



# Glycogen synthase kinase-3 is an intermediate modulator of serotonin neurotransmission

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Serotonin is a neurotransmitter with broad functions in brain development, neuronal activity, and behaviors; and serotonin is the prominent drug target in several major neuropsychiatric diseases. The multiple actions of serotonin are mediated by diverse serotonin receptor subtypes and associated signaling pathways. However, the key signaling components that mediate specific function of serotonin neurotransmission have not been fully identified. This review will provide evidence from biochemical, pharmacological, and animal behavioral studies showing that serotonin regulates the activation states of brain glycogen synthase kinase-3 (GSK3) via type 1 and type 2 serotonin receptors. In return, GSK3 directly interacts with serotonin receptors in a highly selective manner, with a prominent effect on modulating serotonin 1B receptor activity. Therefore, GSK3 acts as an intermediate modulator in the serotonin neurotransmission system, and balanced GSK3 activity is essential for serotonin-regulated brain function and behaviors. Particularly important, several classes of serotonin-modulating drugs, such as antidepressants and atypical antipsychotics, regulate GSK3 by inhibiting its activity in brain, which reinforces the importance of GSK3 as a potential therapeutic target in neuropsychiatric diseases associated with abnormal serotonin function.

**Keywords:** serotonin, GSK3, 5-HT1A, 5-HT1B, 5-HT2A, antidepressants

## SEROTONIN NEUROTRANSMISSION

### SEROTONIN

Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic neurotransmitter that is synthesized from the amino acid tryptophan. Serotonergic neurons arise from the raphe nucleus of the brain stem, and they project upward to most areas of the brain and downward to peripheral nerve terminals. Upon release, 5-HT in the synapse is recycled by reuptake through the 5-HT transporter (5-HTT) and is catabolized by monoamine oxidase (MAO). There are at least 7 families and 14 subtypes of 5-HT receptors that are classified by their sequence homology and associated type of G-proteins and signal transduction pathways (Hoyer and Martin, 1997; Bockaert et al., 2006; **Table 1**). Among these 5-HT receptors, the type 1 (5-HT1) and the type 2 (5-HT2) receptors are the most studied, with rich literatures showing their roles in brain function and diseases (Hannon and Hoyer, 2008).

### SEROTONIN TYPE 1A (5-HT1A) RECEPTORS

5-HT1A receptors are the prototypical 5-HT1 receptors. In addition to coupling to the inhibitory G-protein (Gi) that inhibits adenylyl cyclase, decreases cyclic AMP (cAMP) production, and inactivates protein kinase A (PKA; De Vivo and Maayani, 1986), studies in neurons reveal that 5-HT1A receptors also regulate other protein kinases, such as growth factor-associated Akt and extracellular signal-regulated kinases (Erk; Cowen et al., 1996, 2005). 5-HT1A receptors are widely distributed in major brain areas including the dorsal raphe nucleus, cerebral cortex, hippocampus, striatum, and nucleus accumbens (Varnas et al., 2004). In 5-HT neurons, 5-HT1A autoreceptors are located in the cell bodies and

somatodendrites, and activation of these receptors reduces neuron firing and suppresses 5-HT release (Riad et al., 2000). In non-serotonergic neurons, 5-HT1A heteroreceptors are found in both pre- and post-synaptic locations (Hoyer et al., 2002). Depending on the location and associated neurons, activation of 5-HT1A heteroreceptors modulates neurotransmission of glutamate, GABA, norepinephrine, and acetylcholine (Fink and Gothert, 2007), generally causing inhibition of long-term potentiation (Edagawa et al., 1998; Tachibana et al., 2004), increased axonal and dendritic branching (Yan et al., 1997), and postnatal neurogenesis (Banasz et al., 2004). 5-HT1A receptors mediate many behavioral effects of 5-HT, particularly those related to mood and anxiety (Kennett et al., 1987; Parks et al., 1998).

### SEROTONIN TYPE 1B (5-HT1B) RECEPTORS

The rodent 5-HT1B receptors and its human homolog 5-HT1D receptors share high protein sequence homology and signal transduction processes with 5-HT1A receptors, and 5-HT1B receptors also act as both autoreceptors and heteroreceptors in the brain (Bouhelal et al., 1988; Hoyer and Martin, 1997; Leone et al., 2000; Bockaert et al., 2006). Different from 5-HT1A autoreceptors, 5-HT1B autoreceptors are primarily located on the 5-HT neuron axon terminals that extend to other brain regions. Upon activation, 5-HT1B autoreceptors cause a strong feedback inhibition of 5-HT release (Riad et al., 2000). 5-HT1B receptors also act as heteroreceptors in several brain regions to control release of other neurotransmitters (Sari, 2004). Both 5-HT1B receptor agonists and antagonists may have antidepressant effects; blocking autoreceptors increases extracellular 5-HT which enhances the effect of

**Table 1 | Serotonin receptors.**

Family	Subtypes		Classical signal transduction
5-HT1	1A, 1B, 1D, 1E, 1F	G <sub>i</sub> /G <sub>o</sub> -protein coupled	Inhibit AC, reduce cAMP, inhibit PKA
5-HT2	2A, 2B, 2C	G <sub>q</sub> /G <sub>11</sub> -protein coupled	Increase IP <sub>3</sub> , increase intracellular calcium, increase DAG, activate PKC
5-HT3		Ligand-gated Na <sup>+</sup> and K <sup>+</sup> cation channel	Membrane depolarization
5-HT4		G <sub>s</sub> -protein coupled	Activate AC, increase cAMP, activate PKA
5-HT5	5A, 5B	G <sub>i</sub> /G <sub>o</sub> -protein coupled	Inhibit AC, reduce cAMP, inhibit PKA
5-HT6		G <sub>s</sub> -protein coupled	Activate AC, increase cAMP, activate PKA
5-HT7		G <sub>s</sub> -protein coupled	Activate AC, increase cAMP, activate PKA

serotonin-reuptake inhibitors (Dawson et al., 2006), whereas activation of heteroreceptors contributes to an antidepressant effect, potentially through their effects on dopaminergic neurotransmission (Chenu et al., 2008). Recently, 5-HT1B receptors have been found to interact with an intracellular adaptor protein p11 (Svenningsson et al., 2006), which regulates 5-HT1BR activity and stability, and deletion of which causes depression-like behaviors in animals (Svenningsson and Greengard, 2007).

### SEROTONIN TYPE 2A (5-HT2A) RECEPTORS

5-HT2A receptors are the prototypical type 2 5-HT receptors that couple to G<sub>q</sub> protein to activate phospholipase C (PLC) and its down-stream targets such as protein kinase C (PKC; Conn and Sanders-Bush, 1984; Roth et al., 1986; Takawa et al., 1989). 5-HT2A receptors have also been reported to activate Akt and Erk (Watts, 1998; Johnson-Farley et al., 2005; Zhong et al., 2008), although the receptor-coupling mechanism of this regulation is not completely understood. 5-HT2A receptors also couple to the intracellular scaffolding protein  $\beta$ -arrestin2, which may have unique functions to initiate desensitization of 5-HT2A receptors and to direct ligand-selective signal transduction processes (Schmid et al., 2008; Schmid and Bohn, 2010). 5-HT2A receptors are expressed throughout the brain, most prominently in the cerebral cortex, striatum, and hippocampus (Bockaert et al., 2006), where they are located on soma, dendrites, and axons of pyramidal neurons, interneurons, and monoaminergic neurons (Jakab and Goldman-Rakic, 1998; Cornea-Hebert et al., 1999; Hoyer et al., 2002; Miner et al., 2003). Activation of 5-HT2A receptors modulates levels of other neurotransmitters, such as causing inhibitory control over dopamine release (Schmidt and Fadayel, 1995). In many studies, 5-HT2A receptors are found to counteract the physiological and behavioral effects of 5-HT1A receptors (Marek et al., 2003). For example, Yuen et al. (2008) reported that activation of 5-HT2A/C receptors in prefrontal cortical neurons significantly attenuates the effect of 5-HT1A receptors on NMDA currents and microtubule depolymerization. 5-HT2A receptors have a different behavioral profile as 5-HT1A receptors. Most notably, a group of 5-HT2A receptor agonists are hallucinogens (Nichols, 2004), but 5-HT2A receptors also have significant effects in regulating other neuropsychiatric behaviors, such as mood, cognition, and sleep (Landolt and Wehrle, 2009).

