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A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health

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Mental illnesses, such as bipolar disorder, attention-deficit/hyperactivity disorder, depression and schizophrenia are a major public health concern worldwide. Several pharmacologic agents acting on monoamine neurotransmission are used for the management of these disorders. However, there is still little understanding of the ultimate molecular mechanisms responsible for the therapeutic effects of these drugs or their relations with disease etiology. Here I provide an overview of recent advances on the involvement of the signalling molecules Akt and glycogen synthase kinase-3 (GSK3) in the regulation of behaviour by the monoamine neurotransmitters dopamine (DA) and serotonin (5-HT). I examine the possible participation of these signalling molecules to the effects of antidepressants, lithium and antipsychotics, as well as their possible contribution to mental disorders. Regulation of Akt and GSK3 may constitute an important signalling hub in the subcellular integration of 5-HT and DA neurotransmission. It may also provide a link between the action of these neurotransmitters and gene products, like disrupted in schizophrenia 1 (*DISC1*) and neuregulin (*NRG*), that are associated with increased risk for mental disorders. However, changes in Akt and GSK3 signalling are not restricted to a single disorder, and their contribution to specific behavioural symptoms or therapeutic effects may be modulated by broader changes in biologic contexts or signalling landscapes. Understanding these interactions may provide a better understanding of mental illnesses, leading to better efficacy of new therapeutic approaches.

Introduction

Neurotransmission mediated by the monoamines dopamine (DA) and serotonin (5-HT) is a major target for psychiatric drugs. Reuptake inhibitors that elevate synaptic 5-HT levels are commonly used for the treatment of major depression and other mood disorders,¹ whereas medications blocking 5-HT_{2A} receptors have antipsychotic effects.² Likewise, the first generation of antipsychotic drugs, such as haloperidol and chlorpromazine, are potent blockers of D2-class DA receptors,^{3,4} whereas psychostimulants, like amphetamine and methylphenidate, that are used for the treatment of attention-deficit/hyperactivity disorder (ADHD) elevate DA tones.

Most DA neurons in the brain have their cell bodies in the substantia nigra pars compacta and ventral tegmental area

that project to the striatum, nucleus accumbens and frontal cortex. Most 5-HT neurons are located in the raphe nuclei and send projections to multiple brain regions, including the striatum, hippocampus and frontal cortex.

Multiple control mechanisms regulate the activity of monoaminergic synapses. Briefly, DA and 5-HT are synthesized in presynaptic neurons, stored into vesicles by the vesicular monoamine transporter and released to the synapse in response to action potentials.⁵ In the adult brain, the rate-limiting enzymes for the synthesis of DA and 5-HT are tyrosine hydroxylase and tryptophan hydroxylase 2 (Tph2), respectively.⁶⁻⁹ Following release, neurotransmitter molecules stimulate postsynaptic receptors. The duration of this stimulation and concentration of neurotransmitters at the synapse are tightly regulated by transporters, such as the DA transporter

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(DAT) and the 5-HT transporter, that reuptake neurotransmitters from the extracellular space to the cytoplasm of presynaptic neurons where they will be stored into vesicles.^{5,10} The intensity of neurotransmitter release and synthesis are also regulated by autoreceptors on presynaptic neurons. There are 6 DA receptors and more than 15 5-HT receptors (Table 1). With the exception of 1 class of 5-HT receptors (5-HT₃ receptors), all of these are 7-transmembrane domain proteins coupled to heterotrimeric G proteins. These G protein-coupled receptors (GPCRs) exert most of their known actions on signalling by modulating the production of different second-messenger molecules (Table 1).

Although understanding the effects of psychoactive drugs on DA and 5-HT neurotransmission has allowed the development of drugs targeting key molecules, such as monoamine transporters, more selectively,¹² there is still little information concerning the involvement of these neurotransmitter systems in the etiology of mental disorders. For example, several genetic studies have focused on the role of DA receptor dysfunction in human disorders.^{13–18} However, as noted in 2000 by Wong and colleagues,¹⁹ “while there are some evidences that polymorphisms and mutations in [DA] receptors can alter functional activity and pharmacological profiles, no conclusive data link these gene variants to drug response or disease.” Unfortunately, this situation has not changed much over the past 10 years.

Instead of establishing a clear link with monoamine neurotransmission, genetic studies of mental disorders conducted over the last decade identified several polymorphisms in genes, such as disrupted in schizophrenia 1 (*DISC1*)²⁰ and neuregulin 1 (*Nrg1*),²¹ that are not obviously related to DA or 5-HT neurotransmission. This suggests that drugs acting on 5-HT or DA neurotransmission may exert their therapeutic effects in mental disorders by “normalizing” cell signalling mechanisms that are also affected by genetic or environmental factors in people with these disorders. Recent lines of evidence from my colleagues and I, as well as other groups, have indicated that both DA and 5-HT exert part of their actions on behaviour by modulating the activity of glycogen synthase kinase-3 (GSK3) and signalling molecules, such as

Akt, that are involved in its regulation. Interestingly, this signalling pathway is also regulated by different types of psychiatric drugs, including antipsychotics, antidepressants and lithium.²² Furthermore, several proteins encoded by genes associated with mental disorders affect the activity of this signalling pathway. In this brief review, I provide an overview of research by my colleagues and I on the characterization of the regulation of Akt and GSK3 signalling by DA and 5-HT. I also examine the possible role of these molecules as subcellular integrators for the effects of DA and 5-HT in mental disorders and the action of psychiatric drugs.

