SSRI treatment: a proposed model. *eNeuro* 2:ENEURO.0032-14.2015. doi: 10.1523/ENEURO.0032-14.2015

Pham, T. H., and Gardier, A. M. (2019). Fast-acting antidepressant activity of ketamine: highlights on brain serotonin, glutamate, and GABA neurotransmission in preclinical studies. *Pharmacol. Ther.* doi: 10.1016/j.pharmthera.2019.02.017

Pham, T. H., Mendez-David, I., Defaix, C., Guiard, B. P., Tritschler, L., David, D. J., et al. (2017). Ketamine treatment involves medial prefrontal cortex serotonin to induce a rapid antidepressant-like activity in BALB/cJ mice. *Neuropharmacology* 112, 198–209. doi: 10.1016/j.neuropharm.2016.05.010

Philippe, T. J., Vahid-Ansari, F., Donaldson, Z. R., Le Francois, B., Zahrai, A., Turcotte-Cardin, V., et al. (2018). Loss of MeCP2 in adult 5-HT neurons induces 5-HT1A autoreceptors, with opposite sex-dependent anxiety and depression phenotypes. *Sci. Rep.* 8:5788. doi: 10.1038/s41598-018-24167-8

Pletscher, A. (1991). The discovery of antidepressants: a winding path. *Experientia* 47, 4–8.

Pollak Dorocic, I., Furth, D., Xuan, Y., Johansson, Y., Pozzi, L., Silberberg, G., et al. (2014). A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. *Neuron* 83, 663–678. doi: 10.1016/j.neuron.2014.07.002

Polter, A. M., and Li, X. (2010). 5-HT1A receptor-regulated signal transduction pathways in brain. *Cell. Signal.* 22, 1406–1412. doi: 10.1016/j.cellsig.2010.03.019

Polter, A. M., Yang, S., Jope, R. S., and Li, X. (2012). Functional significance of glycogen synthase kinase-3 regulation by serotonin. *Cell. Signal.* 24, 265–271. doi: 10.1016/j.cellsig.2011.09.009

Portella, M. J., de Diego-Adelino, J., Puigdemont, D., Perez-Egea, R., Alvarez, E., Artigas, F., et al. (2009). Pindolol augmentation enhances response outcomes in first depressive episodes. *Eur. Neuropsychopharmacol.* 19, 516–519. doi: 10.1016/j.euroneuro.2009.04.004

Pratelli, M., Migliarini, S., Pelosi, B., Napolitano, F., Usiello, A., and Pasqualetti, M. (2017). Perturbation of serotonin homeostasis during adulthood affects serotonergic neuronal circuitry. *eNeuro* 4:ENEURO.0376-16.2017. doi: 10.1523/ENEURO.0376-16.2017

Quentin, E., Belmer, A., and Maroteaux, L. (2018). Somato-dendritic regulation of raphe serotonin neurons; a key to antidepressant action. *Front. Neurosci.* 12:982. doi: 10.3389/fnins.2018.00982

Rajkowska, G., Mahajan, G., Legutko, B., Challagundla, L., Griswold, M., and Albert, P. R. (2017). Length of axons expressing the serotonin transporter in orbitofrontal cortex is lower with age in depression. *Neuroscience* 359, 30–39. doi: 10.1016/j.neuroscience.2017.07.006

Ramaker, M. J., and Dulawa, S. C. (2017). Identifying fast-onset antidepressants using rodent models. *Mol. Psychiatry* 22, 656–665. doi: 10.1038/mp.2017.36

Raymond, J. R., Mukhin, Y. V., Gettys, T. W., and Garnovskaya, M. N. (1999). The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.* 127, 1751–1764.

Ren, J., Friedmann, D., Xiong, J., Liu, C. D., Ferguson, B. R., Weerakkody, T., et al. (2018). Anatomically defined and functionally distinct dorsal raphe serotonin sub-systems. *Cell* 175:e20. doi: 10.1016/j.cell.2018.07.043

Riad, M., Watkins, K. C., Doucet, E., Hamon, M., and Descarries, L. (2001). Agonist-induced internalization of serotonin-1a receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). *J. Neurosci.* 21, 8378–8386.

Riccio, O., Jacobshagen, M., Golding, B., Vutskits, L., Jabaudon, D., Hornung, J. P., et al. (2011). Excess of serotonin affects neocortical pyramidal neuron migration. *Transl. Psychiatry* 1:e47. doi: 10.1038/tp.2011.49

Riccio, O., Potter, G., Walzer, C., Vallet, P., Szabo, G., Vutskits, L., et al. (2009). Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. *Mol. Psychiatry* 14, 280–290. doi: 10.1038/mp.2008.89

Richardson-Jones, J. W., Craige, C. P., Guiard, B. P., Stephen, A., Metzger, K. L., Kung, H. F., et al. (2010). 5-HT(1A) autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* 65, 40–52. doi: 10.1016/j.neuron.2009.12.003

