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Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology

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ABSTRACT

Introduction: Adaptogens are natural compounds or plant extracts that increase adaptability and survival of organisms under stress. Adaptogens stimulate cellular and organismal defense systems by activating intracellular and extracellular signaling pathways and expression of stress-activated proteins and neuropeptides. The effects adaptogens on mediators of adaptive stress response and longevity signaling pathways have been reported, but their stress-protective mechanisms are still not fully understood.

Aim of the study: The aim of this study was to identify key molecular mechanisms of adaptogenic plants traditionally used to treat stress and aging-related disorders, i.e., Rhodiola rosea, Eleutherococcus senticosus, Withania somnifera, Rhaponticum carthamoides, and Bryonia alba.

Materials and methods: To investigate the underlying molecular mechanisms of adaptogens, we conducted RNA sequencing to profile gene expression alterations in T98G neuroglia cells upon treatment of adaptogens and analyzed the relevance of deregulated genes to adaptive stress-response signaling pathways using *in silico* pathway analysis software.

Results and discussion: At least 88 of the 3516 genes regulated by adaptogens were closely associated with adaptive stress response and adaptive stress-response signaling pathways (ASRSPs), including neuronal signaling related to corticotropin-releasing hormone, cAMP-mediated, protein kinase A, and CREB; pathways related to signaling involving CXCR4, melatonin, nitric oxide synthase, GP6, Gαs, MAPK, neuroinflammation, neuropathic pain, opioids, renin–angiotensin, AMPK, calcium, and synapses; and pathways associated with dendritic cell maturation and G-coupled protein receptor–mediated nutrient sensing in enteroendocrine cells. All samples tested showed significant effects on the expression of genes encoding neurohormones CRH, GNRH, UCN, G-protein–coupled and other transmembrane receptors TLR9, PRLR, CHRNE, GP1BA, PLXNA4, a ligand-dependent

Abbreviations: AKAP3, A-kinase anchoring protein 3; BA, *Bryonia alba* extracts (EPB-1); BDNF, brain-derived neurotrophic factor; BS, *Boswellia serrata* extract (BosPure); CACNA1I, calcium voltage-gated channel subunit alpha1 I; CACNA2D2, calcium voltage-gated channel auxiliary subunit alpha2delta 2; CACNA2D3, calcium voltage-gated channel auxiliary subunit alpha2delta 3; CAMK1G, calcium/calmodulin dependent protein kinase IG; CHRM4, cholinergic receptor muscarinic 4; CHRNE, cholinergic receptor nicotinic epsilon subunit; CL, *Curcuma longa* extract (Curcugreen, Curamed); CL-BS, fixed combination of CL and BS (Curamin); CNGB1, cyclic nucleotide gated channel beta 1; CREB, cyclic AMP response element binding protein; CREBBP, CREB binding protein; CRH, ccorticotropin releasing hormone; DAG, diacylglycerol; ES, *Etherococcus senticosus* extract (ESE-2); FOS, Fos proto-oncogene, AP-1 transcription factor subunit; FOXO6, forkhead box O6; GNRH1, gonadotropin releasing hormone 1; GPCR, GTP-binding protein coupled receptors; GUCY1A2, guanylate cyclase 1 soluble subunit alpha 2; HSPA6, heat shock protein family A (Hsp70) member 6; IGF1, insulin-like growth factor 1 receptors; IP3, inositol triphosphate; IR, insulin receptors; KRT19, keratin 19; LIPE, lipase E, hormone sensitive type; M, melatonin; MAPK10, mitogen-activated protein kinase 10, *syn.* JNK3, SAPK1b; MAPK13, mitogen-activated protein kinase 13, *syn.* P38 δ, SAPK4; M-WS, fixed combination of M and WS (Adaptra PM); NTFR, neurotrophic factors receptors; NTRK2, neurotrophic receptor tyrosine kinase 2, syn. BDNF receptor, neurotrophic tyrosine kinase, receptor, type 2, Nrh2, TRKB1; PDE3B, phosphodiesterase 3B; PDE9A, phosphodiesterase 9A; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PLC, phospholipase C; PRKCH, protein kinase C eta; PTPRD, protein tyrosine phosphatase, receptor type D; PTPRR, protein tyrosine phosphatase, receptor type R; RAPGEF4, Rap guanine nucleotide exchange factor 4; RC, *Rhaponticum cartamoides* extract (EPL-1)); RORA, RAR related orphan receptor A (*RZR*); RR, *Rhodiola rosea* extract (EPR-7); RR-BA, fixed combination of BA and RR (EP-bar); RR-WS, fixed combination of RR and WS (Adaptra AM); RYR2, ryanodine receptor 2; STAT5A, signal transducer and activator of transcription 5A; STIP1, stress induced phosphoprotein 1; TLR9, toll like receptor 9, member of PI3K (complex); TTN, titin (Member Of: myosin-light-chain kinase; UCN, urocortin (Corticotropin-releasing factor family); VIPR2, vasoactive intestinal peptide receptor 2; WS, *Withania somnifera* extract (BSM-66, 5 mg/ml corresponding to dose of 300 mg); WSL, *Withania somnifera* extract (BSM-66, 1.5 mg/ml corresponding to low dose of 90 mg)

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nuclear receptor RORA, transmembrane channels, transcription regulators FOS, FOXO6, SCX, STAT5A, ZFPM2, ZNF396, ZNF467, protein kinases MAPK10, MAPK13, MERTK, FLT1, PRKCH, ROS1, TTN), phosphatases PTPRD, PTPRR, peptidases, metabolic enzymes, a chaperone (HSPA6), and other proteins, all of which modulate numerous life processes, playing key roles in several canonical pathways involved in defense response and regulation of homeostasis in organisms. It is for the first time we report that the molecular mechanism of actions of melatonin and plant adaptogens are alike, all adaptogens tested activated the melatonin signaling pathway by acting through two G-protein–coupled membrane receptors MT1 and MT2 and upregulation of the ligand-specific nuclear receptor RORA, which plays a role in intellectual disability, neurological disorders, retinopathy, hypertension, dyslipidemia, and cancer, which are common in aging. Furthermore, melatonin activated adaptive signaling pathways and upregulated expression of UCN, GNRH1, TLR9, GP1BA, PLXNA4, CHRM4, GPR19, VIPR2, RORA, STAT5A, ZFPM2, ZNF396, FLT1, MAPK10, MERTK, PRKCH, and TTN, which were commonly regulated by all adaptogens tested. We conclude that melatonin is an adaptation hormone playing an important role in regulation of homeostasis. Adaptogens presumably worked as eustressors ("stress-vaccines") to activate the cellular adaptive system by inducing the expression of ASRSPs, which then reciprocally protected cells from damage caused by distress. Functional investigation by interactive pathways analysis demonstrated that adaptogens activated ASRSPs associated with stress-induced and aging-related disorders such as chronic inflammation, cardiovascular health, neurodegenerative cognitive impairment, metabolic disorders, and cancer.

Conclusion: This study has elucidated the genome-wide effects of several adaptogenic herbal extracts in brain cells culture. These data highlight the consistent activation of ASRSPs by adaptogens in T98G neuroglia cells. The extracts affected many genes playing key roles in modulation of adaptive homeostasis, indicating their ability to modify gene expression to prevent stress-induced and aging-related disorders. Overall, this study provides a comprehensive look at the molecular mechanisms by which adaptogens exerts stress-protective effects.