The Akt–GSK3 signalling pathway

Glycogen synthase kinase-3 and Akt, also known as protein kinase B, are serine threonine kinases that were initially identified as playing a role in the regulation of glycogen synthesis in response to insulin receptor stimulation.^{23–25} Over the years, these molecules were shown to be involved in a host of normal and pathologic processes, including the regulation of glycogenesis, cellular proliferation, programmed cell death, embryogenesis and circadian entrainment.^{26–29}

There are 3 isoforms of Akt that are encoded by separate human genes, *AKT1* (position 14q32.32), *AKT2* (position 19q13.1-q13.2) and *AKT3* (position 1q44). All of these isoforms are activated in response to phosphoinositide-3 kinase (Pi3K)-mediated signalling following the stimulation of various cell surface receptors like GPCRs and receptor tyrosine kinases (e.g., the brain-derived neurotrophic factor receptor TrkB and the Nrg1 receptor ErbB). Activation of Pi3K signalling results in the activation of Akt1 following its phosphorylation on the Thr308 and Ser473 residues.³⁰ Akt is also negatively regulated by protein phosphatases, notably protein phosphatase 2A (PP2A) that can inactivate Akt in vivo.^{31–33}

Glycogen synthase kinase-3 isoforms are among the most extensively studied substrates of Akt. There are 2 isoforms of GSK3 (GSK3 α and GSK3 β) that are encoded by different human genes, *GSK3A* (position 19q13.2) and *GSK3B* (position 3q13.3).³⁴ These proteins are constitutively active serine/threonine kinases that are negatively regulated by Akt and other serine/threonine kinases through phosphorylation of serine residues on their amino-terminal domain — Ser21 for GSK3 α and Ser9 for GSK3 β .^{28,35}

Regulation of Akt and GSK3 by DA

The various functions of DA receptors have been primarily associated with the regulation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) via G protein-dependent signalling (Table 1).¹¹ The D1 class receptors are mostly coupled to G α s and stimulate cAMP production and the activity of PKA. In contrast, D2 class receptors are coupled to G α i/o to inhibit the production of cAMP and thus diminish PKA activity.^{36–39} There is evidence that some neuronal populations can coexpress both receptor types with an effect on signalling responses.^{40–42} However, several independent studies using whole-gene regulated fluorescent reporters in

Table 1: Dopamine and serotonin receptor classes

Receptor class	Receptors	Coupled to	Localization
Dopamine			
D1 class	D1 and D5	G α S (cAMP \uparrow)	Postsynaptic
D2 class	D2L, D2S, D3, D4	G α i/o (cAMP \downarrow) β arrestin-2 (D2, D3)	Pre- and postsynaptic
5-HT ₁	5-HT _{1A-F} , 5-HT ₅	G α i/o (cAMP \downarrow)	Pre- and postsynaptic
5-HT ₂	5-HT _{2A-C}	G α q	Postsynaptic and non-neuronal
5-HT ₃	5-HT _{3A-C}	Ion channel	Postsynaptic and non-neuronal
5-HT others	5-HT ₄ , 5-HT ₆ , 5-HT ₇	G α S (cAMP \uparrow)	Postsynaptic and non-neuronal

5-HT = serotonin; cAMP = cyclic adenosine monophosphate.
For reviews of 5-HT and dopamine receptors see Hoyer et al.¹ and Beaulieu and Gainetdinov.¹¹

bacterial artificial chromosome transgenic mice have shown that striatal neurons appear to express preferentially either D1 or D2 receptors (Fig. 1).^{43–45}

Studies by my colleagues and I of cell signalling in response to elevated DA identified a reduction of Akt phosphorylation/activity along with concomitant activation of both GSK3 α and GSK3 β in the striatum of mice lacking the DAT (DAT knockout mice).^{46,47} Administration of amphetamine, methamphetamine or the nonselective DA receptor agonist apomorphine to normal mice also results in an inhibition of Akt activity and concomitant activation of GSK3, whereas striatal DA depletion has the opposite effect, thus firmly establishing the regulation of the Akt–GSK3 pathway by DA.^{31,46,48,49} Further characterization of these signalling responses using selective D1 and D2 receptor antagonists,⁴⁶ the antipsychotic haloperidol^{50,51} or mice lacking different subtypes of DA receptors have shown that Akt, GSK3 α and GSK3 β are regulated by D2 receptors.⁵² More specifically, D2 receptors appear to be essential for the inhibition of striatal Akt by DA in mice. Interestingly, mice lacking the D3 receptor display a reduced sensitivity of Akt-mediated signalling to dopaminergic drugs but retain the action of these drugs on Akt at higher doses. This suggests that D3 receptors also participate in the regulation of Akt–GSK3 signalling potentially by enhancing the D2 receptor-mediated DA response.⁵²

In the mouse striatum neither Akt nor GSK3 is affected by a direct modulation of cAMP levels, indicating that this signalling pathway is not controlled by canonical D2 receptor signalling⁴⁶ (Fig. 2). Instead, behavioural and biochemical evidence have revealed a role for β -Arrestin-2 (β Arr2), a multifunctional scaffolding/adaptor protein generally involved in GPCR desensitization in the regulation of the Akt–GSK3 pathway by D2 receptors.

Following GPCR stimulation, G protein-mediated signalling is inactivated by mechanisms that result in receptor de-

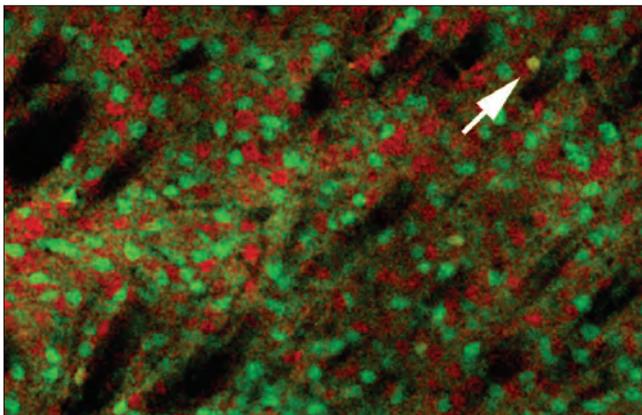


Fig. 1: Segregation of D1 and D2 dopamine (DA) expression in the dorsal striatum. Confocal microscopy images of striatal neurons in double bacterial artificial chromosome transgenic mice expressing the green fluorescent protein reporter gene under the control of the D2 DA receptor gene promoter and the (red) tomato fluorescent protein reporter gene under the control of the DA receptor gene promoter. The arrow points to a cell that expresses both reporter genes.

sensitization, internalization and termination of G protein-mediated signalling. G protein-coupled receptor activation induces the phosphorylation of the receptors and the recruitment of arrestins.^{53–55} Most mammalian tissues express 2 arrestins, β -Arrestin-1 and β Arr2.⁵³ The interaction of arrestins with GPCRs is followed by the recruitment of an endocytic complex, which results in the internalization of receptors.^{53,55–57} However, the role of arrestins in GPCR functions is not limited to desensitization. It has become apparent that in addition to activating G proteins, GPCRs can also elicit cellular responses mediated by the formation of signalling protein complexes held together by arrestins.^{58–60}

Research by my colleagues and I has shown that when administered to β Arr2 knockout mice, the DA drugs amphetamine and apomorphine fail to reduce Akt phosphorylation as they do in wild-type animals.³¹ Similarly, mice lacking both β Arr2 and the DAT do not display an inhibitory action of excessive DA on Akt phosphorylation. This demonstrates that D2 receptors regulate Akt through β Arr2.³¹ Further characterization of the mechanism by which β Arr2 regulates Akt in response to DA showed that stimulation of D2 receptors causes the formation of a protein complex composed at least of Akt, β Arr2 and PP2A, which facilitates the dephosphorylation and/or deactivation³² of Akt by PP2A in

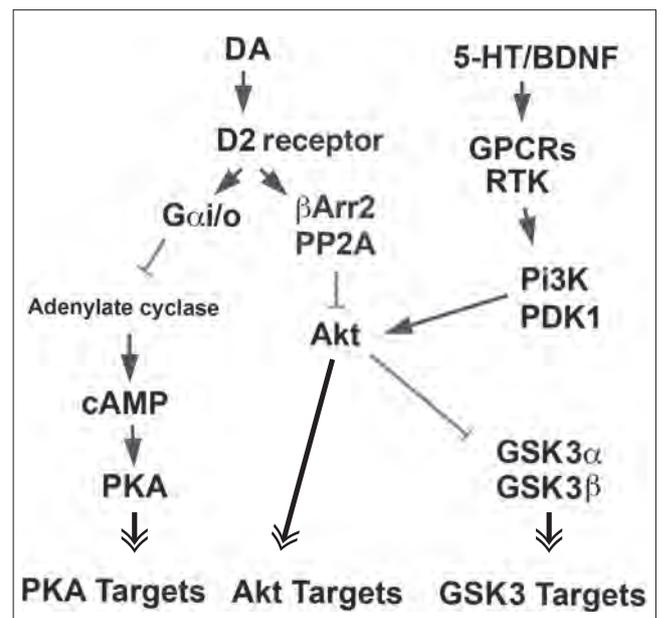


Fig. 2: Signalling networks regulated by dopamine (DA) in neurons responding to D2-class agonists. Regulation of G α s–cAMP–PKA and β Arr2–Akt–GSK3 signalling by D2 receptors. The action of other neurotransmitters, growth factors and neurotrophin has been included to illustrate the role of many of these intermediates as signal integrators. Single arrows: activation. Grey lines: inhibition. Double arrows: actions that can either be activatory or inhibitory in function of specific substrates. 5-HT = serotonin; β Arr2 = β -Arrestin-2; BDNF = brain-derived neurotrophic factor; cAMP = cyclic adenosine monophosphate; GPCR = G protein-coupled receptor; GSK3 = glycogen synthase kinase-3; PDK1 = 3-phosphoinositide-dependent kinase-1; PI3K = phosphoinositide-3 kinase; PKA = protein kinase A; PP2A = protein phosphatase 2A; RTK = receptor tyrosine kinase.

response to DA (Fig. 3A).^{31,46} This reduction of Akt activity leads to an activation of GSK3 in response to D2 receptor stimulation (Fig. 2).

Regulation of behaviour by the D2 receptor- β Arr2-Akt-GSK3 signalling pathway

There are several lines of evidence pointing toward a role of β Arr2, Akt and GSK3 in the regulation of DA-dependent behaviours. Under basal conditions,^{54,61} β Arr2 knockout mice are less active than wild-type littermates when placed in a novel environment, a behaviour that is mediated in part by DA.^{31,62-64} Outside of this habituation period, β Arr2 knockout mice are not overtly impaired in terms of DA functions, as would be the case for mice with more severe disruption of DA neurotransmission.^{11,65,66} However, a lack of β Arr2 affects behavioural responsiveness to different drugs acting on DA receptor functions. Mice lacking β Arr2 have reduced responses to the D1 and D2 receptor agonist apomorphine and to the DA-dependent actions of amphetamine and morphine.^{31,54,64} Furthermore, mice lacking both β Arr2 and the

DAT display a reduction of the typical novelty-induced hyperactivity phenotype of DAT knockout mice.³¹

In addition to behavioural deficits in β Arr2 knockout mice, several other observations support the involvement of the β Arr2-Akt-GSK3 pathway in the regulation of DA-related behaviours. Pharmacologic GSK3 inhibitors can reduce hyperactivity both in DAT knockout mice and in animals treated with amphetamine.^{46,67,68} Furthermore, observations of genetically engineered animals lacking one functional allele of *Gsk3 β* ⁶⁹ revealed that a 50% reduction of brain GSK3 β protein levels⁴⁶ reduces behavioural responsiveness to amphetamine.⁴⁶ Conversely, mutant mice lacking inhibitory phosphorylation sites on both GSK3 α and GSK3 β are hyperresponsive to amphetamine,⁷⁰ whereas transgenic mice overexpressing GSK3 β develop a hyperactivity phenotype reminiscent of hyperdopaminergic DAT knockout mice.⁷¹ Finally, Akt1 knockout mice show enhanced disruption of sensorimotor gating by amphetamine in prepulse inhibition tests.⁵⁰ Disruption of sensorimotor gating by amphetamine is commonly used as a behavioural paradigm to model psychosis in rodents, and this effect can be blocked by antipsychotics.⁷² Since Akt1 is inhibited following the stimulation of D2 receptors, the increased behavioural effect of amphetamine in Akt1 knockout mice supports the involvement of Akt inhibition in DA-related behavioural responses. However, DA regulates more than locomotion and sensorimotor gating.⁷³ Further detailed characterization of DA-related behaviours in more specific tests in rodents or nonhuman primates may thus be warranted to fully understand the contribution of β Arr2, Akt and GSK3 in the expression of DA-associated behaviours.

Regulation of GSK3 by 5-HT

Investigations of *drosophila* have shown that the fly ortholog of the 5-HT_{1A} receptors regulates shaggy, the ortholog of GSK3, in the insect brain.⁷⁴ Regulation of shaggy by 5-HT_{1A} receptors in the fly is important for the control of circadian rhythms. In mice, different classes of drugs acting on 5-HT neurotransmission, such as selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors and tricyclic antidepressants, inhibit GSK3 β by increasing its phosphorylation in the frontal cortex, hippocampus and striatum.⁷⁵⁻⁷⁷ In addition, mice with about 80% reduction in 5-HT synthesis resulting from the expression of a Tph2 R439H loss-of-function mutant (equivalent to the human R441H Tph2 variant^{49,78}) display increased GSK3 activity in the frontal cortex.⁷⁹

Two classes of serotonin receptors, 5-HT₁ and 5-HT₂, appear to be involved and play opposing roles in regulating GSK3 β activity.^{75,76} Administration of the 5-HT₁ agonist 8-OH-DPAT or of the 5-HT₂ antagonist LY53857 to wild-type mice both result in increased GSK3 β phosphorylation in the brain.⁷⁶ This suggests that 5-HT can inhibit GSK3 β by acting through 5-HT₁ receptors, whereas activation of 5-HT₂ receptors would result in GSK3 β activation. However, it should be mentioned that the 5-HT_{2A} agonist DOI and the 5-HT_{1A} antagonist WAY100635 did not affect GSK3 β activity in the brain,⁷⁶ thus leaving the question of the relative contribution of different 5-HT receptors to the regulation of GSK3 β only partially addressed.