Rush, A. J., Warden, D., Wisniewski, S. R., Fava, M., Trivedi, M. H., Gaynes, B. N., et al. (2009). STAR*D: revising conventional wisdom. *CNS Drugs* 23, 627–647. doi: 10.2165/00023210-200923080-00001

Samuels, B. A., Anacker, C., Hu, A., Levinstein, M. R., Pickenhagen, A., and Tsetsenis, T. (2015). 5-HT1A receptors on mature dentate gyrus granule cells are critical for the antidepressant response. *Nat. Neurosci.* 18, 1606–1616. doi: 10.1038/nn.4116

Samuels, B. A., Mendez-David, I., Faye, C., David, S. A., Pierz, K. A., Gardier, A. M., et al. (2016). Serotonin 1A and serotonin 4 receptors: essential mediators of the neurogenic and behavioral actions of antidepressants. *Neuroscientist* 22, 26–45. doi: 10.1177/1073858414561303

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809.

Savitz, J., Lucki, I., and Drevets, W. C. (2009). 5-HT(1A) receptor function in major depressive disorder. *Prog. Neurobiol.* 88, 17–31. doi: 10.1016/j.pneurobio.2009.01.009

Savitz, J. B., and Drevets, W. C. (2009). Imaging phenotypes of major depressive disorder: genetic correlates. *Neuroscience* 164, 300–330. doi: 10.1016/j.neuroscience.2009.03.082

Schiapparelli, L., Del Rio, J., and Frechilla, D. (2005). Serotonin 5-HT receptor blockade enhances Ca(2+)/calmodulin-dependent protein kinase II function and membrane expression of AMPA receptor subunits in the rat hippocampus: implications for memory formation. *J. Neurochem.* 94, 884–895.

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am. J. Psychiatry* 122, 509–522.

Serretti, A., Kato, M., De Ronchi, D., and Kinoshita, T. (2007). Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Mol. Psychiatry* 12, 247–257.

Singh, J. B., Fedgchin, M., Daly, E. J., De Boer, P., Cooper, K., Lim, P., et al. (2016). A double-blind, randomized, placebo-controlled, dose-frequency study of intravenous ketamine in patients with treatment-resistant depression. *Am. J. Psychiatry* 173, 816–826. doi: 10.1176/appi.ajp.2016.16010037

Stockmeier, C. A., Howley, E., Shi, X., Sobanska, A., Clarke, G., Friedman, L., et al. (2009). Antagonist but not agonist labeling of serotonin-1A receptors is decreased in major depressive disorder. *J. Psychiatr. Res.* 43, 887–894. doi: 10.1016/j.jpsychires.2009.01.001

Stockmeier, C. A., Shapiro, L. A., Dilley, G. E., Kolli, T. N., Friedman, L., and Rajkowska, G. (1998). Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J. Neurosci.* 18, 7394–7401.

Stone, J. M., Dietrich, C., Edden, R., Mehta, M. A., De Simoni, S., Reed, L. J., et al. (2012). Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Mol. Psychiatry* 17, 664–665.

Szewczyk, B., Albert, P. R., Burns, A. M., Czesak, M., Overholser, J. C., and Jurjus, G. J. (2009). Gender-specific decrease in NUDR and 5-HT1A receptor proteins in the prefrontal cortex of subjects with major depressive disorder. *Int. J. Neuropsychopharmacol.* 12, 155–168. doi: 10.1017/S1461145708009012

Talbot, J. N., Jutkiewicz, E. M., Graves, S. M., Clemans, C. F., Nicol, M. R., Mortensen, R. M., et al. (2010). RGS inhibition at G(alpha)i2 selectively potentiates 5-HT1A-mediated antidepressant effects. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11086–11091. doi: 10.1073/pnas.1000003107

Teissier, A., Chemiakine, A., Inbar, B., Bagchi, S., Ray, R. S., Palmiter, R. D., et al. (2015). Activity of raphe serotonergic neurons controls emotional behaviors. *Cell Rep.* 13, 1965–1976. doi: 10.1016/j.celrep.2015.10.061

Teissier, A., Soiza-Reilly, M., and Gaspar, P. (2017). Refining the role of 5-HT in postnatal development of brain circuits. *Front. Cell. Neurosci.* 11:139. doi: 10.3389/fncel.2017.00139

Tendler, A., Gersner, R., Roth, Y., and Zangen, A. (2018). Alternate day dTMS combined with SSRIs for chronic treatment resistant depression: a prospective multicenter study. *J. Affect. Disord.* 240, 130–136. doi: 10.1016/j.jad.2018.07.058

Trivedi, M. H., Fava, M., Wisniewski, S. R., Thase, M. E., Quitkin, F., and Warden, D. (2006a). Medication augmentation after the failure of SSRIs for depression. *N. Engl. J. Med.* 354, 1243–1252.

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006b). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28–40.