Introduction

Adaptogens are natural compounds or plant extracts that increase adaptability and survival of organisms to stress (Panossian, 2017). Like vitamins and antioxidants, adaptogens constitute a category of nutritional and herbal medicinal products ((Shikov et al., 2014). They exhibit anti-fatigue effects during stress and show promising preventive and therapeutic potential against age-related disorders (Panossian and Gerbarg, 2016; Panossian and Wikman, 2009, 2010, 2014). Adaptogens stimulate cellular and organismal defense systems, activating intracellular and extracellular signaling pathways and expression of stress-activated proteins and neuropeptides, and enhancing survival against oxidative stress (Panossian et al., 2013, 2014; Schriner et al., 2009, 2013). One strategy of therapeutic intervention in aging-associated diseases addresses adaptive signaling pathways that ameliorate and postpone aging by activation of genes involved in regulating the adaptive stress response (Calabrese et al., 2010a; Hunt et al., 2011; Li and Fukagawa, 2010; Matsson, 2008a; Murugaiyah and Mattson, 2015; Rattan et al., 2013; Son et al., 2008; Calabrese and Mattson, 2011). Our previous studies demonstrated the effects of some adaptogens on key mediators of stress response and modulation of longevity pathways (Panossian and Gerbarg, 2016; Panossian et al., 2013, 2014). Overall, more than 70 plants have been described as adaptogenic ((Panossian, 2017); however, it is unclear which are most characteristic and which molecular mechanisms of action they share (Asea et al., 2013).

The aim of this study was to identify key molecular mechanisms that are common for adaptogenic action and to provide a rationale for the use of adaptogens in stress- and age-related diseases. This work used gene expression profiling by transcriptome-wide mRNA sequencing in human T98G neuroglia cells followed by interactive pathway analysis of regulated genes. We selected five medicinal plants (*Rhodiola rosea* L. [RR], *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim [ES], *Withania somnifera* (L.) Dunal [WS], *Rhaponticum carthamoides* (Willd.) Iljin [RC], and *Bryonia alba* L. [BA]) traditionally used to treat stress- and aging-related disorders and commonly accepted as adaptogenic; two presumably adaptogenic substances (curcumin and melatonin, widely distributed in the plant kingdom); and one plant, *Boswellia serrata* Roxb. ex Colebr. [BS], that has never been considered as adaptogenic, despite multiple effects on the immune system and containing tetra- and pentacyclic terpenoids like those of adaptogenic *Withania, Bryonia*, Ginseng, and *Eleutherococcus*.

The choice of T98G neuroglia cells was justified in previous publications (Panossian et al., 2013; Spencea et al., 2011; Stein, 1979; Guzova et al, 2001; Su et al., 2012). Glia comprising approximately 90% of the CNS is serving as a transportation link between the bloodstream and neurons. It contributes to the neuroprotection of the brain through the expression of the innate immune response, promoting the clearance of neurotoxic proteins and apoptotic cells from the CNS as well as by regulating the entry of inflammatory systemic cells into the brain at the blood brain barrier, reducing axonal loss and gliosis (Spencea et al., 2011; Nguyen et al., 2002; Hauwell et al., 2005). This activates both tissue repair and the rapid restoration of tissue homeostasis. Glial cell expresses a variety of hormonal receptors, which play key roles in stress-induced disorders (Bennett, 2000). Physiological function of neuroglial cells include an uptake of neurotransmitters, synthesis and release of neurotrophic factors, immune regulation, modulation of synaptic activity, metabolic supply of energy and other substances (Henn and Hamberger, 1971; Haydon, 2001; Ullian et al., 2001, maintaining brain homeostasis – a function supposed to be the characteristic for adaptogens by definition.

Material and methods

Drugs and chemicals

Pharmaceutical grade dry extracts of *Curcuma longa* L. rhizome (Curcugreen; Arjuna Natural Ltd.; Keralia, India; $DER_{native,}$ 25:1, extraction solvent - ethylacetate, curcuminoids content - 95%), *Boswellia serrata* Roxb. ex Colebr gum resin (AKBAMAX ®; Arjuna Natural Ltd.; Keralia; DERnative, 10:1, extraction solvent – ethylacetate, consisting of 75% boswellic acids and 10% 3-O-acetyl-11-keto-boswellic acid, *Rhodiola rosea* L. roots and rhizome (EPR-7; $DER_{native,}$ 7:1, extraction solvent - ethanol 70%, rosavin content - 3.7%, salidroside content – 3.1%), *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim roots ($DER_{native,}$ 20:1, extraction solvent - ethanol 70%, eleutherosid E content −1.5%, eleutherosid B content - 1.2%), *Withania somnifera* (L.) Dunal roots (KSM-66; $DER_{native,}$ 12:1, extraction solvent – milk, withanloids content – 5.5%), *Rhaponticum cartamoides* (Willd.) Iljin roots ($DER_{native,}$ 12:1, extraction solvent - water, total ecdysteroids content – 1.0%) and *Bryonia alba* L. roots ($DER_{native,}$ 6.5:1, extraction solvents - ethanol 70% and cucurbitacin's content – 1.2%) were obtained from EuropharmaUSA Inc. (Green Bay, WI, USA). All were manufactured in

accordance with the ICHQ7A and EMEA guidelines for Good Agricultural and Collecting Practice and Good Manufacturing Practice of active pharmaceutical ingredients.

Working samples used in experiments were prepared by dilution of stock solutions Curcugreen (11.6 mg/ml, DMSO) and BosPure (5.0 mg/ml, DMSO) extracts with 10 volumes of phosphate buffered saline solution (PBS) to obtain working solutions. Working solutions of 50 µl were added to 9.9 ml of cell culture to obtain the same final concentrations of genuine extracts as in the incubation. The final concentrations of RR (3.3 µg/ml), WS (5.0 µg/ml), WSL (1.5 µg/ml), ES (1.0 µg/ml), RC (1.0 µg/ml), BA (0.25 µg/ml), CL (5.8 µg/ml), BS (2.5 µg/ml) extracts, melatonin (M, 0.08 µg/ml), the combinations CL-BS (8.3 µg/ml), RR-BA (3.55 µg/ml), RR-WS (8.3 µg/ml), M-WS (5.08 µg/ml), and (DMSO < 0.01%) in cell culture incubation media were compatible in all test samples used to treat T98G neuroglial cells for RNA sequencing experiments. These concentrations of the extracts correspond to human single doses of 5 mg of melatonin, 200 mg of EPR-7 (RR), 300 mg of KSM-66 (WS), 90 mg of KSM-66 (WSL), 305 mg of Adaptra PM (M-WS), 500 mg of Adaptra AM (RR-WS), 16 mg of EPB-1 (BA), 216 mg of EP-bar (RR-BA), 50 mg of EPE-2 (ES), 60 mg of EPL-1 (RC), 350 mg of BSM-95 (CL), 150 mg of BosPure (BS), 500 mg of Curamin (CL-BS). The final concentration of DMSO was the same in all incubated cell cultures (1%). The maximal concentrations of some active compounds of plant extracts in the blood stream and tissues might be 2-5-fold lower after oral single administration due to their limited bioavailability. However, their steady state level can increase after repeated multiple administrations, to the concentrations tested in these *in vitro* experiments.

mRNA sequencing

Transcriptome-wide RNA sequencing was performed as previously described by us (Kadioglu et al., 2016). T98G neuroglia cells were seeded and attached for 24 h prior to drug treatment. Cells were treated for 24 h at various combinations and concentration of drugs or DMSO as solvent control. The final concentrations of test samples are described in the section above. Then, total RNA was isolated using InviTrap Spin Universal RNA Mini kit (250) (Stratec Molecular, Berlin, Germany). Total RNA quality and quantity were evaluated using an Agilent Bioanalyser 2100 and Qubit Fluorometer (Life Technologies, Darmstadt, Germany). Poly A + RNA was isolated, fractionated and double-stranded cDNA was synthesized using the TruSeq RNA sample prep v2 protocol (Illumina Inc., San Diego, CA). End-repaired, A-tailed and adaptor-ligated cDNA was PCR-amplified by 10 cycles. The library was sequenced in paired-end mode (2 × 100 bp) using 0.4 lane of an Illumina HiSeq 2000 flow cell. Gene expressions were quantified using the RPKM measure (Mortazavi et al., 2008; Ferreira et al., 2014). RPKM values for transcripts and the ratios of transcripts were taken into consideration to calculate the overall RPKM value for each gene. The deregulation of genes was calculated by dividing overall RPKM values of genes in treated cells by those in non-treated cells. The technical performance of the RNA-sequencing experiments of the present study has been validated by quantitative real time RT-PCR as recently published (Seo et al., 2018).