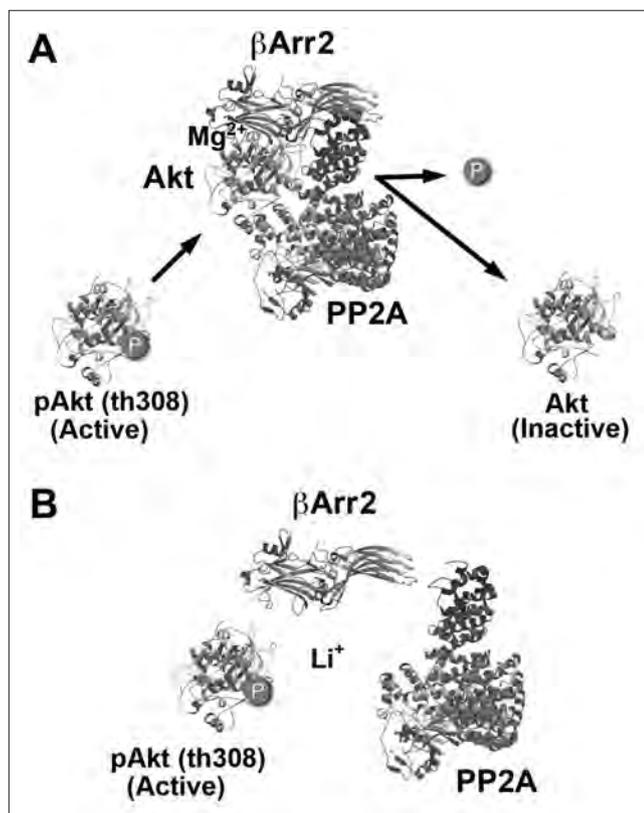


Fig. 3: Model for arrestin-dependent inhibition of Akt and its regulation by lithium (Li^+). **(A)** Under basal conditions, Akt phosphorylation/activation is the result of an equilibrium between activation of Akt by phosphorylation and its inactivation by protein phosphatase 2A (PP2A)-mediated dephosphorylation. Dephosphorylation of Akt is facilitated by formation of the magnesium (Mg^{2+})-dependent scaffolding of Akt and PP2A by β -Arrestin-2 (β Arr2). **(B)** By displacing magnesium, lithium destabilizes the signalling complex formed by Akt, β Arr2 and PP2A, thereby enhancing Akt activity by reducing its dephosphorylation.

There are also contradictory observations concerning the involvement of Akt in the regulation of GSK3 by 5-HT receptors. Some studies reported this regulation mechanism to be independent from Akt following the administration of SSRIs.^{47,75} In contrast, a more recent study has indicated that administration of D-fenfluramine, which increases brain serotonergic tones, activates Akt in the mouse brain.⁸⁰ One possible explanation for these discrepancies is that older studies monitored the phosphorylation of Ser473 as an indicator of Akt activity, whereas the more recent study followed the phosphorylation of the Thr308 residue of Akt. Since the phosphorylation of Akt on Thr308 appears to be critical for the regulation of GSK3 by Akt,⁸¹ it is possible that the more recent study may be more representative of the role of Akt in the regulation of GSK3 by 5-HT. However further studies of the mechanisms through which 5-HT regulates Akt and GSK3 activity *in vivo* may be needed to firmly establish this possibility.

A role for GSK3 in the regulation of behaviour by 5-HT

There is also evidence for a role of GSK3 in the regulation of 5-HT-mediated behaviours. Mice treated with GSK3 inhibitors⁶⁷ or different genetically modified mice with altered GSK3 function display behavioural changes that are reminiscent of normal animals following antidepressant treatment. Glycogen synthase kinase-3 β haploinsufficient mice have been shown to exhibit an antidepressant-like response in the Porsolt forced swimming test and the tail suspension test.^{63,82,83} Furthermore, mutant mice lacking GSK3 α and GSK3 β inhibitory phosphorylation sites display a general reduction of anxiety and increased exploratory behaviour in several tests that are generally used to evaluate the effects of drugs acting on 5-HT neurotransmission, indicating that regulation of GSK3 amino-terminal domain phosphorylation by Akt may play a role in the regulation of these behaviours.⁸⁴

To assess the contribution of GSK3 β to 5-HT-regulated behaviours, mice expressing the R439H Tph2 loss-of-function mutation were bred with haploinsufficient GSK3 β animals⁶⁹ to produce mice carrying a different allelic combination of the wild-type and mutant *Tph2* gene and with mice of the same *Tph2* genotypes with reduced GSK3 β expression. Mice from these different genotypes were evaluated in several behavioural tests designed to analyze 5-HT-mediated emotional states in rodents. Results from these experiments showed that a reduction of about 50% in the expression of GSK3 β (about 25% of total GSK3) is sufficient to rescue behavioural deficits induced by reduced 5-HT synthesis in the tail suspension test and in a test used to measure anxiety-like behaviours in mice.⁷⁹ Interestingly, the reduction of GSK3 β expression also curbed the display of aggressive behaviours in 5-HT-depleted mice,⁷⁹ suggesting a role of GSK3 in the regulation of social behaviours and aggression by 5-HT. That being said, 5-HT and GSK3 have both been implicated in the regulation of several other behavioural manifestations beyond the regulation of mood, anxiety and social interactions. Mice expressing the R439H Tph2 variant with normal or reduced expression of GSK3 β should provide an

interesting tool to clarify the roles played by GSK3 in the regulation of 5-HT-related behaviours in the future. However, this model will need to be combined with other pharmacologic and genetic engineering approaches to firmly establish a link of causality between changes in GSK3 activity and behavioural modifications resulting from changes in 5-HT neurotransmission.