Turcotte-Cardin, V., Vahid-Ansari, F., Luckhart, C., Daigle, M., Geddes, S. D., and Tanaka, K. F. (2019). Loss of adult 5-HT1A autoreceptors results in a

paradoxical anxiogenic response to antidepressant treatment. *J. Neurosci.* 39, 1334–1346. doi: 10.1523/JNEUROSCI.0352-18.2018

Underwood, M. D., Kassir, S. A., Bakalian, M. J., Galfalvy, H., Dwork, A. J., Mann, J. J., et al. (2018). Serotonin receptors and suicide, major depression, alcohol use disorder and reported early life adversity. *Transl. Psychiatry* 8:279. doi: 10.1038/s41398-018-0309-1

Ungerstedt, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand. Suppl.* 367, 1–48.

Vahid-Ansari, F., Daigle, M., Manzini, M. C., Tanaka, K. F., Hen, R., Geddes, S. D., et al. (2017). Abrogated freud-1/Cc2d1a repression of 5-HT1A autoreceptors induces fluoxetine-resistant anxiety/depression-like behavior. *J. Neurosci.* 37, 11967–11978. doi: 10.1523/JNEUROSCI.1668-17.2017

Veerakumar, A., Challis, C., Gupta, P., Da, J., Upadhyay, A., Beck, S. G., et al. (2014). Antidepressant-like effects of cortical deep brain stimulation coincide with pro-neuroplastic adaptations of serotonin systems. *Biol. Psychiatry* 76, 203–212. doi: 10.1016/j.biopsych.2013.12.009

Vidal, B., Fieux, S., Redoute, J., Villien, M., Bonnefoi, F., Le Bars, D., et al. (2018). In vivo biased agonism at 5-HT1A receptors: characterisation by simultaneous PET/MR imaging. *Neuropsychopharmacology* 43, 2310–2319. doi: 10.1038/s41386-018-0145-2

Wallace, J. A., and Lauder, J. M. (1983). Development of the serotonergic system in the rat embryo: an immunocytochemical study. *Brain Res. Bull.* 10, 459–479.

Walther, D. J., Peter, J. U., Bashammakh, S., Hortnagl, H., Voits, M., Fink, H., et al. (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 299:76.

Waselus, M., Galvez, J. P., Valentino, R. J., and Van Bockstaele, E. J. (2006). Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum. *J. Chem. Neuroanat.* 31, 233–242.

Weissbourd, B., Ren, J., DeLoach, K. E., Guenthner, C. J., Miyamichi, K., and Luo, L. (2014). Presynaptic partners of dorsal raphe serotonergic and GABAergic neurons. *Neuron* 83, 645–662. doi: 10.1016/j.neuron.2014.06.024

Willner, P., and Belzung, C. (2015). Treatment-resistant depression: are animal models of depression fit for purpose? *Psychopharmacology* 232, 3473–3495. doi: 10.1007/s00213-015-4034-7

World Health Organization [WHO] (2017). *Depression and Other Common Mental Disorders: Global Health Estimates*. Geneva: World Health Organization.

Yatham, L. N., Liddle, P. F., Shiah, I. S., Scarrow, G., Lam, R. W., Adam, M. J., et al. (2000). Brain serotonin2 receptors in major depression: a positron emission tomography study. *Arch. Gen. Psychiatry* 57, 850–858.

Yokoyama, M., Suzuki, E., Sato, T., Maruta, S., Watanabe, S., and Miyaoka, H. (2005). Amygdalic levels of dopamine and serotonin rise upon exposure to conditioned fear stress without elevation of glutamate. *Neurosci. Lett.* 379, 37–41.

Young, S. N., and Leyton, M. (2002). The role of serotonin in human mood and social interaction. Insight from altered tryptophan levels. *Pharmacol. Biochem. Behav.* 71, 857–865.

Yu, H., Wang, D. D., Wang, Y., Liu, T., Lee, F. S., and Chen, Z. Y. (2012). Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. *J. Neurosci.* 32, 4092–4101. doi: 10.1523/JNEUROSCI.5048-11.2012

Yuen, E. Y., Jiang, Q., Chen, P., Gu, Z., Feng, J., and Yan, Z. (2005). Serotonin 5-HT1A receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. *J. Neurosci.* 25, 5488–5501.

Zanderigo, F., Pantazatos, S., Rubin-Falcone, H., Ogden, R. T., Chhetry, B. T., Sullivan, G., et al. (2018). In vivo relationship between serotonin 1A receptor binding and gray matter volume in the healthy brain and in major depressive disorder. *Brain Struct. Funct.* 223, 2609–2625. doi: 10.1007/s00429-018-1649-6

Zanos, P., and Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Mol. Psychiatry* 23, 801–811. doi: 10.1038/mp.2017.255

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* 533, 481–486. doi: 10.1038/nature17998

Zarate, C. A. Jr., Brutsche, N. E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., et al. (2012). Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol. Psychiatry* 71, 939–946. doi: 10.1016/j.biopsych.2011.12.010

Zarate, C. A. Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856–864.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Vahid-Ansari, Zhang, Zahrai and Albert. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership