Glycogen synthase kinase 3 beta alters anxiety-, depression-, and addiction-related behaviors and neuronal activity in the nucleus accumbens shell

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**ABSTRACT**

Psychiatric disorders such as anxiety, depression and addiction are often comorbid brain pathologies thought to share common mechanistic biology. As part of the cortico-limbic circuit, the nucleus accumbens shell (NAcSh) plays a fundamental role in integrating information in the circuit, such that modulation of NAcSh circuitry alters anxiety, depression, and addiction-related behaviors. Intracellular kinase cascades in the NAcSh have proven important mediators of behavior. To investigate glycogen-synthase kinase 3 (GSK3) beta signaling in the NAcSh in vivo, we knocked down GSK3beta expression with a novel adeno-associated viral vector (AAV2) and assessed changes in anxiety- and depression-like behavior and cocaine self-administration in GSK3beta knockdown rats. GSK3beta knockdown reduced anxiety-like behavior while increasing depression-like behavior and cocaine self-administration. Correlative electrophysiological recordings in acute brain slices were used to assess the effect of AAV-shGSK3beta on spontaneous firing and intrinsic excitability of tonically active interneurons (TANs), cells required for input and output signal integration in the NAcSh and for processing reward-related behaviors. Loose-patch recordings showed that TANs transduced by AAV-shGSK3beta exhibited reduction in tonic firing and increased spike half width. When assessed by whole-cell patch clamp recordings these changes were mirrored by reduction in action potential firing and accompanied by decreased hyperpolarization-induced depolarizing sag potentials, increased action potential current threshold, and decreased maximum rise time. These results suggest that silencing of GSK3beta in the NAcSh increases depression- and addiction-related behavior, possibly by decreasing intrinsic excitability of TANs. However, this study does not rule out contributions from other neuronal sub-types.

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1. Introduction

Mood disorders such as anxiety and depression have high comorbidity with drug addiction in humans (Pettinati et al., 2013). Dysregulation of the reward system may constitute a common molecular mechanism of these neuropsychiatric disorders and therefore understanding complex neuroadaptations common to neuropsychiatric disorders constitutes a promising avenue for therapeutics.

The nucleus accumbens is heavily implicated in the control of emotional behavior and reward (Pontieri et al., 1995; Pliakas et al.,...
2001; Green et al., 2006; Nestler and Carlezon, 2006; Larson et al., 2011). As part of the ventral striatum, the nucleus accumbens has as its sole output two major populations of medium spiny neurons (MSNs) whose activity is modulated by a population of tonically active interneurons (TANs), which are mostly cholinergic (Lenz and Lobo, 2013). Despite comprising not more than 5% of the total population of neurons in the NAc, TANs play important roles in reward prediction, task attention, memory, addiction, and aversive behaviors (Aosaki et al., 1994; Apicella, 2002; Anagnostaras et al., 2003; Furey et al., 2008; Williams and Adinoff, 2008; Lenz and Lobo, 2013). TANs control MSN activity and are particularly responsive to salient reward-related stimuli (Morris et al., 2004). Early studies have provided evidence for a role of TANs in cocaine addiction with immunotoxin-mediated cell ablation resulting in increased sensitivity to cocaine (Hikida et al., 2001) and preventing behavioral abnormalities associated with cocaine induced by centrally active acetylcholinesterase inhibitors in the NAc (Hikida et al., 2003). More recently, studies using optogenetics confirmed that selective inhibition of TANs results in suppression of cocaine induced behaviors (Witten et al., 2010), further confirming the pivotal role of these cells in reward behavior and addiction. At the circuit level, activation of TANs has been shown to elicit both fast glutamatergic transmission (Higley et al., 2011) and GABAergic inhibition of MSNs, the latter coincident with synchronous cholinergic activation and sufficient to pause MSNs firing (English et al., 2012). These studies suggest a complex role of these cells in the NAc circuit that deserves further investigation.

Intracellular kinase signaling cascades, activated through a variety of mechanisms, have proven important mediators of NAcSh function, and by extension, the etiology of neuropsychiatric disorders. Specifically, the ERK/MAPK, PKA, and PKC signaling cascades have been studied in the NAcSh with success (Self et al., 1998; Schroeder et al., 2008; Ortinski et al., 2015). The AKT/GSK3β pathway has also garnered particular attention for its role in dopamine signaling, the actions of antipsychotic drugs, and even responses to addictive drugs, especially in the nucleus accumbens (Perrine et al., 2008; Beaulieu et al., 2009; Nwaneshiudu and Unterwald, 2010; Beaulieu et al., 2011; Wilkinson et al., 2011; Miller et al., 2014).

GSK3β was originally discovered for its role in glycogen synthesis but has since been implicated in a variety of cellular processes (Wildburger and Laezza, 2012), and dysregulation of this kinase has been implicated in bipolar disorder and neurodegenerative disorders (Grimes and Jope, 2001; Jope, 2011). One of the mechanisms of action of lithium, the commonly prescribed mood stabilizer, is inhibition of GSK3β (Klein and Melton, 1996; Stambolic et al., 1996). Heterozygous GSK3β knockout mice show reductions in depression-like behavior similar to the effects of lithium (O’Brien et al., 2004). Drugs of abuse, especially cocaine, can modulate levels of GSK3β in the NAc (Perrine et al., 2008) and GSK3β is involved in cocaine-induced hyperactivity, cocaine sensitization, cocaine reward memory, and cocaine conditioned place preference (Miller et al., 2009, 2014; Shi et al., 2014). Previous studies indicate that the role of GSK3β is highly dependent on brain region and even cell type as global knockdown may not have the same effects as regional or even cell-type specific knockdown (Latapy et al., 2012; Urs et al., 2012; Zhou et al., 2012). Thus, GSK3β has therapeutic potential for comorbid depression and addiction, but knowledge gaps exist on its brain region specific mechanism of action. The environmental enrichment manipulation combines novelty, exercise, and social contact to produce robust protective depression and addiction phenotypes (Green et al., 2002, 2010). Enrichment increases the ratio of phosphorylated (inactive) to total GSK3β in the hippocampus and cortex (Hu et al., 2013) and environmental enrichment is able to reverse cognitive deficits caused by constitutively active expression of GSK3 in mice (Pardo et al., 2015). Therefore GSK3 may be involved in protecting against depression and addiction phenotypes.

The role of GSK3β in cocaine self-administration, the addiction paradigm with the most face validity, is so far lacking. Additionally, few studies have examined anxiety and depression behaviors along with addiction-related behaviors in the same animals. The current study therefore explores anxiety-like, depression-like, and addiction-related behaviors in the same animals following knockdown of GSK3β in the NAcSh of rats.

In order to analyze the role of GSK3β specifically in the NAcSh in behavior relevant to affective disorders and drug addiction, we designed and constructed a novel adeno-associated viral vector (AAV2) which uses RNA interference to knockdown GSK3β in adult rats and allows for prolonged knockdown of GSK3β in the adult brain. The AAV2 serotype infects neuronal cells in vivo but is not specific to any one neuronal cell type. To provide correlative functional outcomes to the behavioral studies we investigated the role of GSK3β on spontaneous firing and intrinsic excitability of tonically active neurons (TANs), comparing electrophysiological properties of these neurons between GSK3β knockdown vs. control.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan, Houston, TX) were obtained at 21–days-old (electrophysiology) or 225–250 g (behavior) and maintained in a controlled environment (temperature, 22 °C; relative humidity: 50%; 12 h light/dark cycle, lights on 0600 h) in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved colony in standard polycarbonate cages with ad libitum access to food and water except during surgery and behavioral experiments. All surgical procedures and experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and approved by The University of Texas Medical Branch Institutional Animal Care and Use Committee.

2.1.1. Timeline of behavior following GSK3β knockdown

Rats in the behavioral cohort underwent stereotaxic vector injection one week after arrival. Three weeks later, rats underwent a battery of behavioral tests beginning with spontaneous behaviors (order of tests: elevated plus maze, sucrose neophobia, locomotor activity, social contact, sucrose preference, cold stress defecation) for two weeks, followed by food regulation to 85% body weight over one week and sucrose pellet responding for three weeks. Rats were subjected to one behavioral test at a time and anxiety and appetitive tests occurred prior to drug tests. Rats were then implanted with an indwelling jugular vein catheter and after one week of recovery, behavioral experiments resumed with drug self-administration (acquisition, maintenance, dose response, progressive ratio, and reinstatement). Rats were between 225 and 250 g on arrival and the average weight on the day of the stereotaxic vector injections was 295 g (average 293 g controls, 296 g for shGSK3β). Average weight after spontaneous behaviors and before food restriction prior to sucrose pellet responding was 425 g (avg. 424 g for controls, 425 g for shGSK3β). Average rat weight on the day of catheter implantation was 445 g (447 g for controls, 445 g for shGSK3β). Following drug-self administration, animals were anesthetized, decapitated, and the placement of the vector was verified. See Fig. 2A for timeline of behavioral testing.

2.1.2. In vivo knockdown of GSK3β

In order to knockdown GSK3β in vivo, rats were anesthetized with isoflurane (VetEquip, Pleasanton, CA) and injected bilaterally
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