Akt and GSK3 signalling in the actions of antipsychotics

Several psychoactive drugs have been shown to modulate the activity of the Akt-GSK3 signalling pathway (Fig. 4).²² Drugs like SSRIs and MAO inhibitors that elevate 5-HT synaptic transmission have been shown to inhibit GSK3 *in vivo*.^{75,76} Conversely, drugs that elevate DA neurotransmission (e.g., amphetamine) reduce the inhibitory phosphorylation of GSK3 α and GSK3 β and therefore increase the activity of these kinases.^{46,70,85} By blocking D2 receptors, classic antipsychotics can prevent the inhibition of Akt and concomitant activation of GSK3 by DA. Such regulation of Akt and GSK3 activity has been reported in mice after chronic treatment with haloperidol.⁵⁰

Acute and chronic treatments with atypical antipsychotics, including clozapine, olanzapine, quetiapine, ziprasidone and risperidone, have also been shown to result in an inhibition of GSK3 in the rodent brain.^{75,86} The effect of atypical antipsychotics on GSK3 activity can be explained in part by the D2 receptor antagonist action of atypical antipsychotics. An elegant study conducted using bioluminescence energy transfer in cell lines has suggested that although the effects of different atypical antipsychotics on G protein-mediated D2 receptor signalling may differ, all of these drugs share the common property of blocking the recruitment of β Arr2 to this GPCR.⁸⁷ Unfortunately, this study did not directly address the effect of these different drugs on the β Arr2-mediated regulation of Akt and GSK3 by DA in the brain, and a few alternative mechanisms may also explain the modulation of GSK3 activity by these drugs *in vivo*. For instance, in addition to their effects on D2 receptors, atypical antipsychotics are also antagonists of 5-HT_{2A} receptors¹² and may interfere with the regulation of GSK3 by 5-HT.^{75,77} In addition, a handful of studies also suggest a role for the disheveled Wnt signalling pathway in the regulation of GSK3 activity by antipsychotics.^{51,88} Therefore, there may be more than 1 mechanism through which antipsychotic drugs affect GSK3 activity in a given cell or in different neuronal populations. Further characterizations of the role of these mechanisms and of possible functional cross-talk between them at the cellular and system levels may thus be important to understand the possible implication of GSK3-mediated signalling in the effects of these drugs.

Akt and GSK3 signalling in the actions of lithium

Akt and GSK3 have also been associated with the action of the mood stabilizer lithium (Fig. 4). Lithium concentrations of

2 mM are known to inhibit GSK3 β in vitro.^{89,90} However, these lithium concentrations are higher than the 0.5–1.5 mM lithium serum concentrations used for the treatment of bipolar disorders.⁹¹ In the mouse brain, both acute and chronic lithium treatments inhibit GSK3 indirectly by activating Akt and increasing the regulatory amino-terminal domain phosphorylation of GSK3.^{46,92} We have shown that in the mouse striatum, this indirect effect of lithium on the activity of GSK3 can result from the disruption of a signalling complex composed of Akt, β Arr2 and PP2A.⁶³ This protein complex mediates, among other potential roles, some of the D2 receptor signalling functions (Fig. 2, Fig. 3B).^{31,63} When administered to β Arr2 knockout mice, lithium does not affect Akt–GSK3 signalling as it does in normal animals. Furthermore, several behavioural effects of chronic lithium treatments in tests used to model mania or antidepressant drug effects in rodents are abolished in mice lacking β Arr2.⁶³

It has long been suggested that lithium may exert some of its pharmacologic effects by competing with magnesium,

which acts as a cofactor for several enzymes, including kinases such as GSK3.^{93,94} The molecular mechanism through which lithium interferes with the formation of the Akt– β Arr2–PP2A signalling complex appears to involve an interference with a biologic function of magnesium ions (Fig. 3).⁶³ Initial investigations by my colleagues and I have shown that increased concentrations of magnesium, but not of other monovalent or divalent cations, can prevent the disruption of preassembled Akt– β Arr2–PP2A complexes by lithium in vitro.⁶³ Furthermore, magnesium salts were shown to be required for the interaction between recombinant Akt1 and β Arr2, whereas addition of lithium disrupted this same interaction in the presence of magnesium.⁶³ This suggests that magnesium contributes to the interaction of Akt and β Arr2 and that lithium competition with magnesium can affect not only enzymatic activity but also the stability of higher-order protein complexes, thus providing a potential mechanism for the destabilization of the Akt– β Arr2–PP2A complex by lithium.