Ingenuity pathway analysis

RNA sequencing data were analyzed using IPA (Ingenuity Systems®, www.ingenuity.com). IPA software relies on the Ingenuity Knowledge Base, a routinely updated database containing biological and chemical interactions and functional annotations gathered from the literature. For obtaining information about cellular functions, networks, and affected pathways, IPA offers the Core Analysis tool, which was used for all datasets. The predicted (z-score > 2) effects are based on changes of gene expression in the experimental samples relative to the control.

Results

Effects of herbal extracts on gene expression profiles in isolated neuroglia cells

Cultivated neuroglia T98G cells were treated with test substances for 24 h, and total RNA was isolated. We performed transcriptome-wide mRNA expression analyses to identify 3516 significantly deregulated genes (more than two-fold change compared to control) from 25 molecular networks (see Supplementary data 1. Molecules, Tables 1–3). The number of up- and downregulated genes varied in different test samples, ranging from 985 to 1907 (Supplementary data 1. Molecules, Table 4). Expression of 218 genes changed in response to almost all test extracts, (Supplementary data 1. Molecules, Table 5), including 88 genes specifically in response to RR, ES, and WS, which are generally accepted as adaptogenic (Supplementary data 1. Molecules, Table 8). A total of 75 genes encoded neurohormones (*CRH, GNRH1, UCN*), G-protein–coupled (*GPR158, GPR19, VIPR2*) and other transmembrane receptors (*TLR9, PRLR, CHRNE, GP1BA, PLXNA4*), ligand-dependent nuclear receptor (*RORA*), transmembrane channels (*RYR2, CACNA2D2, CNGB1, GPM6A, KCNAB1, KCNAB3, KCNB1, KCNIP4, JPH3*), transcription regulators (*FOS, FOXO6, SCX, STAT5A, ZFPM2, ZNF396, ZNF467*), protein kinases (*MAPK10, MAPK13, MERTK, FLT1, PRKCH, ROS1, TTN*), phosphatases (*PTPRD, PTPRR*), peptidases (*PAPPA2, TLL1*), metabolic enzymes (*GUCY1A2, LDHD, LIPE, PDE3B, PDE9A, PRKN, AOC3, CEL, DNAH11, GALNT6, RNF43, TRMT9B*), a chaperone (*HSPA6*), and other proteins (*e.g., KRT19, LAMA2, RAPGEF4, STIP1, TMEFF2, TNNC2, ANXA8L1, CRYGS, DEFB1, DNAH6, FLG, MAGEC1, MATN4, MUC20, MXRA5, PPP1R9A, PROK2, ZBTB37, ZNF483, ZNF571, ZNRD1ASP*) (Tables 1 and 2 and Supplementary data 1. Molecules, Table 7). Of these, 37 genes (Supplementary data 1a. Molecules in pathways and Table 9 in Supplementary data 1. Molecules. Table 9) are involved in adaptive stress-response signaling pathways (Tables 3 and 4). Thus, the core analysis of datasets obtained from experiments with test samples showed an overlap of molecules in the datasets ($p > 1.3$) to the 177 canonical pathways (Supplementary data 1b, 2, 2a, and 2b) and 25 molecular networks (Supplementary data 1. Molecules Table 3) linked to various cellular and physiological functions and diseases (Supplementary data 1b) in the QIAGEN Knowledge Database.

Further analysis revealed that adaptogens altered several adaptive stress-response pathways, suggesting that adaptogens are likely to interfere with signaling involving corticotropin releasing hormone (CRH), cAMP, protein kinase A (PKA), glucocorticoid receptor (GR), CREB in neurons, AMPK, NRF2 oxidative stress, dendritic cell maturation, melatonin, eNOS, GP6, GPCR-mediated nutrient sensing in enteroendocrine cells, Gαs, MAPK, neuroinflammation, neuropathic pain, opioid, renin–angiotensin, and synapses (Fig. 1, Table 4 and Supplementary data 2c). Table 3 shows the top canonical pathways that contained significant numbers of deregulated genes from datasets obtained in experiments with test samples, while Table 4 shows predicted activation ($z > 2$) or inhibition ($z < 2$) of the pathways. Table 4 shows that generally accepted adaptogens activate all adaptive signaling pathways except for CRH pathways, which can be activated or inhibited depending on test sample concentration. In contrast, curcumin and *Boswellia* extracts inhibited neuroinflammation (CL, BS), neuropathic pain (BS), opioids (CL), cAMP (CL), calcium (CL), and melatonin (BS, CL) signaling pathways (Table 4). It is noteworthy that melatonin activated all adaptive signaling pathways (Table 4) and upregulated expression of *UCN, GNRH1, TLR9, GP1BA, PLXNA4, CHRM4, GPR19, VIPR2, RORA, STAT5A, ZFPM2, ZNF396, FLT1, MAPK10, MERTK, PRKCH*, and *TTN*, which were commonly regulated by all adaptogens (Table 2 and Supplementary data 2c). Furthermore, all adaptogens activated the melatonin signaling pathway, while BS inhibited this pathway (Table 4), presumably through a lack of effect on expression of the gonadotropin-releasing hormone–encoding gene *GNRH* (Fig. 2). Similar effects of melatonin and “classical” adaptogen ES were observed on canonical

Table 1
Genes regulated by generally recognized adaptogen - ES, RR and WS. Upregulated genes are in red color while down-regulated genes – in blue color text (See References: Hahm et al., 2014; Han et al., 2003; He et al., 2017; Jung et al., 2007; Kim et al., 2012; Li et al., 2016; Mondal et al. 2010; Sa et al., 2015; Tu et al., 2018; Wang et al., 2013; Xia et al., 2016; Yan et al., 2014; Yu and Kim, 2013; Zang et al. 2016; Zeng et al., 2015;).

Type(s)	Symbol	Entrez Gene Name	Path-ways	Plant name	Reference
Hormones	*CRH*	corticotropin releasing hormone		Schisandra chinensis and Rhodiola rosea	Xia et al.,2016
	UCN	**urocortin (Corticotropin-releasing factor family)**			
	GNRH1	**gonadotropin releasing hormone 1**			
Transmembrane receptor	*TLR9*	**toll like receptor 9, member of PI3K (complex),**	**152**	Eleutherococcus	Han et al., 2003
	CHRNE	**cholinergic receptor nicotinic epsilon subunit**			
	PRLR	**prolactin receptor**			
	GP1BA	glycoprotein Ib platelet alpha subunit			
	PLXNA4	plexin A4			
G-protein coupled receptors	*CHRM4*	**cholinergic receptor muscarinic 4**			
	GPR19	G protein-coupled receptor 19			
	VIPR2	**vasoactive intestinal peptide receptor 2**			
Nuclear receptor	*RORA*	**RAR related orphan receptor A (RZR)**			
Transcription regulators	*STAT5A*	**signal transducer and activator of transcription 5A**			
	FOS	**Fos proto-oncogene, AP-1 transcription factor subunit**	**21**	Schisandra chinensis and Rhodiola rosea	Xia et al., 2016
	FOXO6	**forkhead box O6**			
	SCX	scleraxis bHLH transcription factor			
	ZFPM2	zinc finger protein, FOG family member 2			
	ZNF396	zinc finger protein 396			
	ZNF467	zinc finger protein 467			
Kinases	*FLT1*	**fms related tyrosine kinase 1**			
	MAPK10	**mitogen-activated protein kinase 10, JNK-3**	**77**	Withaferin A Withanolide D Rhapontic um Eleutherococcus Notoginseno side R1	Hahm et al., 2014; Yu &Kim, 2013 Mondal et al. 2010 He et al., 2017 Jung et al., 2007 Tu et al., 2018
	MAPK13	**mitogen-activated protein kinase 13, p-38**	**59**	Withaferin A Withanolide D Acanthoic acid Ginseng Schisantherin A Notoginseno side R1 Salidroside	Hahm et al., 2014; Yu &Kim, 2013 Mondal et al. 2010 Kim et al., 2012 Li et al., 2016 Sa et al., 2015 Tu et al., 2018 Wang et al., 2013; Yan et