### THERAPEUTIC IMPLICATIONS OF 5-HT MODULATORS

For decades, modulation of 5-HT neurotransmission has been the primary pharmacological target for the treatment of major

neuropsychiatric diseases, particularly depression and anxiety. The monoamine oxidase inhibitors (MAOI) enhance 5-HT neurotransmission by blocking 5-HT metabolism (Youdim and Bakhle, 2006), while tricyclic antidepressants (TCA; Klerman and Cole, 1965), serotonin-selective reuptake inhibitors (SSRI), and serotonin-norepinephrine reuptake inhibitors (SNRI) all enhance synaptic 5-HT action by blocking reuptake of 5-HT. Global enhancement of serotonin neurotransmission may activate all subtypes of serotonin receptors in brain, while each 5-HT receptor subtype has different and specific functions in defined brain regions. Activation of 5-HT1A receptors is thought to contribute to the effect of antidepressants in regulating mood and anxiety (Parks et al., 1998; Ramboz et al., 1998; Leonardo and Hen, 2008; Akimova et al., 2009; Savitz et al., 2009; Polter and Li, 2010; Price and Drevets, 2010), whereas acute activation-induced down-regulation of 5-HT1A autoreceptors may be necessary for the antidepressant and anxiolytic effects during long-term 5-HT reuptake inhibitor treatment (Bluer and Ward, 2003; Albert and Lemonde, 2004). The specific roles of other 5-HT receptor subtype in the antidepressant action remain to be largely unknown.

In addition to enhancing 5-HT neurotransmission for treatment of anxiety and depression, agents with 5-HT2A receptor antagonistic property appear to have a unique role in treating neuropsychiatric diseases. Several antidepressants, including mirtazapine, nefazodone, and trazodone have 5-HT2A receptor antagonistic properties that may contribute to their unique antidepressant effect. Atypical antipsychotics not only are antagonists of dopamine D2 receptors (as with the conventional antipsychotics), but they also block 5-HT2A and 5-HT2C receptors (Meltzer et al., 1989; Markowitz et al., 1999). In addition to treating psychosis, most atypical antipsychotics have indications for bipolar mania, and some have shown efficacy in ameliorating symptoms of depression (Tohen et al., 2003; Calabrese et al., 2005; Berman et al., 2007; Marcus et al., 2008; Bauer et al., 2009). Although the exact mechanism remains to be unknown, facilitating dopamine and norepinephrine neurotransmission (Schmidt and Fadayel, 1995; Zhang et al., 2000) and/or enhancing 5-HT1A receptor action (Marek et al., 2003) may contribute to this therapeutically relevant action of 5-HT2A receptor antagonists.

The brain's serotonergic system is highly complex, in part due to its diffusely distributed multiple receptor subtypes and the signal transduction pathways regulated by these receptors. Therefore, identifying intracellular signaling molecules that direct 5-HT-regulated signals to specific physiological and behavioral effects may facilitate our understanding of brain 5-HT function and its

role in neuropsychiatric diseases. Below we review current findings on the interrelationship between 5-HT and glycogen synthase kinase-3 (GSK3), with an emphasis on the type 1 and type 2 5-HT receptors.

## EFFECTS OF 5-HT ON REGULATING GSK3

### 5-HT REGULATES GSK3 BY PHOSPHORYLATION

5-hydroxytryptamine was first found to regulate GSK3 in mouse brain. When wild type mice received an acute administration of d-fenfluramine, a drug that enhances 5-HT release and blocks 5-HT reuptake, phosphorylation of GSK3 $\beta$  at serine-9 residue was significantly increased in the cerebral cortex, hippocampus, and striatum (Li et al., 2004). Phosphorylation of this residue transforms the N-terminal of GSK3 into a pseudosubstrate which blocks other GSK3 substrates from entering the active site of the enzyme, thus, d-fenfluramine treatment results in inhibition of GSK3 $\beta$  in the brain. However, even in the presence of a MAOI that blocks the metabolism of 5-HT, the effect of a single intraperitoneal injection of d-fenfluramine is transient, peaking at 1 h, and gradually returning to baseline level by 4 h. This could be due to rapid metabolism of the drug in mice, but rapid release of 5-HT may cause negative feedback inhibition of 5-HT release as well as 5-HT receptor desensitization; both may also contribute to the transient effect of d-fenfluramine on GSK3 phosphorylation.

The inhibitory control of GSK3 by 5-HT is further demonstrated by an elegant study (Beaulieu et al., 2008b) in a mouse model where mice carry a mutation in the tryptophan hydroxylase-2 (TPH-2) gene that is equivalent to a rare human variant (R441H) identified in a few individuals with major depressive disorder (Zhang et al., 2005). The homozygous mutant mice, when compared to littermate wild type mice, have significantly lower levels of the 5-HT precursor 5-hydroxytryptophan (5-HTP), 5-HT, and its metabolite 5-Hydroxyindoleacetic acid (5-HIAA) in the frontal cortex, hippocampus, and striatum. Meanwhile, the level of phosphorylated serine-9 of GSK3 $\beta$  is significantly lower, and GSK3 activity is elevated in the same brain regions of the mutant mice (Beaulieu et al., 2008b). Therefore, a sustained deficiency of 5-HT may reset the activity level of GSK3 in brain to higher level, compared to wild type mice where the constitutively active GSK3 is likely under inhibitory regulation (Doble and Woodgett, 2003). The overactive GSK3 in the R441H mutant mice may be involved in the behavioral abnormalities of these mice, since the depressive- and anxiogenic-like behavioral phenotypes of these mice were largely reversed by a GSK3 inhibitor, and by genetically reducing the level of GSK3 $\beta$ .

### BEHAVIORAL SIGNIFICANCE OF GSK3 REGULATION BY 5-HT

Regulation of GSK3 by 5-HT may have functional significance in maintaining brain physiology and behaviors that are regulated by serotonergic neurotransmission. The 5-HT reuptake inhibitor antidepressant fluoxetine has been shown to increase phospho-Ser9-GSK3 $\beta$  in the cerebral cortex, hippocampus, striatum, and cerebellum of mouse brain (Li et al., 2004, 2007; Beaulieu et al., 2008b) and phospho-Ser21-GSK3 $\alpha$  in the hippocampus (Polter et al., 2011). Several studies also suggest that inhibition of GSK3 $\beta$  is an important intermediate step in the antidepressant effect of

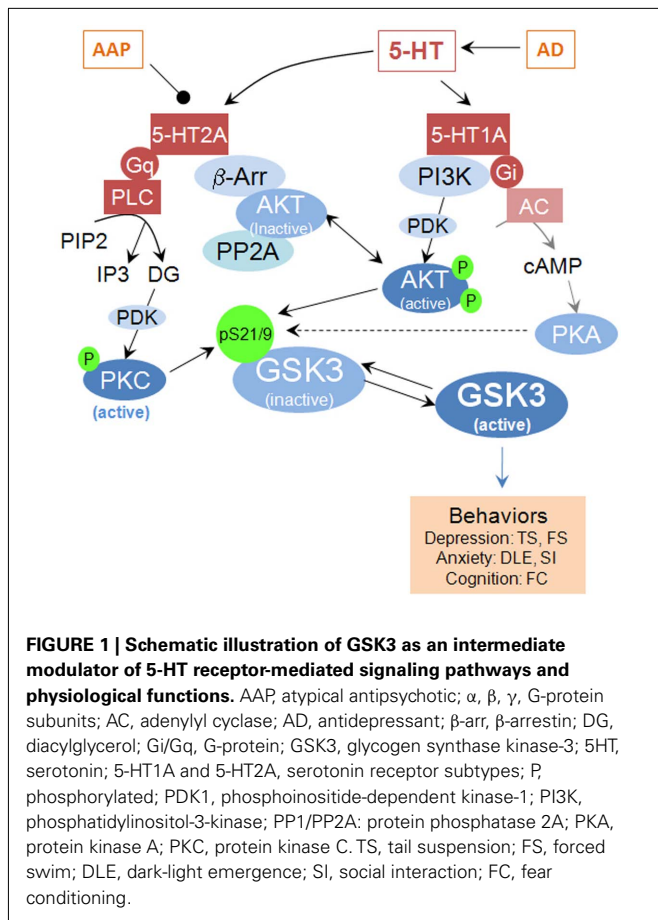
fluoxetine. In the TPH-2 mutant mice, abnormal behavior associated with 5-HT deficiency is mediated by GSK3 $\beta$  (Beaulieu et al., 2008b). Additionally, inhibition of GSK3 by small molecule inhibitors or in GSK3 $\beta$ -deficient mice have been shown to reduce immobility in the forced swim test (Gould et al., 2004; Kaidanovich-Beilin et al., 2004; O'Brien et al., 2004; Beaulieu et al., 2008a; Rosa et al., 2008), similarly to the antidepressant fluoxetine (Page et al., 1999; Bianchi et al., 2002). We have recently tested this behavioral effect of fluoxetine in mutant GSK3 knock-in (KI) mice in which serine-9 of GSK3 $\beta$  or serine-21 of GSK3 $\alpha$  was substituted with alanine (S9A-GSK3 $\beta$ -KI and S21A-GSK3 $\alpha$ -KI; McManus et al., 2005; Polter et al., 2010). These mice have normal levels of GSK3 $\beta$  and GSK3 $\alpha$ , but the N-terminal serine cannot be regulated by phosphorylation. In the forced swim test (Porsolt et al., 1977) the baseline immobility (saline treatment) was not significantly different between S9A-GSK3 $\beta$ -KI, S21A-GSK3 $\alpha$ -KI, and littermate wild type mice. As expected, wild type mice responded to fluoxetine (20 mg/kg, i.p., 30 min) with a significant 56% reduction in immobility when compared to saline-treated mice. However, the anti-immobility effect of fluoxetine in S9A-GSK3 $\beta$ -KI mice was markedly diminished, with only 17% non-significant reduction of immobility (Polter et al., 2011). Although fluoxetine was able to increase phospho-Ser21-GSK3 $\alpha$  in the hippocampus, S21A-GSK3 $\alpha$ -KI mice partially responded to fluoxetine in the forced swim test. Therefore, phosphorylation of GSK3 $\beta$  predominantly mediates the acute antidepressant-like effect of fluoxetine, which reinforces the importance of phosphorylation of GSK3 $\beta$  in the therapeutic action of fluoxetine (Figure 1).

Since the antidepressant effect of fluoxetine in human only appears after chronic administration, to associate GSK3 regulation by fluoxetine to a therapeutic effect, it is important to determine if chronic fluoxetine treatment also regulates GSK3 $\beta$ . This has recently been reported by Okamoto et al. (2010) who found that chronic fluoxetine administration for 3 weeks significantly increased phospho-Ser9-GSK3 $\beta$  in mouse hippocampus. Although this is the only selective 5-HT reuptake inhibitor thus far shown to increase phospho-Ser9-GSK3 $\beta$  upon chronic treatment, a dual-acting antidepressant venlafaxine (blocks both 5-HT and norepinephrine transporters) has also been shown to increase phospho-Ser9-GSK3 $\beta$  after chronic administration (Okamoto et al., 2010). When investigating the effect of chronic treatment with imipramine, a TCA that inhibits reuptake of both 5-HT and norepinephrine on activation of Akt and downstream transcription factor FoxO3a (Polter et al., 2009), we also noticed a significant increase in the level of phospho-Ser9-GSK3 $\beta$  in the cerebral cortex, hippocampus, and striatum of mouse brain after 4 weeks of intraperitoneal administration (Figure 2). It is not yet verified if the effect of venlafaxine and imipramine on GSK3 $\beta$  is a combined action via both 5-HT and norepinephrine, as regulation of brain GSK3 $\beta$  by selective adrenergic-enhancing drugs has not been studied.

## REGULATION OF GSK3 BY 5-HT<sub>1A</sub> RECEPTORS

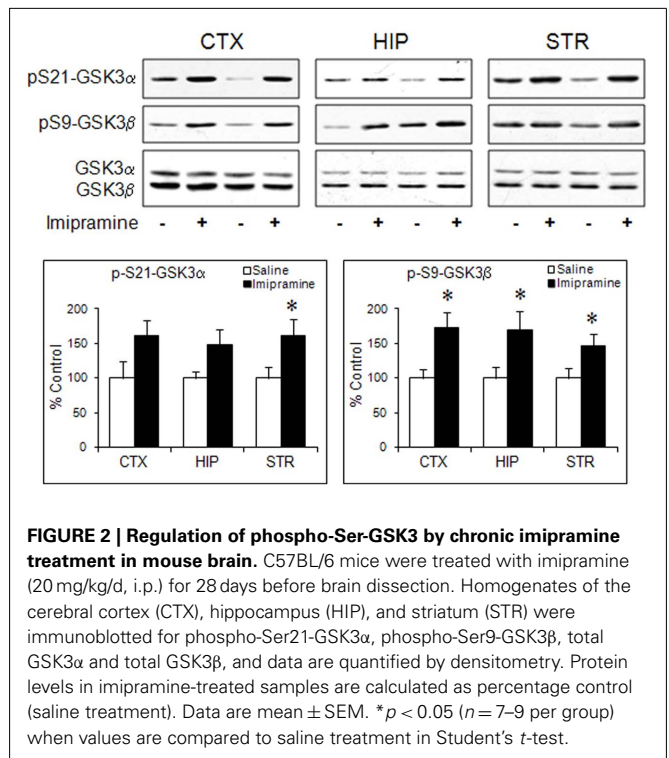
### ACTIVATION OF 5-HT<sub>1A</sub> RECEPTORS REGULATES GSK3 BY PHOSPHORYLATION

With many physiological actions of brain 5-HT that are divergently mediated by 5-HT receptor subtypes, it is critically important to



determine the receptor subtypes that transduce 5-HT signal to inhibition of GSK3. In mice treated with d-fenfluramine, the 5-HT1A receptor antagonist WAY100635 was able to block over 60% of d-fenfluramine-induced increase in phospho-Ser9-GSK3 $\beta$ , suggesting that 5-HT1A receptors have a major role in mediating the GSK3-regulating effect of 5-HT. Indeed, when mice receive a single systemic administration of 5-HT1A receptor agonist 8-hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT), the level of phospho-Ser9-GSK3 $\beta$ , but not total GSK3 $\beta$ , is significantly increased in the cerebral cortex, hippocampus, and striatum (Li et al., 2004). 8-OH-DPAT is also able to increase the level of phospho-Ser21-GSK3 $\alpha$  in the hippocampus, but the effect is less robust (Polter et al., 2011).

In the hippocampus, a brain area that is enriched in 5-HT1A receptors (Hoyer et al., 2002), GSK3 $\beta$  is ubiquitously expressed with high level of immunoreactivity in neuronal cell bodies and dendritic processes (Peineau et al., 2007; Perez-Costas et al., 2010). 8-OH-DPAT-induced increase in phospho-Ser9-GSK3 $\beta$  is particularly prominent in the dendrites and cell bodies of CA3, and is observable in the cell bodies and projections of dentate granule cells and the dendrites of CA1 (Polter et al., 2011). The quantified ratio of phospho-Ser9-GSK3 $\beta$  to nuclear marker in the subfields of the hippocampus reveals significant increases in the stratum pyramidale and the stratum radiatum/stratum lucidum of CA3 and in the hilus of the dentate gyrus. Therefore, the response of GSK3 $\beta$



to systemic administration of 8-OH-DPAT in the hippocampus is preferentially in the pyramidal glutamatergic neurons and their dendrites, suggesting that regulation of GSK3 $\beta$  by 5-HT1A receptors may have functional impact on the glutamatergic circuits of the hippocampus. GSK3 $\beta$  has been shown to be a mediator of glutamate receptor activity and synaptic plasticity in the hippocampus (Hooper et al., 2007; Peineau et al., 2007; Du et al., 2010), thus regulation of GSK3 $\beta$  by 5-HT1A receptors in the hippocampus may further link this 5-HT signaling mechanism to hippocampal regulation of learning and memory.

**THE ROLE OF GSK3 IN 5-HT1A RECEPTOR-REGULATED BEHAVIORS AND OTHER PHYSIOLOGICAL FUNCTIONS**

Among 5-HT1A receptor-regulated behaviors, inhibition of contextual fear conditioning, a form of associative learning (Kim and Jung, 2006), is a hippocampus-dependent function (Stiedl et al., 2000; Tsetsenis et al., 2007; Ogren et al., 2008). To determine the role of GSK3 $\beta$  in 5-HT1A receptor-regulated fear conditioning, we tested the expression of contextual and cued fear responses in S9A-GSK3 $\beta$ -KI, S21A-GSK3 $\alpha$ -KI, and littermate wild type mice. In wild type mice, 8-OH-DPAT (1 mg/kg, i.p.) injected 30 min prior to contextual test completely suppressed a standard fear conditioning training-induced contextual freezing (Polter et al., 2011). Compared to wild type mice, S9A-GSK3 $\beta$ -KI mice also exhibited increased freezing in contextual tests, but 8-OH-DPAT had no significant effect in reducing the context freezing. In contrast to S9A-GSK3 $\beta$ -KI mice, S21A-GSK3 $\alpha$ -KI mice responded to 8-OH-DPAT in contextual freezing similarly as wild type mice. Thus, inhibition of GSK3 $\beta$ , but not GSK3 $\alpha$ , via 5-HT1A receptors is a necessary intermediate process for 5-HT1A receptor-regulated inhibition of contextual fear learning. This function of GSK3 $\beta$  is

selective to the contextual content since the response of cued fear learning to 8-OH-DPAT was similar in S9A-GSK3 $\beta$ -KI mice and wild type mice.

The functional significance of GSK3 $\beta$  in 5-HT1A receptor-mediated physiological functions is not limited to contextual fear learning. In cultured rat hippocampal neurons, 5-HT1A receptor activation by 8-OH-DPAT increases phospho-Ser9-GSK3 $\beta$  and stimulates mitochondrial movements in the axons, an effect mimicked by a GSK3 inhibitor (Chen et al., 2007). This finding suggests the importance of GSK3 $\beta$  in 5-HT1A receptor-mediated regulation of energy distribution in neurons. In *Drosophila*, over-expressing the mammalian 5-HT1A receptor ortholog d5-HT1B receptor increases the level of phosphorylated serine of SHAGGY (SGG), the *Drosophila* GSK3 $\beta$  (Yuan et al., 2005). This inhibitory regulation of SGG by d5-HT1B receptor prevents SGG from phosphorylating timeless (TIM) protein for light-induced degradation. Therefore, d5-HT1B receptor reduces behavioral phase shifts in *Drosophila* by increasing phospho-Ser-SGG. The role of GSK3 in other 5-HT1A receptor-mediated functions remains to be elucidated, but this could be an exciting area in therapeutic drug development, as GSK3 inhibitors, when applied appropriately, may rescue abnormal physiology and behaviors due to functional deficiency of 5-HT1A receptors in brain.

#### **SIGNALING MECHANISMS MEDIATING THE EFFECT OF 5-HT1A RECEPTORS ON GSK3**

5-HT1A receptors activate Gi-coupled signal pathways. In a recent study, Talbot et al. (2010) found that mice expressing regulators of G protein signaling (RGS)-insensitive G $\alpha$ 2 have increased sensitivity to 8-OH-DPAT-induced activation, and exhibit elevated levels of cortical and hippocampal phospho-Ser9-GSK3 $\beta$ . This effect of RGS-insensitive active G $\alpha$ 2 was blocked by the 5-HT1A receptor antagonist WAY100635. This finding suggests that regulation of GSK3 $\beta$  by 5-HT1A receptors is mediated by a Gi-coupled signaling pathway (Figure 1). However, activation of G $\alpha$ 2 results in inhibition of adenylyl cyclase and inactivation of PKA. Although PKA is one of the several protein kinases that phosphorylate GSK3 $\beta$  on the serine-9 residue (Fang et al., 2000; Li et al., 2000), it is unlikely that this conventional G $\alpha$ -coupled signaling pathway is responsible for direct phosphorylation of GSK3 $\beta$ . Alternatively, 5-HT1A receptor agonists have consistently shown to increase Akt phosphorylation in neuronal cells, including hippocampal derived HN2-5 cells (Adayev et al., 1999), primary hippocampal neurons (Cowen et al., 2005; Chen et al., 2007), and primary fetal rhombencephalic neurons (Druse et al., 2005). Regulation of Akt by 5-HT1A receptors is mediated by phosphoinositide 3-kinase (PI3K; Cowen et al., 2005; Hsiung et al., 2005, 2008), and is sensitive to inhibition of G $\alpha$  activity by pertussis toxin (Cowen et al., 2005). Furthermore, activation of Akt by 5-HT1A receptors can be inhibited by cAMP and restored after inactivation of PKA (Hsiung et al., 2008). Therefore, 5-HT1A receptor-induced activation of Akt likely follows 5-HT1A receptor-induced activation of the G $\alpha$ -adenylyl cyclase-cAMP-PKA signaling pathway. Since Akt is another major protein kinase that regulates phospho-Ser9-GSK3 $\beta$  (Cross et al., 1995), Akt may mediate 5-HT1A receptor-induced GSK3 $\beta$  phosphorylation. Indeed, systemic treatment of mice with the 5-HT1A receptor agonist 8-OH-DPAT significantly increased

the active phospho-Thr308-Akt in the hippocampus, and intra-hippocampal infusion of the PI3K inhibitor LY294002 blocked both phospho-Thr308-Akt and phospho-Ser9-GSK3 $\beta$  in response to 8-OH-DPAT (Polter et al., 2011).

#### **SELECTIVITY OF GSK3 REGULATION BY 5-HT1A RECEPTORS**

As discussed above, activation of 5-HT1A receptors increases both phospho-Ser9-GSK3 $\beta$  and phospho-Ser21-GSK3 $\alpha$  in the hippocampus, however, the magnitude of response in GSK3 $\alpha$  is smaller than GSK3 $\beta$  (Polter et al., 2011). Additionally, regulation of GSK3 $\alpha$  phosphorylation by 5-HT1A receptors has less impact in fear conditioning (Polter et al., 2011). These pilot studies suggest different roles of GSK3 isoforms in mediating the physiological and behavioral functions of 5-HT1A receptors. Additional studies are needed to differentiate the response of GSK3 $\alpha$  and GSK3 $\beta$  to 5-HT1A receptor agonists in different brain regions, and to compare the impact of each GSK3 isoform in other 5-HT1A receptor-regulated behaviors, which may provide valuable information on the physiological and behavioral impacts of the two GSK3 isoforms in 5-HT neurotransmission.

A caveat of studying 5-HT1A receptor-regulated signaling in brain is that the differential functions of 5-HT1A autoreceptors and heteroreceptors in different brain regions have divergent functions. Thus, systemic treatment of animals with 5-HT1A receptor agonists can activate 5-HT1A autoreceptors to reduce firing of raphe 5-HT neurons projected to other brain regions, but simultaneously activate 5-HT1A heteroreceptors in those brain regions, such as the hippocampus. Therefore, the effect seen after global activation of 5-HT1A receptors may involve indirect response of GSK3 to activation or inhibition of other neurotransmitters. Therefore, additional studies of GSK3 regulation by systemically and regionally applied 5-HT1A receptor agonists in specific neuron populations in combination with studies in isolated primary neuron cultures will further elucidate the sophisticated mechanisms underlying the GSK3-regulating effect of 5-HT1A receptors. Nevertheless, the effect of global activation of 5-HT1A receptors should be appreciated since systemic drug treatment is likely more relevant to therapeutic implications.