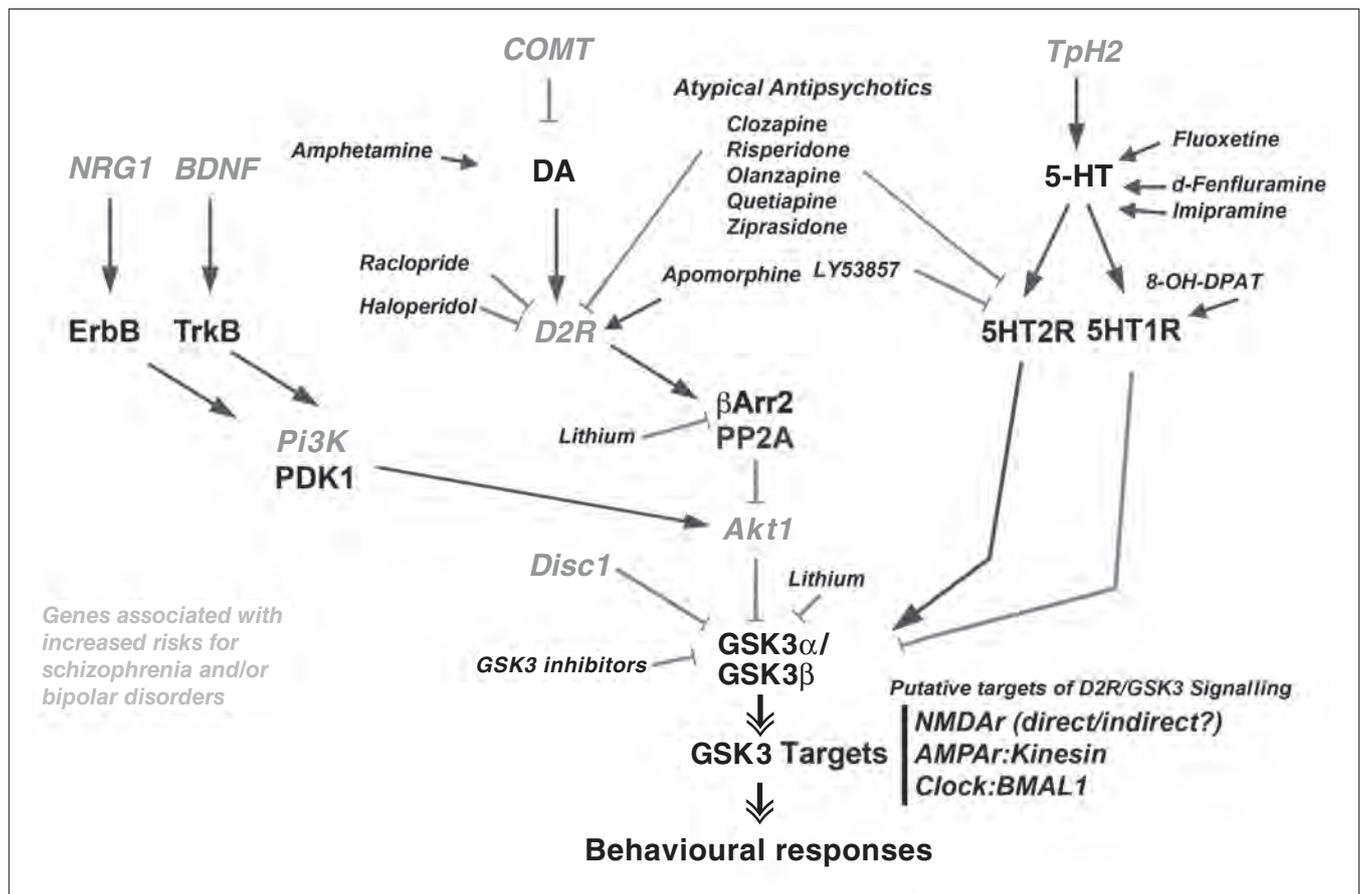


Fig. 4: Regulation of Akt–GSK3 signalling by psychoactive drugs and gene products associated with mental disorders. Proteins are the product of genes associated with increased risk for schizophrenia and/or bipolar disorders. Behavioural changes in dopaminergic responses have been reported in Akt1 knockout and β -Arrestin-2 (β Arr2) knockout mice and in GSK3 β haploinsufficient mice. Single arrows = activation; solid lines = inhibition; double arrows = actions that can either be activatory or inhibitory in function of specific substrates. 5-HT = serotonin; BDNF = brain-derived neurotrophic factor; BMAL1 = brain and muscle Arnt-like protein-1; Clock = circadian locomotor output cycles kaput; COMT = catechol-O-methyltransferase; DA = dopamine; Disc1 = disrupted in schizophrenia 1; GSK3 = glycogen synthase kinase-3; NMDA = *N*-methyl-D-aspartate; NRG1 = neuregulin 1; PDK1 = 3-phosphoinositide-dependent kinase-1; Pi3K = phosphoinositide-3 kinase; PP2A = protein phosphatase 2A; TPH2 = tryptophan hydroxylase 2.

Regulation of Akt–GSK3 signalling in psychiatric disorders

There is converging evidence indicating that genetic risk factors for mental illnesses may affect Akt–GSK3 signalling. Significant association of *AKT1* haplotypes with schizophrenia and/or bipolar disorder has been reported in several independent cohorts.^{22,50,95–98} Lymphocytes and postmortem brain samples from patients with schizophrenia have also been reported to have reduced Akt protein levels compared with those of controls.⁵⁰ In contrast, the direct association of *GSK3A* and *GSK3B* with psychiatric disorders is less clear. There is no clinical evidence demonstrating an association between *GSK3A* and psychiatric disorders. As for *GSK3B*, isolated reports have indicated an association between a –50T/C polymorphism in the *GSK3B* promoter and responsiveness to lithium therapy or the occurrence of psychotic symptoms in patients with mood disorders.^{22,96} Recent evidence also indicates that temporal lobe grey matter volume in people with schizophrenia is associated with a *GSK3B* polymorphism.⁹⁹

Although the evidence for a direct role of *GSK3A* or *GSK3B* mutations is scarce, there are several indications that the regulation of GSK3 is altered in psychiatric disorders. Reduced levels of phosphorylated GSK3 β have been found in post-mortem frontal cortex samples from some individuals with schizophrenia.^{50,100} These variations in phosphorylation can be caused in part by reduced Akt expression. However, other products from genes associated with mental disorders are also involved in the regulation of GSK3. Genetic variants of *TPH2*, *DISC1* and *NRG1* have been associated with the pathogenesis of schizophrenia and/or bipolar disorder^{101,102} and can affect the Akt–GSK3 signalling pathway in various experimental models.^{79,103–105} In addition, significant epistatic interaction has been found between an *AKT1* variant and a functional polymorphism (Val158Met) in the catechol-O-methyltransferase (*COMT*) gene that is associated with schizophrenia,¹⁰⁶ therefore suggesting complex interactions between genetic variants associated with mental disorders and proteins involved in Akt–GSK3 signalling.