(*continued on next page*)

Table 1 (*continued*)

					al., 2014; Zang et al. 2016; Zeng et al., 2015;
	MERTK	MER proto-oncogene, tyrosine kinase			
	PRKCH	**protein kinase C eta**	**72**		
	ROS1	ROS proto-oncogene 1, receptor tyrosine kinase			
	TTN	**titin (Member Of: myosin-light-chain kinase_**			
Phosphatases	***PTPRD***	**protein tyrosine phosphatase, receptor type D**			
	PTPRR	**protein tyrosine phosphatase, receptor type R**			
Metabolic enzymes	***GUCY1A2***	**guanylate cyclase 1 soluble subunit alpha 2**	**19**		
	HSPA6	**heat shock protein family A (Hsp70) member 6**			
	LDHD	**lactate dehydrogenase D**			
	LIPE	**lipase E, hormone sensitive type**			
	PDE3B	**phosphodiesterase 3B**	**16**		
	PDE9A	**phosphodiesterase 9A**			
	PRKN	**parkin RBR E3 ubiquitin protein ligase**			
	AOC3	**amine oxidase, copper containing 3**			
	CEL	**carboxyl ester lipase**			
	DNAH11	dynein axonemal heavy chain 11			
	GALNT6	polypeptide N-acetylgalactosaminyltransferase 6			
	RNF43	ring finger protein 43			
	TRMT9B	tRNA methyltransferase 9B (putative)			
Peptidases	*PAPPA2*	pappalysin 2			
	TLL1	tolloid like 1			
Transporter	*CPLX1*	complexin 1			
Ion channels	***RYR2***	**ryanodine receptor 2**			
	CACNA2D 2	**calcium voltage-gated channel auxiliary subunit alpha2delta 2**	**16**		
	CNGB1	**cyclic nucleotide gated channel beta 1**			
	GPM6A	glycoprotein M6A			
	KCNAB1	potassium voltage-gated channel subfamily A member regulatory beta subunit 1			
	KCNAB3	potassium voltage-gated channel subfamily A regulatory beta subunit 3			
	KCNB1	potassium voltage-gated channel subfamily B member 1			
	KCNIP4	potassium voltage-gated channel interacting protein 4			
	JPH3	junctophilin 3			
Other proteins	***KRT19***	**keratin 19**			
	LAMA2	**laminin subunit alpha 2**			

(*continued on next page*)

Table 1 (*continued*)

	RAPGEF4	**Rap guanine nucleotide exchange factor 4**			
	STIP1	**stress induced phosphoprotein 1**			
	TMEFF2	**transmembrane protein with EGF like and two follistatin like domains 2**			
	TNNC2	**troponin C2, fast skeletal type**			
	ANXA8L1	annexin A8 like 1			
	CRYGS	crystallin gamma S			
	DEFB1	defensin beta 1			
	DNAH6	dynein axonemal heavy chain 6			
	FLG	filaggrin			
	MAGEC1	MAGE family member C1			
	MATN4	matrilin 4			
	MUC20	mucin 20, cell surface associated			
	MXRA5	matrix remodeling associated 5			
	PPP1R9A	protein phosphatase 1 regulatory subunit 9A			
	PROK2	prokineticin 2			
	ZBTB37	zinc finger and BTB domain containing 37			
	ZNF483	zinc finger protein 483			
	ZNF571	zinc finger protein 571			
	ZNRD1ASP	zinc ribbon domain containing 1 antisense, pseudogene			

Up regulated genes are shown in red color, while down regulated genes – in blue color.

melatonin signaling pathways (Fig. 2). The figures in Supplementary data 2c–2h show the effects of all test samples on gene expression and predicted activation of adaptive stress response signaling pathways in detail.

Fig. 3 and Table 5 show the effects of two concentrations of WS on gene expression in the canonical CRH signaling pathway, while Tables 2 and 5 depict their effects on other genes and signaling pathways. The concentration-dependent differences in the effects of the *Withania* extract on other signaling pathways are shown in the figures in Supplementary data 2i.

Fig. 1 in the Supplementary data 3 shows diseases and disorders (metabolic, cardiovascular, inflammatory, immune, neurological, behavioral, cancer, skeletal, muscular and connective tissue, endocrine system, and organismal injuries and abnormalities) associated with the effects of adaptogens on gene expression observed in the human T98G neuroglia cell culture. The height of the bars indicates the significance of overlap of the molecules in the dataset to the pathways in the QIAGEN Knowledge Database. The extent of interaction on tested extracts with genes and molecular networks associated with various cellular (cellular maintenance and compromise, DNA replication recombination and repair) and physiological functions is shown in Fig. 2 of Supplementary data 3.

The core analysis of the datasets obtained from experiments with RR, WS, and their combination RR-WS (Adaptra) revealed that the molecules in the dataset overlapped with the molecular networks associated with the development of neurons in the QIAGEN Knowledge Base (Table 5 in Supplementary data 1). Using currently available knowledge collected in the QIAGEN Knowledge Base, our analyses predicted that RR-WS activates neuronal development (Fig. 4; z-score 2.86, overlap p-value 7.29E-03) because 22 of 57 genes had a measurement direction consistent with decreased development of neurons (Table 6). Of these 22 genes, 10 (*MBP, RELN, ADGRL1, PRKCZ, CDK5R1, CDKL3, TENM4, ROR2, APOE, GRIN3A*) contribute in their overall effect to the development of neurons because of synergistic interaction of RR and WS in Adaptra, resulting in expression of 21 genes (*MBP, RELN, ADGRL1, PRKCZ, CDK5R1, CDKL3, TENM4, ROR2, APOE, GRIN3A, NTF4, HAP1, POU3F2, WNT7B, MYO16, NKX2-1, NEFH, CHRNA7, ELFN1, GHSR, LRRK2*) that were not affected by RR and WS (Table 6).

Another synergistic interaction of WS was observed in the combination with melatonin M-WS/Adaptra PM (Table 7), which may inhibit glucagon expression (Fig. 5). The quantity of glucagon was predicted to be decreased (z-score −2, overlap p-value 1.4E-02; -log p = 1.94) by Adaptra PM because 4 of 4 genes (*CD36, PCSK2, UCN, CACNA1E*) had measurement direction consistent with decreased glucagon, while WS or melatonin had no impact on the expression of *PCSK2* or *CACNA1E*.

Discussion

The results of this study suggest that adaptogens exert a polyvalent biological activity and provoke multiple effects at the transcriptional level of regulation of cellular metabolism and homeostasis. Stress response modifiers have many molecular targets (Fig. 6) because stress response and adaptation to environmental challenge are multistep processes involving intracellular and extracellular signaling pathways at differing levels of stress regulation (Panossian, 2017). Mechanisms in complex biological systems and conditions/states such as adaptation, inflammation, and aging likely cannot be distilled into one or few chemical reactions that occur in the brain or other tissues supporting adaptive homeostasis.