#### **REGULATION OF GSK3 BY 5-HT2A RECEPTORS**

##### **THE PARADOXICAL EFFECTS OF 5-HT2A RECEPTOR AGONISTS AND ANTAGONISTS ON GSK3**

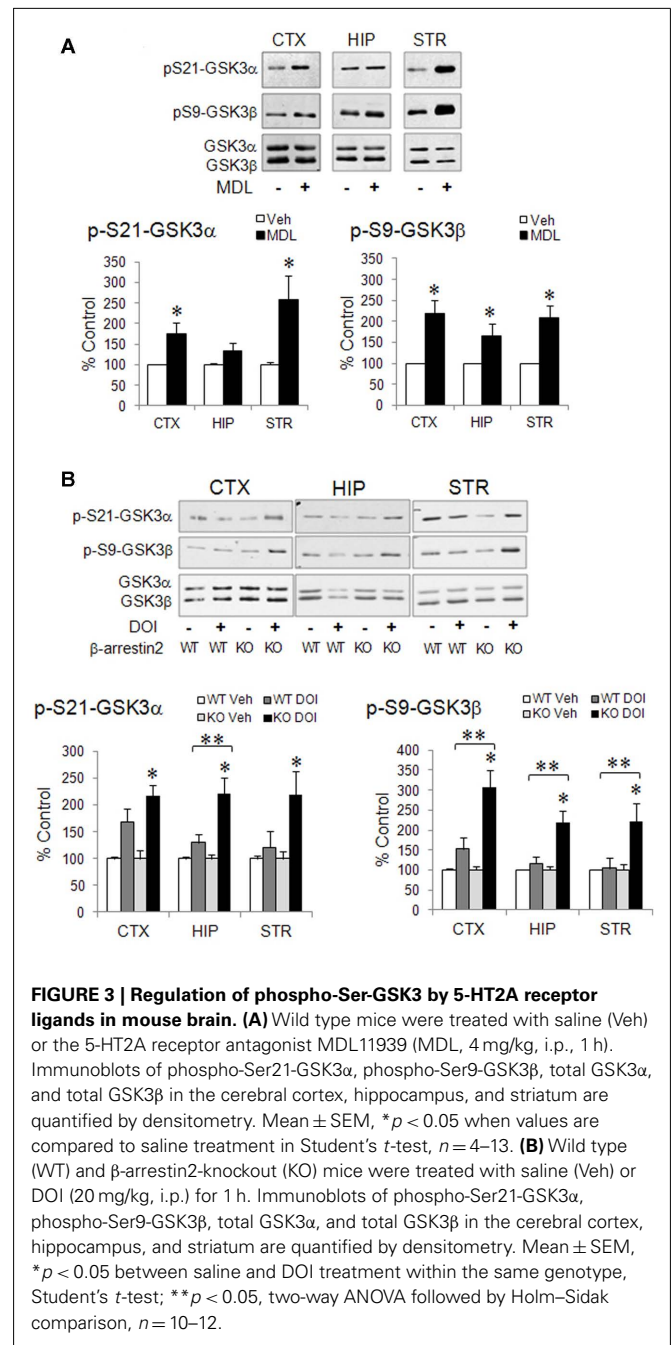
Although 5-HT1A receptors have a prominent regulatory effect on GSK3, more than one 5-HT receptor subtype should be activated upon elevated brain 5-HT. Among them, 5-HT2A receptors have been found to regulate GSK3 with rather sophisticated and yet unidentified mechanisms. We previously reported that activation of 5-HT2A receptors by systemic administration of 1-(2,5-dimethoxy-4-iodophenyl)-propan-2-amine (DOI) for 1 h (a time point that maximally increases phospho-Ser9-GSK3 $\beta$  by d-fenfluramine, fluoxetine, and 8-OH-DPAT), had little effect on phospho-Ser9-GSK3 $\beta$  in the cerebral cortex, hippocampus, or striatum (Li et al., 2004). However, Abbas et al. (2009) later reported that a brief 15-min DOI treatment caused an increase in phospho-Ser9-GSK3 $\beta$  in the mouse brain, although this report did not specify the brain region in which this effect was seen. If the time length of treatment is the major difference of the discrepant

findings, one possibility is that 1 h treatment might have caused desensitization of 5-HT<sub>2A</sub> receptors. Therefore a thorough kinetic study of 5-HT<sub>2A</sub> receptor agonists may further clarify their effect on GSK3, especially the effects in different brain regions.

Since activation of 5-HT<sub>2A</sub> receptors by the hallucinogen DOI is somewhat different from 5-HT-induced activation of 5-HT<sub>2A</sub> receptors (Schmid et al., 2008), the exact action of 5-HT<sub>2A</sub> receptor activation on GSK3 phosphorylation should be further elucidated using endogenous 5-HT<sub>2A</sub> receptor agonists and selective 5-HT<sub>2A</sub> receptor antagonists. However, this has been difficult to study with 5-HT<sub>2A</sub> receptors, because it is already known that endogenous 5-HT increases phospho-Ser-GSK3 by activating other 5-HT receptors, particularly 5-HT<sub>1A</sub> receptors (Li et al., 2004); whereas selective blocking 5-HT<sub>2A</sub> receptors with their antagonists, one of the commonly used manipulations to determine a receptor-selective effect, paradoxically elicit robust increase in the level of phospho-Ser-GSK3 in mouse brain. This phenomenon was first observed with the non-selective 5-HT<sub>2</sub> receptor antagonist LY53857 (Li et al., 2004). Systemic LY53857 treatment not only caused a prolonged elevation of phospho-Ser9-GSK3 $\beta$ , but it also potentiated the GSK3 $\beta$ -regulating effect of *d*-fenfluramine and 8-OH-DPAT. This effect has also been observed using the 5-HT<sub>2A</sub> receptor-selective antagonist MDL11939, which elevates both phospho-Ser21-GSK3 $\alpha$  and phospho-Ser9-GSK3 $\beta$  in mouse brain, with the effect more prominent in the cerebral cortex and striatum and less in the hippocampus (Figure 3A).

### THE EFFECT OF ATYPICAL ANTIPSYCHOTICS ON GSK3

Despite the paradoxical nature of 5-HT<sub>2A</sub> receptors in regulating GSK3, the effect of 5-HT<sub>2A</sub> receptor antagonists may have clinical significance. Several independent studies have found that antipsychotic drugs, including both conventional and atypical antipsychotics, regulate GSK3 in animal brain. The conventional antipsychotic haloperidol was reported to either alter the phosphorylation state or increase the protein level of GSK3 (Alimohamad et al., 2005; Kozlovsky et al., 2006; Roh et al., 2007). A group of atypical antipsychotics, including risperidone, olanzapine, clozapine, quetiapine, and ziprasidone, were consistently found to increase phospho-Ser-GSK3 in mouse brain (Alimohamad et al., 2005; Li et al., 2007; Roh et al., 2007). Although the dopamine D<sub>2</sub> receptor-blocking property of these agents may be involved in their GSK3-regulating effect (Beaulieu et al., 2009), one of the major pharmacological difference between conventional antipsychotics and atypical antipsychotics is that the latter group has a dual antagonistic action on both 5-HT<sub>2</sub> receptors and dopamine D<sub>2</sub> receptors (Schotte et al., 1995). In our study, a low dose haloperidol that binds to D<sub>2</sub> receptors but not 5-HT<sub>2A</sub> receptors had no effect on mouse brain GSK3 (Li et al., 2007). In contrast, systemic administered risperidone increases brain phospho-Ser9-GSK3 $\beta$  at a dose as low as 0.1 mg/kg (Li et al., 2007). As the average dose of risperidone used to attain a 50% *in vivo* blockade of D<sub>2</sub> receptors in rats is about 0.3 mg/kg (Kapur et al., 2003), and the binding affinity of risperidone to 5-HT<sub>2A</sub> receptors is at least 3 times higher than to D<sub>2</sub> receptors (Weiner et al., 2001), it is likely that the increase of phospho-Ser9-GSK3 $\beta$  by the low dose risperidone involves blocking 5-HT<sub>2A</sub> receptors. It remains to be determined if putative 5-HT<sub>2A</sub> receptor antagonists have similar clinical implications



as atypical antipsychotics, and if such effects are dependent on regulation of GSK3.

Clinically, atypical antipsychotics have indications in both psychotic disorders and mood disorders (Derry and Moore, 2007; Philip et al., 2008). Some atypical antipsychotics, either as monotherapy or augmentation, have shown efficacy in ameliorating symptoms of depression (Tohen et al., 2003; Calabrese et al., 2005; Berman et al., 2007; Marcus et al., 2008; Bauer et al., 2009). Interestingly, a combination treatment with risperidone and fluoxetine enhance the effect of either agent alone on phosphorylation of GSK3 $\beta$  in the rodent brain (Li et al., 2007). This effect agrees

with the finding that the 5-HT<sub>2</sub> receptor antagonist LY53857 potentiated the effect of d-fenfluramine and 8-OH-DPAT on GSK3 phosphorylation. However, the role of GSK3 in this clinically relevant combination effect between antidepressants and atypical antipsychotics remains to be determined.

### SIGNALING MECHANISMS INVOLVED IN REGULATION OF GSK3 BY 5-HT<sub>2A</sub> RECEPTORS

Few studies have investigated the signaling mechanisms that mediate the effects of either 5-HT<sub>2A</sub> receptor agonists or antagonists on GSK3 phosphorylation. Classically, the 5-HT<sub>2A</sub> receptors couple to Gq protein that activates PLC and its down-stream PKC (Carr et al., 2002; **Figure 1**). PKC was reported to mediate DOI-induced Erk phosphorylation in the brain (Schmid et al., 2008). Since PKC is one of the protein kinases that regulate GSK3 phosphorylation (Goode et al., 1992), it would be straightforward if the PLC/PKC signaling mechanism is found to mediate 5-HT<sub>2A</sub> receptor-regulated GSK3. However, this mechanism has not been reported, especially since the kinetic correlation between 5-HT<sub>2A</sub> receptor activation and phosphorylation of GSK3 has not been characterized. In a study of dopamine D<sub>2</sub> receptor-regulated signaling (Beaulieu et al., 2004), the intracellular scaffolding protein  $\beta$ -arrestins were found to negatively affect GSK3 phosphorylation in mouse brain, wherein activation of dopamine D<sub>2</sub> receptors reduces phospho-Ser9-GSK3 due to inactivation of Akt by protein phosphatase-2 (PP2A) in a  $\beta$ -arrestin2-driven protein complex (Beaulieu et al., 2005). D<sub>2</sub> receptor antagonists block this effect of dopamine and increase phospho-Ser-GSK3 by disrupting the effect of  $\beta$ -arrestins. 5-HT<sub>2A</sub> receptors also interact with  $\beta$ -arrestins in a cell type- and ligand-selective manner (Gelber et al., 1999; Allen et al., 2008; Schmid et al., 2008; Schmid and Bohn, 2010), but the role of  $\beta$ -arrestins in 5-HT<sub>2A</sub> receptor-regulated signal pathways is less understood. It should be noted that in a previous study by Schmid et al. (2008), DOI-induced head twitch, receptor internalization, and Erk phosphorylation are not dependent on the presence or absence of  $\beta$ -arrestins. However, we have recently found that DOI treatment for 1 h induced a significant over twofold increase in phospho-Ser21-GSK3 $\alpha$  and phospho-Ser9-GSK3 $\beta$  in the cerebral cortex, hippocampus, and striatum in  $\beta$ -arrestin2-knockout mice, an effect that is not seen in wild type mice (**Figure 3B**). This observation suggests that  $\beta$ -arrestin2 may influence how 5-HT<sub>2A</sub> receptors regulate GSK3. Future studies investigating signal transduction mechanisms mediating the effect of 5-HT<sub>2A</sub> receptors on GSK3 should include not only PLC/PKC signaling, but also  $\beta$ -arrestin-mediated signaling. Additionally, since 5-HT<sub>2A</sub> receptors are involved in modulating dopamine neurotransmission (Alex and Pehek, 2007; Di Giovanni et al., 2008; Esposito et al., 2008), a potential indirect effect of 5-HT<sub>2A</sub> receptors on D<sub>2</sub> receptor-regulated GSK3 should also be considered.

### GSK3 SELECTIVELY REGULATES 5-HT<sub>1B</sub> RECEPTOR-MEDIATED SIGNAL TRANSDUCTION AND FUNCTION

Glycogen synthase kinase-3 phosphorylates many protein substrates that distribute in both neural and peripheral tissues (Woodgett, 2001; Jope and Johnson, 2004). As a result, *in vivo* inhibition

of GSK3 can have a variety of physiological effects. Thus, identifying GSK3 substrates that have specific functions in brain circuits could be critical in developing GSK3-targeting treatment for therapeutics. With each clearly identified substrate, substrate-targeting inhibition of brain GSK3 activity may lead to selective modulation of physiological function of the brain. Most 5-HT receptor subtypes, except 5-HT<sub>3</sub> receptors, are G-protein coupled receptors (GPCRs) that represent the largest group of drug targets. Besides activation by receptor ligands, the activity of GPCRs can be modulated by posttranslational modifications, such as phosphorylation by protein kinases (Tobin, 2008), or interaction with intracellular proteins (Ritter and Hall, 2009; Bockaert et al., 2010). However, little evidence has shown GPCR regulation by GSK3.

### DIRECT INTERACTION BETWEEN GSK3 $\beta$ AND 5-HT<sub>1B</sub> RECEPTORS

In an effort to identify GSK3 substrates within the 5-HT neurotransmission system, we searched GSK3 consensus phosphorylation sequence for primed substrates – pre-phosphorylated by a serine/threonine kinase to allow access of GSK3 to the serine/threonine located four amino acids N-terminal of the primed site (Doble and Woodgett, 2003). Somewhat surprisingly, both human and mouse 5-HT<sub>1B</sub> receptors were found to contain eight GSK3 consensus phosphorylation sites spreading within intracellular loops 1, 2, and 3 of the receptor. In contrast, the highly homologous 5-HT<sub>1A</sub> receptors have no GSK3 consensus sites in intracellular loops 1 and 2, and the two sites in intracellular loop-3 of human 5-HT<sub>1A</sub> receptors are not homologous with mouse 5-HT<sub>1A</sub> receptors. In cultured heterologous cells expressing 5-HT<sub>1B</sub> or 5-HT<sub>1A</sub> receptors, GSK3 $\beta$  was found to directly associate with 5-HT<sub>1B</sub> receptors (Chen et al., 2009), which was detected using the bioluminescence resonance energy transfer (BRET) assay that measures the proximity of two proteins within a distance of 1–10 nm (Angers et al., 2000), and confirmed by co-immunoprecipitation of 5-HT<sub>1B</sub> receptors and GSK3 $\beta$ . Intriguingly, this interaction of GSK3 $\beta$  is selective to 5-HT<sub>1B</sub> receptors, as GSK3 $\beta$  does not interact with 5-HT<sub>1A</sub> receptors (Chen et al., 2009). Mutation of every potential GSK3 consensus phosphorylation site of 5-HT<sub>1B</sub> receptors by replacing the serine with alanine further shows that GSK3 $\beta$  associates with 5-HT<sub>1B</sub> receptors at the [pS(154)AKRpT(158)] sequence located in the i2-loop of 5-HT<sub>1B</sub> receptors (Chen et al., 2009).

### GSK3 $\beta$ DIFFERENTIALLY INFLUENCES 5-HT<sub>1B</sub> RECEPTOR ACTIVITY AND ASSOCIATED SIGNALINGS

Both 5-HT<sub>1B</sub> and 5-HT<sub>1A</sub> receptors couple to Gi-protein, activation of which causes inhibition of adenylyl cyclase and reduction of cAMP. Disrupting the interaction between 5-HT<sub>1B</sub> receptors and GSK3 $\beta$  by mutating the GSK3 $\beta$ -interactive Ser-154 residue of the receptor (S154A–5-HT<sub>1B</sub> receptor) effectively abolishes 5-HT-induced conformational change between 5-HT<sub>1B</sub> receptors and Gi $\alpha$ 2 (Chen et al., 2011). In accordance with altered activity of Gi $\alpha$ 2, GSK3 $\beta$ -insensitive S154A–5-HT<sub>1B</sub> receptor does not respond to 5-HT in assay of 5-HT-induced inhibition of cAMP (Chen et al., 2009). These data highly suggest that GSK3 $\beta$  has a functional impact on 5-HT<sub>1B</sub> receptor-mediated signaling. Indeed, several small molecule GSK3 inhibitors and the clinically used lithium are able to disrupt 5-HT-induced conformational

change between 5-HT<sub>1B</sub> receptors and G $\alpha$ 2 and inhibition of cAMP. This is a highly selective function of the GSK3 $\beta$  isoform, as it only occurs in cells with GSK3 $\beta$ -knockdown or over-expressing inactive GSK3 $\beta$ , but GSK3 $\alpha$ -knockdown does not affect 5-HT<sub>1B</sub> receptor-associated G $\alpha$ -cAMP signaling (Chen et al., 2009, 2011). This is another example showing that despite GSK3 $\alpha$  and GSK3 $\beta$  are highly homologous and are regulated via similar upstream mechanisms, the two isoforms of GSK3 have different substrates and mediate different physiological functions (Wang et al., 1994; Liang and Chuang, 2006). In agreement with a selective interaction between GSK3 $\beta$  and 5-HT<sub>1B</sub> receptors, GSK3 inhibitors and molecular manipulation of intracellular GSK3 $\beta$  do not alter 5-HT-induced 5-HT<sub>1A</sub> receptor-G $\alpha$ 2 interaction or 5-HT<sub>1A</sub> receptor-mediated inhibition of cAMP.

G $\alpha$ -mediated cAMP production is not the only GSK3-dependent signaling pathway of 5-HT<sub>1B</sub> receptors as activation of Akt by 5-HT or the selective 5-HT<sub>1B</sub> receptor agonist anpirtoline is also significantly diminished in the mutant S154A-5-HT<sub>1B</sub> receptor-expressing cells (Chen et al., 2011). The mechanisms of regulating Akt by 5-HT<sub>1B</sub> receptors are not fully understood, but since both  $\alpha$ - and  $\beta\gamma$ -subunits of G-proteins may be linked to GPCR-induced activation of Akt (DeWire et al., 2007; New et al., 2007; Yang et al., 2009), the effect of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptor-induced Akt activation could be a consequence of the prominent effect of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptor-associated Gi-protein. In addition, since Akt is one of the upstream GSK3-regulating protein kinases that inactivates GSK3 (Cross et al., 1995), the GSK3-dependent activation of Akt by 5-HT<sub>1B</sub> receptors could function as a feedback regulation to prevent prolonged effect of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptors, but this postulation remains to be examined.

As a GPCR, 5-HT<sub>1B</sub> receptors are expected to interact with  $\beta$ -arrestins that have been recognized to interact with many GPCRs and play important roles in GPCR internalization and alternative signaling (Lefkowitz and Shenoy, 2005). Indeed, we found that  $\beta$ -arrestin2 can be recruited to 5-HT<sub>1B</sub> receptors in response to 5-HT. However, in contrast to the prominent effect of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptor-coupling to G $\alpha$ 2, removing, or inhibiting GSK3 $\beta$  did not affect  $\beta$ -arrestin2 recruitment to 5-HT<sub>1B</sub> receptors (Chen et al., 2011). As a chaperone protein, recruitment of  $\beta$ -arrestins to GPCRs initiates receptor internalization and desensitization (Bohn et al., 1999, 2000). 5-HT was able to internalize both wild type and S154A-5-HT<sub>1B</sub> receptors in 5-HT<sub>1B</sub> receptor-expressing cells (Chen et al., 2009). If this internalization event is directed by  $\beta$ -arrestin2, it is in agreement with our finding that  $\beta$ -arrestin2 recruitment to 5-HT<sub>1B</sub> receptors is independent of GSK3 $\beta$ . However, the time course of 5-HT<sub>1B</sub> receptor internalization is drastically different between wild type and the mutant S154A-5-HT<sub>1B</sub> receptors, wherein wild type receptors rapidly reappear on the cell membranes within 60 min of 5-HT treatment, while mutant GSK3 $\beta$ -insensitive receptors continue to be absent from the cell surface 2 h after 5-HT treatment. This suggests a potentially important function of GSK3 $\beta$  in an unidentified mechanism that is crucial for 5-HT<sub>1B</sub> receptor synthesis and membrane recycling.

Interestingly, the effect of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptors appears to facilitate activity and functional recovery, which is opposite

from other GPCR-regulating protein kinases (GRKs) that typically facilitate desensitization and termination of GPCR activity (Gainetdinov et al., 2004). The underlying importance of GSK3 $\beta$  on 5-HT<sub>1B</sub> receptor-mediated Gi signaling and  $\beta$ -arrestin recruitment is that GSK3 $\beta$  does not function as a general 5-HT<sub>1B</sub> receptor activator, instead, GSK3 $\beta$  selectively modulates signaling-specific actions of 5-HT<sub>1B</sub> receptors. This effect of GSK3 $\beta$  could be attractive when developing GSK3-targeting therapeutic agents, as inhibition of GSK3 $\beta$  would not make inert 5-HT<sub>1B</sub> receptors, but to selectively shift 5-HT<sub>1B</sub> receptor-mediated physiological and behavioral functions toward a Gi signaling-independent direction. This postulation is an important area of future research especially when  $\beta$ -arrestin-associated 5-HT<sub>1B</sub> receptor signaling pathways are identified.

### EFFECT OF GSK3 INHIBITORS ON THE PHYSIOLOGICAL FUNCTIONS OF 5-HT<sub>1B</sub> RECEPTORS

Noticeably, several earlier studies showed that lithium selectively inhibits 5-HT binding to 5-HT<sub>1B</sub> receptors, reduces 5-HT<sub>1B</sub> receptor-induced GTP $\gamma$ s binding, and abolishes 5-HT<sub>1B</sub> receptor-reduced adenylyl cyclase activity (Massot et al., 1999). Similar results have been observed in human platelets from both healthy and depressed subjects where lithium dose-dependently reverses the inhibitory effect of a 5-HT<sub>1B</sub> agonist on adenylyl cyclase (Januel et al., 2002). In animal behavior studies, lithium selectively regulates the behavioral effect of 5-HT<sub>1B</sub> receptor agonists, but not the effect of 5-HT<sub>1A</sub> receptor agonists (Redrobe and Bourin, 1999). With lithium being a selective GSK3 inhibitor (Klein and Melton, 1996), it is highly likely that the selective effect of lithium on 5-HT<sub>1B</sub> receptor function is the result of its inhibition of GSK3. Since lithium is a therapeutic drug that has been found to inhibit GSK3 *in vivo*, perhaps by both its direct and indirect actions on GSK3 (Klein and Melton, 1996; Chalecka-Franaszek and Chuang, 1999; De Sarno et al., 2002), disrupting the interaction between 5-HT<sub>1B</sub> receptors and GSK3 $\beta$  may have significant impact in the physiological functions of 5-HT<sub>1B</sub> receptors and relevant therapeutic effects in neuropsychiatric diseases.

The above postulation has been tested now in mouse brain tissues using small GSK3 inhibitors. When mouse cerebral cortical slices are pre-treated with the GSK3 inhibitors 6-bromindirubin-3'-oxime (BIO) or kenpaullone, both inhibitors concentration-dependently abolish the inhibitory effect of 5-HT<sub>1B</sub> receptor agonist anpirtoline on forskolin-stimulated cAMP, whereas neither affects the effect of 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT on cAMP (Chen et al., 2009). This finding in brain tissues is consistent with findings in cultured cells, where ablation or inhibition of GSK3 $\beta$  selectively abolishes 5-HT<sub>1B</sub> receptor-mediated G $\alpha$ -cAMP signaling. Furthermore, pre-treatment of cortical slices with the GSK3 inhibitors AR-A014418 (Bhat et al., 2003) and BIP-135 (Gaisina et al., 2009) completely abolish the inhibitory effect of anpirtoline on potassium-evoked <sup>3</sup>H-5-HT release (Chen et al., 2011). Since negatively regulating 5-HT release is a characteristic function of 5-HT<sub>1B</sub> autoreceptors in the axon terminals of 5-HT neurons (Trillat et al., 1997; Riad et al., 2000; Sari, 2004), this finding suggests that active GSK3 plays a role to rapidly resets the surge of 5-HT to baseline, which may result in termination



of a physiological or behavioral action of 5-HT. In reverse, inhibition of GSK3 may be important in maintaining 5-HT at a sufficient level that allows 5-HT to deliver sustained physiological action. The signaling pathways mediating 5-HT<sub>1B</sub> autoreceptor-induced inhibition of 5-HT release have never been confirmed. In a study conducted in 5-HT<sub>1B</sub> receptor-expressing cardiac ventricle myocytes, inhibition of 5-HT release by 5-HT<sub>1B</sub> receptors was found to be a pertussis toxin-sensitive G<sub>i</sub>-mediated effect, where the effect was not mediated by cAMP, but by an inwardly rectifying potassium channel (Ghavami et al., 1997). However, pertussis toxin-dependent 5-HT release by 5-HT<sub>1B</sub> receptors in the brain has not been confirmed. It should therefore be noted that although GSK3 inhibitors abolish the effect of 5-HT<sub>1B</sub> receptors on G<sub>i</sub>-associated signalings and 5-HT release, the latter is not necessarily due to the impaired 5-HT<sub>1B</sub> receptor-G<sub>i</sub>α coupling.

### EFFECT OF GSK3 INHIBITORS ON 5-HT<sub>1B</sub> RECEPTOR-REGULATED BEHAVIORS

If GSK3 $\beta$  differentially affects 5-HT<sub>1B</sub> receptor-mediated signal transduction and brain physiological function, it would be expected that GSK3 inhibitors may also differentially affect 5-HT<sub>1B</sub> receptor-regulated behaviors. Indeed, intracerebroventricular infusion of GSK3 inhibitor AR-A014418 or BIP-135 prior to systemic administration of anpirtoline significantly facilitates the anti-immobility effect of the 5-HT<sub>1B</sub> receptor agonist in the tail suspension test (Chen et al., 2011). In this behavioral test, low doses of GSK3 inhibitors and anpirtoline are used so that GSK3 inhibitors alone has no effect and anpirtoline alone only mildly reduces the immobility, which highlights the combination effect of GSK3 inhibition and 5-HT<sub>1B</sub> receptor activation. Finding of this study suggests that GSK3 inhibitors facilitate the anti-immobility effect of 5-HT<sub>1B</sub> heteroreceptors by abolishing 5-HT<sub>1B</sub> autoreceptor-mediated inhibition of serotonin release. Interestingly, the anpirtoline-induced increase in horizontal locomotor activity was not altered by GSK3 inhibitors, but is largely diminished in  $\beta$ -arrestin2-knockout mice (Chen et al., 2011), which is in agreement with the *in vitro* findings showing that association of  $\beta$ -arrestin2 with 5-HT<sub>1B</sub> receptors does not depend on GSK3 $\beta$ . Therefore, these initial behavior data further suggest that GSK3 has differential effects in modulating 5-HT<sub>1B</sub> receptor functions in brain.

