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Rho-family GTPases, Neuronal Plasticity, and Depression

Depression constitutes a spectra of symptoms that adversely affect an individual's cognitive, emotional, motivational, and physiological well-being; this collection of heterogenous symptoms and pathologies is termed major depressive disorder (MDD), and for as many as 40% of individuals suffering from MDD, medications are not able to provide sufficient and/or long-lasting therapeutic relief[1,2]. Functional imaging studies and neuropathological studies with post-mortem human brains implicate dysfunction in the nucleus accumbens (NAc) and ventral tegmental area (VTA), critical nuclei in the brain's dopaminergic (DAergic) reward pathways[2-6]. The VTA consists of a major population of DA neurons which primarily innervates the NAc[2,5]. Dysfunctional neurophysiology and plasticity in these nuclei likely contribute to some of the symptoms of MDD, specifically loss of pleasure and motivation (anhedonia)[2-6]. Anhedonia is studied using chronic social defeat stress (CSDS) which elicits depression-associated behaviors (e.g., reduced social interactions, increased anhedonia, negative body weight changes) in 70% of mice (i.e., termed stress-susceptible) while the remaining 30% do not undergo these adverse behavioral changes (i.e., termed stress-resilient)[2,7].

The structure and function of DAergic neurons in VTA and the DA receptor 1 (D1R)- and D2R-expressing NAc neurons are remodeled during CSDS[2,8-16]. Stress-induced anhedonia to model depression-like behavior results in reduced spontaneous excitatory inputs to D1R-expressing MSNs, whereas D2R-expressing MSNs undergo the opposite change[17,18]. D1R-expressing (but not D2R) accumbal neurons also display reduced dendritic arborization (morphological plasticity), an important parameter in excitatory neurotransmission for the MSNs as dendritic spines are the primary site of excitatory synapses[8,10-12,19,20].

Stress-induced models of MDD involve changes (i.e., plasticity) in dendritic spine morphology, density, and/or synaptic function[2,12,21,22]. Indeed, CSDS reduces the dendritic complexity and total dendritic length of D1R-expressing, but not D2R-expressing, MSNs in the NAc[23] (Fig. 1). Such changes in dendrite and spine morphologies require dynamic remodeling of the actin cytoskeleton. Rho-family GTPases (e.g., Rho, Rac, and Cdc42) regulate the morphogenesis and remodeling of actin-based neuronal structures such as spines[24-28]. In general, Rho inhibits, whereas Rac/Cdc42 promote, spine morphogenesis and development[2,24,29,30]. The above observations prompted the natural question: what role do Rho-family GTPases have in CSDS-induced neuronal plasticity in NAc neurons? In D1R-expressing MSNs, gene expression of RhoA, its primary downstream effector, Rho-

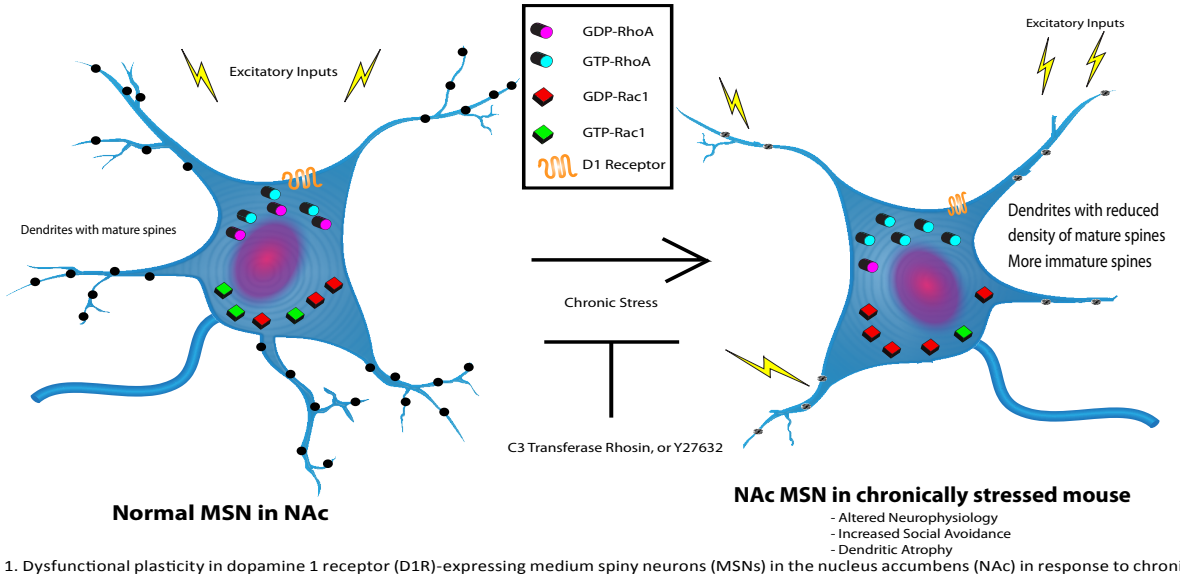

Fig. 1. Dysfunctional plasticity in dopamine 1 receptor (D1R)-expressing medium spiny neurons (MSNs) in the nucleus accumbens (NAc) in response to chronic social defeat stress (CSDS). Following CSDS exposure, D1R-expressing MSNs in NAc display elevated levels of active RhoA, decreased excitatory inputs, hyperexcitability, dendritic atrophy (loss of complexity and mature spines, sometimes decreased density), increased social avoidance in animals containing these neurons, and decreased levels of active Rac1. The negative neurophysiological, behavioral, and structural spine changes are blocked or reversed by Rho and ROCK inhibitors (e.g., C3 transferase, rhosin, Y27632).

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kinase (ROCK), and the Rho GEF Arhgef1 significantly increased[23]. Notably, no other GTPase's gene profile changes. Intra-accumbal inhibition of RhoA with the C3 transferase enzyme prevents stress-induced social avoidance while intra-accumbal activation of RhoA enables a subthreshold stress treatment to produce social avoidance[23]. Similarly, the small-molecule, selective RhoA inhibitor rhosin reverses chronic stress-induced increases in RhoA activity and hyperexcitability and reduces spontaneous excitatory inputs to D1R-expressing MSNs in NAc[31] (Fig. 1). In addition, rhosin enhanced the density of dendritic spines (sites of excitatory neurotransmission)[31]. Prevention of RhoA over-activation confers resistance to stress-induced deficiencies in neuronal function and dendritic structure[31] (Fig. 1). Concomitant with stress-induced social avoidance and altered electrophysiological activity in D1R-expressing MSNs is the loss of dendritic arborization, total dendritic length, and reduced number of branch points along the dendrites. Over-activation of RhoA following CSDS is also responsible for these deleterious changes in overall dendrite morphology[23]. Furthermore, in CSDS-treated mice, similar restoration of social behavior and dendritic complexity to that of control mice is observed following systemic injection of the ROCK inhibitor, Y27632, for 7 days[23] (Fig. 1).

Contrary to a CSDS-mediated increase in RhoA expression and activity in the NAc, Rac1, another Rho-family GTPase, is down-regulated at the transcriptional and translational level specifically in the NAc after CSDS[19]. Of the Rho-family GTPases examined, only Rac1 displays stress-induced transcriptional regulation in CSDS-susceptible (but not resilient) mice that correlates with increased social avoidance behavior[19] (Fig. 1). Rac1 transcript levels are also reduced in the NAc of post-mortem brains of non-medicated MDD patients[19]. The CSDS-mediated reduction in Rac1 transcript and protein levels is via epigenetic regulation with a decrease in permissive acetylation of the Rac1 promoter in susceptible, but not resilient, mice. Furthermore, susceptible mice also have enhanced methylation directly upstream of the promoter and resilient mice have decreased methylation[2,19]. Intra-accumbal inhibition of class 1 HDACs reverses the Rac1 down-regulation and the associated social avoidance behavior. Similarly, in NAc from post-mortem brains of depressed human subjects, Rac1 gene expression is reduced[19]. Rac1 is responsible for the formation and maturation of spines (i.e., morphogenesis) on the MSNs of NAc via its regulation of cofilin-mediated remodeling of the actin cytoskeleton[2,24,28-30]. Concomitant with down-regulation of Rac1 mRNA and protein, social avoidance, and anhedonia following CSDS is formation of immature stubby dendritic spines on accumbal MSNs that co-localize with cofilin[19]. Rescue of Rac1 protein levels is correlated with reversing social avoidance behavior and the development of such spines[19].

Summary

Successful treatment of MDD is hampered by several factors, including treatment resistance, multi-symptoms and neurobiological systems involved, and a high rate of remission[1,2]. It is unlikely that a one-size-fits-all-therapeutic-approach will yield success, thus necessitating the need to explore signaling cascades associated with all of the neurobiological systems involved in depression. To this end, Cytoskeleton offers purified cytoskeletal proteins, functional assays, signal transduction reagents, GTPase activation assays, antibodies, live cell imaging probes, and kits to quantify endogenous levels of post-translational modifications in cells and tissues.

G-LISA Activation Assay Kits

Product	Assays	Cat. #
RhoA G-LISA Activation Assay (Luminescence format)	96	BK121
RhoA G-LISA Activation Assay Kit (Colorimetric format)	96	BK124
Rac1,2,3 G-LISA Activation Assay (Colorimetric format)	96	BK125
Rac1 G-LISA Activation Assay (Luminescence format)	96	BK126
RhoA / Rac1 / Cdc42 G-LISA Activation Assay Bundle 3 Kits	3 kits 24 per kit	BK135
Total RhoA ELISA	96	BK150

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Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol each	CY-SC006
SiR700-Actin Kit Includes SiR-Actin and Verapamil	690 / 720 nm	35 nmol	CY-SC013

Acti-Stain Phalloidins

Product	Amount	Cat #
Acti-stain 488 phalloidin	300 Slides	PHDG1-A
Acti-stain 555 phalloidin	300 Slides	PHDH1-A
Acti-stain 670 phalloidin	300 Slides	PHDN1-A
Rhodamine Phalloidin	1 x 500 ul	PHDR1