Gene expression analysis has contributed significantly to our understanding of adaptive stress response signaling and molecular mechanisms of action of adaptogenic plants (Panossian et al., 2013, 2014, 2015). This understanding has led to the identification of specific signaling pathways that are activated during the adaptive stress response and are possibly predictive for assessment of pharmacological activity of herbal extracts and health claims of herbal preparations (Fig. 7). Key among these are classic/conventional/canonical stress responses activated to maintain cellular homeostasis (Fig. 7) (Evans et al., 2002; Mattson, 2008a; Panossian, 2017).

Transcriptome-wide gene-expression profiling provides information about the effects of adaptogenic herbal extracts on up- or down-regulated genes and corresponding proteins that interact in molecular networks by well-known canonical signaling pathways. The present study accomplished two tasks: (1) identification of common molecular targets and mediators of adaptive stress response regulated by

Table 2
Extension of Table 1. Fold change values vs. control of genes regulated by generally recognized adaptogen - ES, RR and WS. Upregulated genes are in red color while downregulated genes – in blue color text.

Gene Symbol	RR-WS	RR	WS	BA	WSL	ES	RC	CL-BS	BS	CL	M
CRH		-2.1			-2.8	-2.5			-2.4		
UCN	2.4		2.4	8.9	5.0	2.4		3.3	5.9	3.6	2.2
GNRH1	2.0	4.3	3.5	3.5	3.2	2.4		3.8		4.0	4.5
TLR9	**2.3**	**2.9**	**3.9**	**2.6**	**2.8**	**3.2**	**9.4**	**3.2**	**5.8**	**2.7**	**9.0**
CHRNE	-2.6		-2.6		-2.7	-2.2		-3.0	-2.3	-4.3	
PRLR	-11.9	-9.4	2.3		-2.1	-11.1	-7.7		2.5		2.3
GP1BA	2.3	3.5	3.6	4.7	2.3	2.4	2.7	2.1	3.6		6.7
PLXNA4	2.3	9.5	11.0	9.2	9.9	4.0	8.3	12.9	11.6	10.3	4.5
CHRM4	2.3			2.6	2.3	3.2	2.8	3.2		3.0	2.6
GPR19	2.3	2.9	2.2	2.1	2.1	3.1	2.4	3.4	2.2	4.1	2.6
VIPR2		3.5	2.6	3.2	4.0	3.8	3.9	5.0	2.9		2.3
RORA	3.9	3.1	3.7	4.2	4.7	3.4	4.2	2.2	2.1	5.5	4.5
STAT5A	**2.3**	**2.1**	**2.1**			**2.1**	**3.0**	**2.1**			
FOS				-2.1	-2.7	-3.1	-2.8	-3.7	-2.1	-5.0	
FOXO6	-7.9	-2.1	-3.9	-4.6	-4.3		-5.1		-2.4	-3.4	
SCX		-2.1	-2.6	-4.7	-2.8	-2.5			-3.6	-2.2	-2.7
ZFPM2		16.4	3.0	13.4	5.6	4.7		5.2	3.0	4.5	7.7
ZNF396		2.9	2.3	3.0	2.1	2.4				2.4	
ZNF467	7.6	7.6	4.6	3.9	10.5	6.5	5.9	4.3	7.4	2.7	10.1
FLT1	2.0	2.6	2.8	2.1	2.6	2.1	2.2	2.9	2.2	2.2	2.4
MAPK10	**2.5**	**6.3**	**2.9**	**5.2**	**4.0**				**4.6**	**2.5**	**5.3**
MAPK13	**2.3**	**12.3**	**4.7**	**10.3**	**7.7**	**7.3**	**3.5**	**6.4**	**2.5**	**9.8**	
MERTK	4.5	5.7	7.7	6.5	3.9	2.8	4.1	8.0	5.8	4.4	7.9
PRKCH	**3.0**		**6.2**		**2.8**		**4.7**	**6.4**	**4.9**	**11.5**	**2.3**
ROS1		-4.2	-5.2		-5.7	-5.0			-2.4	-2.3	
TTN	4.8	3.2	4.1	4.3	3.7	3.0	2.3	3.2		5.0	6.8
PTPRD	-4.3	-3.4	-2.1		-4.6		-2.8	-3.0	-3.9	-4.5	
PTPRR	-2.6	-2.8	-5.2		-5.7				-2.4	-3.0	-2.4
GUCY1A2		2.1		2.6	2.5		2.1	2.4	2.1		4.5
HSPA6		3.6			2.6	2.8		9.9		7.6	2.2
LDHD	3.8	2.9	5.4	2.6	2.8	2.4	3.5	3.2	3.2		

(*continued on next page*)

Table 2 (*continued*)

LIPE	-2.7		-3.2	-2.6	-2.4	-2.5	-4.3		-2.4		-4.4
PDE3B		2.9	4.6	2.6	2.8		2.3				6.8
PDE9A	3.0		-2.1	2.6	2.6	2.0	4.2	4.3	2.5		2.8
PRKN	5.5	2.6	6.9		2.1	2.9	5.3		2.2		4.1
AOC3	4.4	4.0	2.6		2.4	-2.5	4.0	6.3		3.0	
CEL		2.9	2.7	3.9	3.2	2.4	4.1	2.7		2.2	
DNAH11		3.8	3.8		3.5	2.8	2.3	5.4	2.5	5.8	3.4
GALNT6	8.3	2.9	5.4	6.5	2.8	2.4	2.3	4.3		2.7	
RNF43		2.4	2.3	6.5	2.8	5.4	4.7	3.6	3.4		
TRMT9B	3.6	4.7	3.6	3.0	4.0	2.9	3.1	2.1	2.7		2.3
PAPPA2	7.5	4.7	6.9	11.6	9.1	8.9	7.0		11.5	6.2	5.4
TLL1	-2.7	-5.3	-6.5	-2.6	-4.3	-2.1	-4.3	-2.8		-11.3	
PTPRD	-4.3	-3.4	-2.1		-4.6		-2.8	-3.0	-3.9	-4.5	
PTPRR	-2.6	-2.8	-5.2		-5.7				-2.4	-3.0	-2.4
CPLX1	-5.9	-4.7	-5.8		-2.1	-5.6		-8.4	-2.7	-5.1	-4.0
RYR2	3.8	2.8	3.1	7.8	7.7	12.1	2.3	2.1	5.8	4.4	11.3
CACNA2D2	3.8		6.9	2.6	2.8	4.9	2.3	2.1	2.5	3.5	
CNGB1	6.5	3.6			4.9	4.7	4.7	9.2	2.5		2.3
GPM6A		2.1	3.1	3.5	2.8	2.5	3.5		2.2		3.4
KCNAB1	9.8	7.7	4.6	19.5	8.4	10.5	7.0	8.6	7.4	11.5	26.0
KCNAB3	5.0	6.0	2.8	3.4	2.8	3.2	2.7	2.9	3.6	2.4	3.4
KCNB1	3.3	2.5	2.7	5.3	2.2	2.2	3.3		3.0		3.9
KCNIP4	2.8	2.2	3.6	3.0	2.3	3.0	3.9	3.9	2.5	3.0	2.6
JPH3	-3.3	-2.1	-2.2	-7.7	-2.7	-2.4	-8.5	-9.3		-5.6	
KRT19	-3.9	-2.1	-2.6	-4.7	-8.5	-3.7	-2.5			-3.4	
LAMA2	2.4	2.8	2.9	4.2	3.7	2.6	2.9	3.7	2.9	5.1	3.1
RAPGEF4	6.3	11.3	3.9	2.2	3.0	3.4	2.3	2.1	4.9	2.3	
STIP1		2.9	3.3		2.2			2.2		2.7	4.2
TMEFF2	-6.6	-5.2	-6.5	-3.9	-3.6	-3.1				-2.8	-2.2
TNNC2	-3.4	-2.0	-2.5	-6.3	-3.7	-2.5	-6.6	-3.7	-3.2		
ANXA8/ANXA8L1		-5.6	-3.5	-2.1	-2.1	-3.7		-5.5	-3.3	-6.8	-2.4
CRYGS	3.9	4.9	7.9	5.1	2.1	4.9		3.3	3.4		2.2
DEFB1	2.4	5.0	5.7	2.5	2.9	3.3	5.0	3.3			3.3
DNAH6	2.0	3.8	3.3	4.3	2.8	2.2	2.7	3.2	2.5		