Taken together, molecular and *in vivo* studies support a prominent effect of GSK3 $\beta$  to selectively regulate 5-HT<sub>1B</sub> receptor function (Figure 4). The effect of GSK3 $\beta$  is elicited by directly interacting with 5-HT<sub>1B</sub> receptors at the intracellular loop-2. This interaction functions to facilitate 5-HT<sub>1B</sub> receptor-regulated G<sub>i</sub>α-mediated signaling. In the absence of GSK3 $\beta$ , 5-HT<sub>1B</sub> receptors are dissociated from the conventional G<sub>i</sub>α-mediated signaling, but still able to respond to agonist-induced recruitment of  $\beta$ -arrestin2 and receptor internalization. Regulation of 5-HT<sub>1B</sub> receptors by GSK3 $\beta$  has functional significance since it impacts 5-HT<sub>1B</sub> receptor-dependent 5-HT release. Although this and other physiological functions of the highly selective modulation of 5-HT<sub>1B</sub> receptors by GSK3 $\beta$  remain to be investigated in further detail, initial evidence supports a significant behavioral impact of this GSK3 action that may be important for therapeutic development. Several important questions remain to be addressed before the

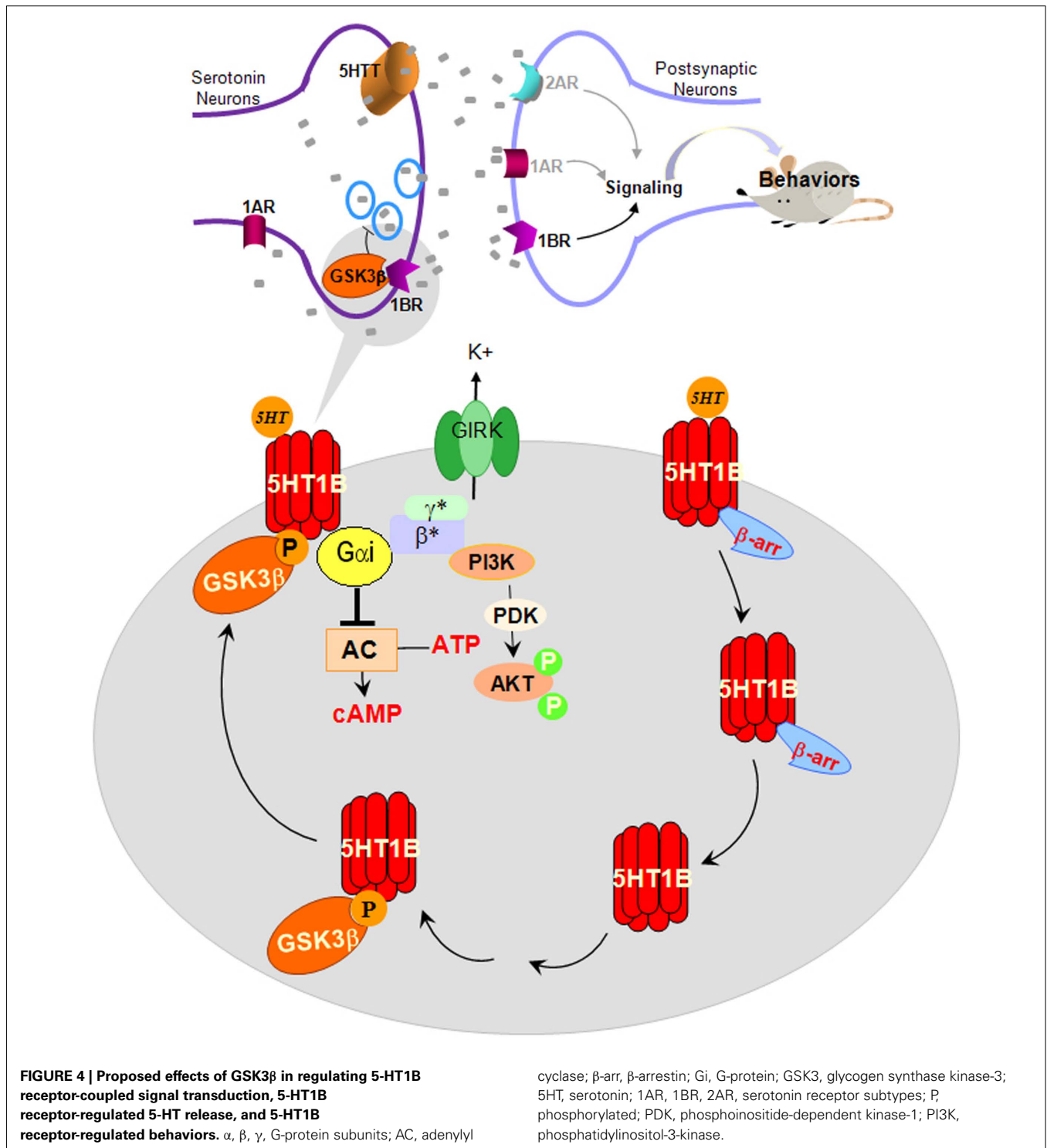
importance of this unique effect of GSK3 $\beta$  is completely elucidated. Particularly important is to understand if the effect of GSK3 $\beta$  is selective to 5-HT<sub>1B</sub> autoreceptors only or both auto- and hetero-receptors, which 5-HT<sub>1B</sub> receptor-regulated signaling mechanisms in the brain are affected by GSK3 $\beta$ , and if the GSK3 $\beta$ -dependent signaling pathways correlate with the effect of GSK3 $\beta$  on the physiological and behavioral functions of brain 5-HT<sub>1B</sub> receptors.

### SUMMARY

Cumulative evidence from *in vitro* measurements, pharmacological studies, and animal behavioral tests strongly support GSK3 as an integrative mediator of 5-HT neurotransmission. First, GSK3 is an early intracellular responder to a surge of brain 5-HT, which results in GSK3 phosphorylation at an N-terminal serine and inactivation of its constitutive activity. Phosphorylation of GSK3 by increasing brain 5-HT may be therapeutically important as the behavioral effect of fluoxetine is mediated by GSK3 phosphorylation. Second, GSK3 is a down-stream target of 5-HT<sub>1A</sub> receptor signaling, and enhancing GSK3 phosphorylation by 5-HT<sub>1A</sub> receptors in the hippocampus is associated with its inhibitory effect on contextual fear learning. Third, 5-HT<sub>2A</sub> receptors elicit sophisticated regulatory effect on GSK3, as both agonist and antagonist of 5-HT<sub>2A</sub> receptors may inactivate it, but the effects likely depend on the receptor activation state and the presence of other receptor interactive proteins. Regulation of GSK3 by 5-HT<sub>2A</sub> receptors may have therapeutic implication as evidenced by the prominent inhibitory effect of atypical antipsychotics on GSK3, presumably related to the 5-HT<sub>2A</sub> receptor antagonistic effect of these drugs. Finally, GSK3 $\beta$  interacts with and modulates 5-HT<sub>1B</sub> receptor activity in a receptor subtype- and signaling pathway-selective manner. Although the physiological impact of this action in intact brain remains to be elucidated, it is predicted that this action of GSK3 $\beta$  is to rapidly reset the level of 5-HT in certain brain areas to ameliorate prolonged effect of 5-HT, whereas the therapeutic implication of this unique action of GSK3 remains to be further studied.

The prominent effect of GSK3 on serotonin neurotransmission may partially explain the many findings of behavioral actions of GSK3 as well as it being a converging target of mood stabilizers, antidepressants, and antipsychotics (Li and Jope, 2010). Integrating 5-HT neurotransmission by GSK3 may be particularly indicative in the pathophysiological roles of GSK3 in mood, anxiety, and cognitive disorders that involve dysregulation of 5-HT neurotransmission, and in the therapeutics targeting GSK3 for the treatment of these diseases.

However, our current knowledge on this potentially important function of GSK3 is limited. GSK3 has only been found to associate with 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, and 5-HT<sub>2A</sub> receptors. Evidence showing GSK3 as a down-stream target or a modulator of other 5-HT receptor subtypes is still lacking. As discussed in this review, the two isoforms of GSK3 appear to have common and unique relations to different 5-HT receptors, but it is not yet clear if the divergent relationship is receptor subtype- or brain region-specific. Additional investigations are also necessary to fully understand how altered activity of GSK3 affects 5-HT-regulated behaviors, and to determine the mechanisms of how each behavior is affected by



altered GSK3 activity. Since GSK3 is a protein kinase, an important task is to identify specific protein substrates that mediate the divergent physiological and behavioral effects of GSK3 within and beyond 5-HT neurotransmission. Human studies are needed to determine if clinical findings support the ample preclinical evidence suggesting a role of GSK3 in 5-HT dysregulation and related brain disorders. Finally, all the ongoing and future research tasks

should converge to developing GSK3 modulators that are able to substitute, facilitate, or supplement therapeutic drugs that are known to modulate 5-HT neurotransmission.

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