Conclusion

Research conducted by my colleagues and I, as well as other groups, has uncovered several biochemical, genetic, behavioural and pharmacologic evidence for a contribution of the Akt–GSK3 signalling pathway in the treatment and etiology of psychiatric disorders. However, our understanding of this role is certainly far from complete. For instance, there is still little knowledge about the exact nature of the neural circuits involved in the regulation of behaviour by these kinases. The possible involvement of Akt and GSK3 in signalling evoked by the monoamines norepinephrine and histamine or by active monoamine degradation products such as 3-methoxytyramine¹⁰⁷ has also remained unexplored. Furthermore, the molecular mechanisms through which this pathway exerts its various actions in the brain are not well understood beyond the simple control of GSK3 activity.

Several recent research efforts have established a link be-

tween ionotropic glutamate receptor functions and GSK3 activity in the hippocampus and frontal cortex.^{108–110} This is particularly intriguing since altered glutamate receptor functions have long been suspected to play a role in mental disorder pathology, mostly in schizophrenia.^{73,111} However, it would be premature to conclude that the role of Akt–GSK3 signalling is restricted to the regulation of glutamate receptor functions. Over the last 5 years, Akt and GSK3 have also been associated with several other mechanisms that can potentially be involved in schizophrenia, depression or bipolar disorders. Among these, it is of interest that Akt is involved in the regulation of the mammalian target of rapamycin¹¹² and of the forkhead box transcription factors.⁸⁰ Furthermore, there is strong evidence for a role of GSK3 in the regulation of circadian rhythm, epigenetic gene regulation²⁹ and 5-HT_{1B} receptor cell surface trafficking.^{113,114}

Another intriguing observation is that changes in the regulation of Akt and GSK3 activity so far are similar across different drug categories. Overall, antipsychotics, antidepressants acting on 5-HT neurotransmission and lithium have all been reported to activate Akt and inhibit GSK3 in vivo. Interestingly, the mood stabilizers valproate and lamotrigine have also been reported to inhibit GSK3 under certain conditions, but the mechanisms underlying this effect and their behavioural significance are not clear.^{91,115} Furthermore, direct inhibition of GSK3 isoforms, either through pharmacologic or genetic means, has been shown to have effects that are similar to some of those of antidepressants, lithium or antipsychotics in different behavioural tests conducted in rodents.^{22,68,82} Yet these drugs do not share the same clinical profile in humans. It is possible that inhibition of GSK3 in specific neuronal populations in response to a given drug could be associated with distinct behavioural outcomes. Activation of Akt and inhibition of GSK3 may also represent core effects for some shared action of psychoactive drugs, which may explain their increased efficacy in the context of combination therapies.^{22,91,116,117} In this case, a modulation of Akt–GSK3 signalling may contribute to the effect of a drug while not being its exclusive component. The complete clinical profile of a given drug would then result not from the inhibition or activation of a single signalling pathway but from a more complex modulation of the overall cell signalling landscape^{118,119} in different neuronal populations.

A similar observation can be made for the potential contribution of Akt and GSK3 to the etiology of mental disorders. Several studies suggest that activation of GSK3 can be a shared outcome of several susceptibility genes for mental disorders. However, whereas dysregulation of these genes can lead to a reduced expression of Akt or affect the inhibition of GSK3 by *DISC1*, the disease specificity of such changes is unclear (e.g., association with schizophrenia, bipolar disorders and depression in the case of *DISC1*¹²⁰ and with bipolar disorders and schizophrenia in the case of *AKT1*⁹⁷), whereas their contribution to disease development is not always confirmed across studies.^{120,121} It is a truism that several factors must simultaneously contribute to psychiatric illnesses.^{122–124} It can be postulated that a modulation of GSK3 activity may represent a core risk factor that increases the probability for a psychiatric

illness in a given individual under certain genetic and environmental conditions. An analogous situation exists in cancer biology where “double hit” or “multiple hit” models predict that accumulation of germinal and somatic mutations in some genes regulating cell proliferation and survival is ultimately responsible for disease progression.^{125,126} In these models, the gain or loss of function of a single signalling protein contributes to disease causation in a nonobligatory and nonexclusive fashion. Similar types of models involving the contribution of various genetic and environmental “hits” have also been put forward for schizophrenia.^{122,123} Therefore, in the present state of knowledge, it is probable that an activation of GSK3 is one of several factors that can contribute to mental disorders. However, it is not possible at the moment to conclude that the deregulation of a given signalling protein or pathway is essential or sufficient for the occurrence of a specific psychiatric disease.

Whereas many questions remain to be answered about the role of Akt and GSK3 in mental disorders, it remains important that existing data strongly indicate that regulation of Akt and GSK3 constitute an important signalling hub in the subcellular integration of 5-HT and DA neurotransmission with the functions of genes that are associated with mental disorders. Understanding these interactions may provide a better understanding of mental illnesses in general, leading to new therapeutic approaches having superior efficacy and lesser side effects.

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