(*continued on next page*)

Table 2 (*continued*)

FLG	3.8	3.3	3.1	4.5	2.1	-2.5		6.4		6.2	3.4
MAGEC1	3.0	8.6	3.8	3.9	4.2	4.8	4.7	11.8	3.3	9.8	3.4
MATN4	2.1	5.7	3.9		2.8	4.5		5.4		5.3	2.2
MUC20	4.5	4.8	3.1	10.3	4.2	6.5	7.1	12.9	8.3	5.3	3.4
MXRA5	2.1	2.5	3.5	2.3	2.2	2.3	2.8		3.0		
PPP1R9A	4.5	2.8	5.4	5.2	9.1	6.5	2.1	2.1	3.1	7.1	10.2
PROK2	8.0	3.8	3.1	7.7	3.4	3.2	2.3	3.2	4.1		3.4
ZBTB37	3.5	3.1	2.9	4.6	3.0	2.5	2.2	4.7	3.8	3.9	3.9
ZNF483	2.5	2.6	3.1	2.6	3.2	2.0		2.1	2.7	2.7	
ZNF571	2.6	3.8	2.4	6.1	2.6		2.9	3.7	2.4	4.0	6.2
ZNRD1ASP	4.6	4.3	2.3	8.4	3.5	2.4	3.5	5.9	3.3	6.2	6.7

*Up-regulated genes are shown in red color, while down-regulated genes – in blue color.

adaptogens (Fig. 8 and Table 1), and (2) prediction of cellular and physiological functions and diseases associated with these targets (Figs. 5 and 8 and Supplementary data 3). These results have led to the identification of signaling pathways (Fig. 1) that adaptogens activate or inhibit in cultivated neuroglia cells and to the prediction of their potential pharmacological activity.

Effects herbal extracts and their fixed combination on transcriptome wide gene expression profiles and genes involved in regulation of adaptive stress response

Adaptogens modulated 75 genes encoding expression of proteins playing key regulatory functions in the adaptive stress response, vital functions, and survival of cells and organisms (Table 1). Many of these genes have various functions in cells (Supplementary data 2 and 2b), but a clear majority play important roles in the stress system (neuroendocrine immune complex) that regulates development, aging, circulatory, digestive, and other organismal systems. Functionally, these proteins are neurohormones, G-protein–coupled and other transmembrane receptors, ligand-dependent nuclear receptor, transmembrane channels, transcription regulators, protein kinases, phosphatases, peptidases, metabolic enzymes, a chaperone, and other proteins (Fig. 8).

In this study, we for the first time describe the effects of adaptogens on gene expression of three neurohormones, which are upstream in the neuroendocrine hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal axes, i.e., corticotropin-releasing hormone, urocortin (UCN), and gonadotropin-releasing hormone 1, playing key roles in the regulation of homeostasis and various physiological functions associated with stress-induced defense response and reproductive status (Oyola and Handa, 2017). Furthermore, we found that all tested adaptogens significantly affected the expression of the *PRLR* and *TLR9* genes, respectively encoding the prolactin (PRL) receptor and Toll-like receptor, which contribute to the regulation of stress responses through regulation of the HPA axis.

Thus, CRH, a 41 amino acid neuropeptide, plays a major role in coordinating the behavioral, endocrine, cardiovascular, autonomic, and immune mechanisms that allow mammals to adapt under both basal and stressful conditions (see Supplementary data 1b). Under stress, CRH stimulates the release of adrenocorticotropic hormone (ACTH) into the blood stream, which in turn stimulates the adrenal cortex to release cortisol and other steroid hormones (Rivier and Vale, 1983; Ulrich-Lai and Herman, 2009). These steroid hormones bind mineralocorticoid receptors and GR receptors in different brain regions. This negative feedback process prevents a stress-induced over-reaction and reestablishes the baseline homeostatic state. All of these responses are known as adaptive to activate defense for the neuroendocrine immune system and re-establish homeostasis (McEwen Gianaros, 2010). Although the acute HPA response to stressors is beneficial, constant activation of this circuitry by chronic or traumatic stressful episodes may lead to a dysregulation of the HPA axis and to pathology. Dysregulation of these orchestrated interactions can result in pathologies such as immunodeficiency, memory impairment, obesity, and cardiometabolic disorders (Levine et al., 2014; Munck et al., 1984; Sapolsky et al., 2000).

Urocortins (UCNs; Ucn 1, Ucn 2, and Ucn 3) are 40, 38, and 38 amino acid peptides of the CRH family, which have 2–140-fold higher affinity for CRF receptor 2 (CRF-R2) than CRH. They play an important role in the regulation of the HPA axis and stress response (regarding its duration, intensity and restoration of homeostasis). However, their specific functions in the stress response differ: CRH actions are stressing and pro-inflammatory, whereas those of Ucn 1 are stress-coping and protective. Urocortins also act as regulatory factors of the cardiovascular, gastrointestinal, reproductive, and immune systems. CRF receptors are coupled to multiple G proteins, resulting in the regulation of diverse intracellular networks involving numerous effectors such as cAMP and protein kinases. Common signaling pathways used by these peptides include MAPK pathways. As a result, UCNs are involved in numerous pathophysiological states, including mood disorders, cardiovascular and immune system diseases, neurodegenerative diseases, and disorders of the skeletal system (Lawrence et al., 2015). Ucn1 downregulates pro-inflammatory cytokines in the inflamed joint including TNF-α, IFN-γ, IL-6, IL-1β, and IL-12 and increases production of the anti-inflammatory cytokines IL-10 and TGF-β.

GnRH (gonadoliberin) is a neurohormone produced and secreted by GnRH neurons in the hypothalamus. GnRH is released into tiny blood vessels that carry this hormone from the brain to the pituitary gland, which contains the gonadotrope cells, where GnRH activates its own receptor, gonadotropin-releasing hormone receptor (GnRHR), a seven-transmembrane G-protein–coupled receptor that stimulates the β-isoform of the phosphoinositide phospholipase C, which goes on to mobilize calcium and PKC. This action results in activation of proteins involved in synthesis and secretion of two more gonadotropins – follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH are released into the general circulation and act on the testes and ovaries to initiate and maintain reproductive function. FSH and LH control levels of hormones produced by the testes and ovaries (such as testosterone, estradiol, and progesterone) and are important in controlling sperm production and maturation and release of the oocyte during the menstrual cycle. Thus, a single hormone, GnRH1, controls a complex process of follicular growth, ovulation, and corpus luteum maintenance, and spermatogenesis. GnRH activity influences a variety of sexual behaviors. Increased levels of GnRH facilitate sexual displays and behavior in females. An elevation of GnRH raises testosterone capacity beyond the average range in males (Handa and Weiser, 2014;

Table 3
Effect of adaptogens on canonical pathways commonly involved in regulation of adaptive stress response signaling. The values indicate the significance of the overlap of the molecules in the dataset to the pathways in the QIAGEN Knowledge Base calculated by Fisher's right tailed exact test and the -log (*p*-value). The higher is the value, the more significant the overlap of the dataset with the pathway.

Canonical Pathway	RR	WS	BA	WSL	ES	RC	BS	CL	M	RR-WS	WS-M
Corticotropin Releasing Hormone Signaling	0.760	**2.023**	**1.502**	**2.697**	1.160	**1.881**	**2.774**	**3.218**	0.572	**1.286**	0.592
Nitric Oxide Signaling in the Cardiovascular System	0.774	**2.144**	1.132	**1.934**		**1.564**	0.816	**2.222**	0.932	0.455	0.338
Calcium Signaling	0.847	0.768	0.706	**1.748**	**3.121**	**1.840**	**2.185**	1.132	1.007	**1.905**	**1.589**
LPS-stimulated MAPK Signaling	0.261	0.914	1.062	**1.630**	0.291	0.527	0.839	0.968		0.741	0.555
eNOS Signaling	1.236	1.116	**1.423**	**1.591**	**1.720**	1.013	0.733	**2.440**	1.276	**1.785**	0.000
Synaptic Long Term Depression	0.292	**1.417**	0.511	**1.559**	0.525	**2.430**	**2.847**	0.812	0.580	0.596	0.775
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells	**1.936**	1.278	0.627	**1.521**	**2.080**	**3.644**	**2.014**	**1.888**	0.475	1.397	1.242
GP6 Signaling Pathway	0.816	**1.301**	**1.302**	**1.502**	0.902	0.599	0.863	0.807	0.439	1.033	1.261
Dendritic Cell Maturation	0.520	0.643	1.073	1.285	0.826	0.570	**1.339**	**1.881**	0.752	0.880	0.614
Opioid Signaling Pathway	0.397	0.513	0.861	1.265	1.115	**1.646**	**1.324**	**2.129**	0.473	0.340	1.148
Neuropathic Pain Signaling In Dorsal Horn Neurons	1.071	0.873	0.821	1.086	**2.499**	1.136	**2.423**	**1.794**	1.187	0.685	1.192
Melatonin Signaling	0.674	1.181	1.078	1.068	1.134	**1.578**	**1.098**	**1.384**	1.154	**1.422**	1.153
AMPK Signaling	0.00	0.69	1.20	1.01	0.29	0.42	0.42	0.80	0.00	**1.43**	0.30
Renin-Angiotensin Signaling	**1.317**	1.120	0.716	0.983	0.743	1.031	0.710	**2.282**	0.378	**1.593**	0.494
Protein Kinase A Signaling	1.152	**1.457**	0.553	0.792	0.914	**2.338**	**1.485**	0.913	**2.407**	0.815	0.460
cAMP-mediated signaling	**2.080**	**1.688**	0.666	0.682	**2.288**	**2.606**	0.720	0.832	**1.409**	0.586	0.790
CREB Signaling in Neurons	0.402	0.511	1.257	0.592	0.664	**1.419**	1.107	1.247	0.564	0.348	0.686
CXCR4 Signaling	0.338	**1.552**	0.600	0.534	0.389	0.813	0.801	**1.697**	0.344	0.464	0.611
Dopamine-DARPP32 Feedback in cAMP Signaling	**1.350**	0.258		0.351	0.601	0.578	0.811	1.191		0.301	0.408
Gαs Signaling	**1.984**	0.939	0.447	0.315	0.588	**1.626**	1.205		0.719	1.064	0.910
NRF2-mediated Oxidative Stress Response	0.00	0.44	0.00	0.36	0.00	0.57	0.00	1.13	0.42	0.00	0.00
Glutamate Receptor Signaling	0.54	0.00	0.23	0.25	**1.50**	0.00	0.56	1.14	1.18	0.87	0.59
Neuroinflammation Signaling Pathway	0.00	0.00	0.43	0.36	0.30	0.00	0.90	**1.997**	0.40	0.00	0.00

Oyola and Handa, 2017).

Table 2 shows that ES and WSL upregulated expression of stress-protective and neuroprotective UCN and at the same time downregulated expression of stress-mimetic and proinflammatory CRH that results in predicted inhibition of inflammation (Figs. 2 and 4 in Supplementary data 2d), while *Rhodiola* exhibited its stress-protective effect only via inhibition of CRH (Fig. 1 in Supplementary data 2d). At higher doses, *Withania* did not downregulate the stress-mimetic and proinflammatory effect of CRH, resulting in overall activation of the CRH signaling pathway (Fig. 2). Because Ucn1 may have a potential anti-inflammatory or cytoprotective function, mediated by CRF-R2 (Lawrence et al., 2015), upregulation of UCNs by apoptogens is a possible explanation for their cytoprotective and anti-inflammatory effects.

PRL, one of the most versatile hormones known, is a pleiotropic pituitary hormone with more than 300 known physiological effects. This protein hormone exerts regulatory control in reproduction, immunomodulation, angiogenesis, energy metabolism, osmotic balance, and development. It is considered an adaptive hormone because of the key roles it plays in the modulation of the stress response and during pregnancy and lactation. In addition to its peripheral functions, PRL also plays many important roles as a neuropeptide, promoting physiological responses in the brain related to reproduction, stress adaptation, neurogenesis, and neuroprotection. PRL, which crosses the blood–brain barrier, is secreted from the pituitary in response to a few stressors, and local hypothalamic production reaches several brain regions, producing strong modulatory effects, including on anxiety and depressive-like behaviors. PRL also regulates neurogenesis (the generation of new neurons) (Torner, 2016) and contributes to regulation of the stress response through the inhibition of the HPA axis. Initial studies suggested that PRL may counteract glucocorticoid actions on the immune system during the stress response. However, more recent studies showed that

Table 4
Effect of adaptogens on canonical pathways commonly involved in regulation of adaptive stress response signaling. Predicted activation (in red color) or inhibition (in blue color) based on currently available knowledge collected in QIAGEN Knowledge Base. Significance values calculated based on the z-score calculation.

Canonical Pathway	RR	WS	BA	WSL	ES	RC	BS	CL	M	RR-WS	WS-M
Calcium Signaling	0.33	0.38	**2.71**	0.63	0.53	1.26		-0.28	**2.53**	0.30	-0.63
cAMP-mediated signaling	1.07	1.39	**2.50**	**2.53**	1.07	1.81	1.00	-0.47	**2.18**	1.00	1.26
Corticotropin Releasing Hormone Signaling	0.38	1.00	0.28	-0.01	-1.41	0	-0.30	0.23	1.00	-1.00	0.82
CREB Signaling in Neurons	0	-0.45	2.53	0	0.82	1.00	0	1.15	1.41		1.00
CXCR4 Signaling	0.45	-0.33	1.67	0.82	**2.24**	**2.00**	0	1.81	0.71	0.82	**2.24**
Dendritic Cell Maturation	1.41	**2.12**	**2.32**	0.90	**2.33**	0.71	0.90	1.09	**3.05**	1.67	**2.12**
Dopamine-DARPP32 Feedback in cAMP Signaling	-0.33	0.45	**2.12**	0	0.82	0	0	-0.53	1.41	-1.63	0
GP6 Signaling Pathway	0.38	**2.12**	1.51	1.67	1.89	**2.24**	1.89	0.58	**2.83**	2.12	0.71
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells	0.33	0.38	**2.12**	0.71	1.00	1.73	0.33	0	1.89	0.71	1.13
Gαs Signaling	0.38	1.34	**2.65**		1.00	1.63	0.82	0	1.89	1.34	2.24
Melatonin Signaling	1.00	0.45	1.34	1.00	**2.24**	0.82	-0.45	0	1.63	0.45	2.00
Neuropathic Pain Signaling In Dorsal Horn Neurons	1.13	0	1.67	0.38	0.63	1.13	-0.63	0.53	**2.53**	-0.82	0.38
Nitric Oxide Signaling in the Cardiovascular System	1.34	**2.12**	1.67	0.71	0	1.13	1.34	0	**2.83**	1.00	0
Opioid Signaling Pathway	**2.12**	1.00	**2.18**	0.83	1.73	1.07	0.83	-1.57	**2.14**	1.67	1.73
Protein Kinase A Signaling	1.39	1.39	1.79	0.83	1.73	1.89	0	1.18	**2.65**	0.53	1.51
Renin-Angiotensin Signaling	1.89	1.13	1.00	0.38	0.82	1.89	0.82	0.50	1.89	**2.12**	**2.24**
Role of NFAT in Cardiac Hypertrophy	1.26	1.67	**2.67**	1.15	1.90	1.39	0.90	1.09	**2.11**	1.90	1.51
Synaptic Long Term Depression	0.82	1.26	1.51	0.30	1.89	1.39	0	-0.77	**2.71**	1.41	0.71
AMPK Signaling	0	**2.24**	**2.53**	0.71	**2.00**	1.00	1.00	0.53	1.00	0.82	0.00
Neuroinflammation Signaling Pathway	1.67	1.00	2.36	0.63	0.63	0.82	-0.53	-0.19	2.00	1.41	1.00
NRF2-mediated Oxidative Stress Response	0	0	0.45	1.00	0	0	0	1.26	-0.45	0	0
Glutamate Receptor Signaling	0	0	0	0	0	0	0	0	1.00	0	0

*Activated pathways are shown in red color, while inhibited pathways – in blue color.

preventing PRL-R expression in the brain via an antisense probe strongly increases stress-induced ACTH secretion in virgin and lactating rats, suggesting that PRL plays an inhibitory role in the HPA axis. It has been hypothesized that PRL modulates the activity of the HPA axis through reduced neural inputs to the paraventricular nucleus. Both acute and chronic PRL intracerebroventricular administration in virgin female rats reduces neuronal activation in the amygdala and CRH hypothalamic expression in response to stress. Additionally, PRL is locally released from the PVN and MPOA in response to stress (Torner, 2016).

Downregulation of PRLR by adaptogens (Tables 1 and 2) suggests

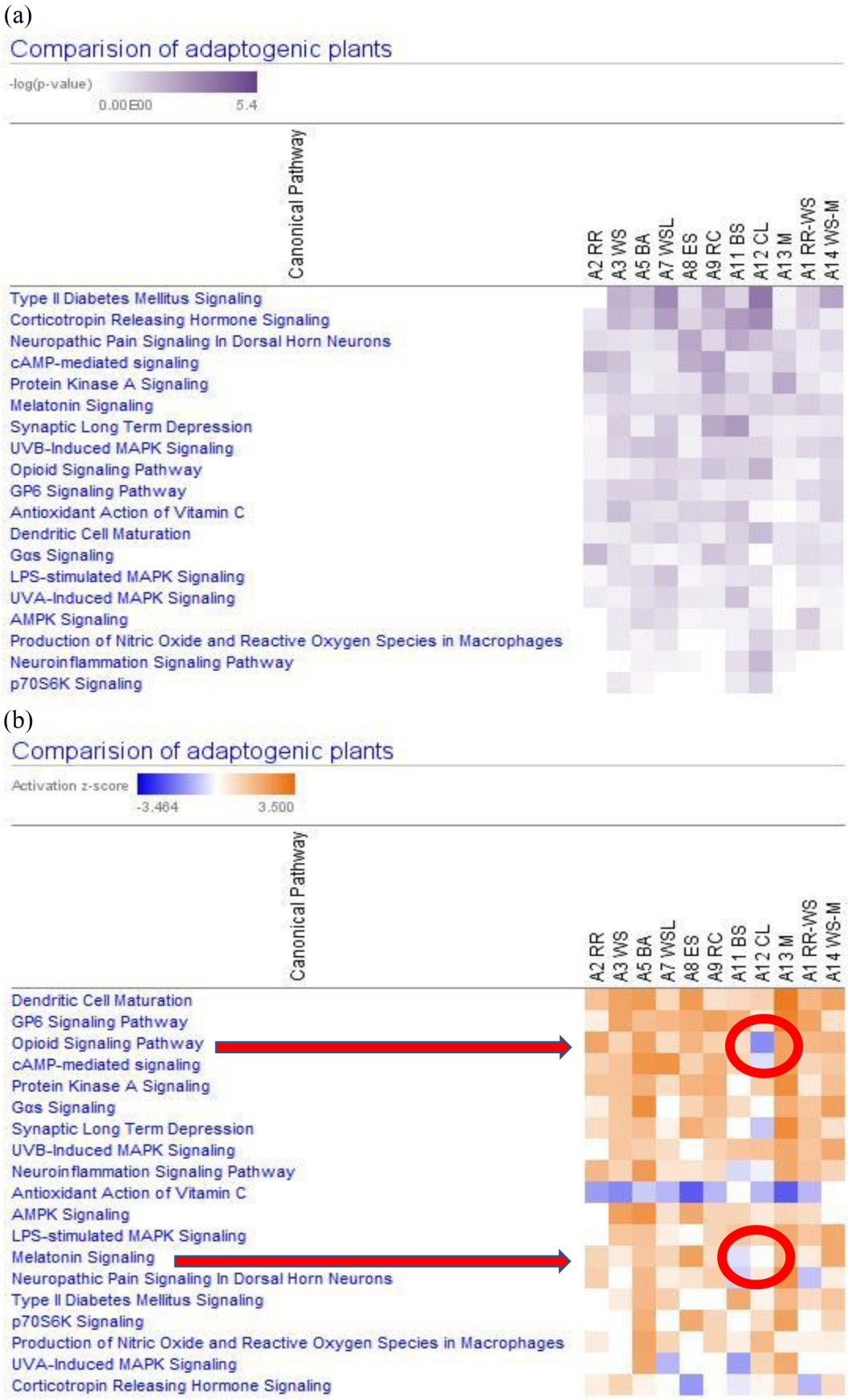

Fig. 1. (a) Heatmap; intensity of blue squares indicates significance of the overlap of the molecules in the dataset to the pathways in the QIAGEN Knowledge Base. Significance values were calculated based on results of Fisher's exact tests (right tailed), and the -log (*p*-value) are displayed above of the heat map. The deeper the intensity of the color in the square, the more significant the overlap of the dataset with the pathway. (b) Indicates whether the pathway is predicted to be activated (orange squares) or inhibited (blue squares) based on currently available knowledge collected in QIAGEN Knowledge Base. Significance values were calculated based on the z-score calculation displayed above the heatmap.

activation of HPA axis signaling and reactivity. Table 2 and Fig. 2a show that all tested adaptogenic extracts except RC significantly increased (from 2 to 5-fold compared to control) GnRH1. This observation is in line with the number of publications reporting the gonadotropic effects of *WS* (Abdel-Magied et al., 2001; Ahmad et al., 2010; Al-Qarawi et al., 2000; Bhattarai et al., 2010; Kataria et al., 2015; Rahmati et al., 2016.)

Nine genes that encode transmembrane and G-protein–coupled receptors (Table 1) mediate a plethora of signaling pathways (Supplementary data 1a) playing key roles in the adaptive stress response and stress-induced and aging-related diseases (Supplementary data 1, 1a and 1b). These include cholinergic receptors, PRL receptor, TLR9 (Toll-like receptor 9), and a member of PI3K (complex), which plays a fundamental role in pathogen and virus recognition and activation of

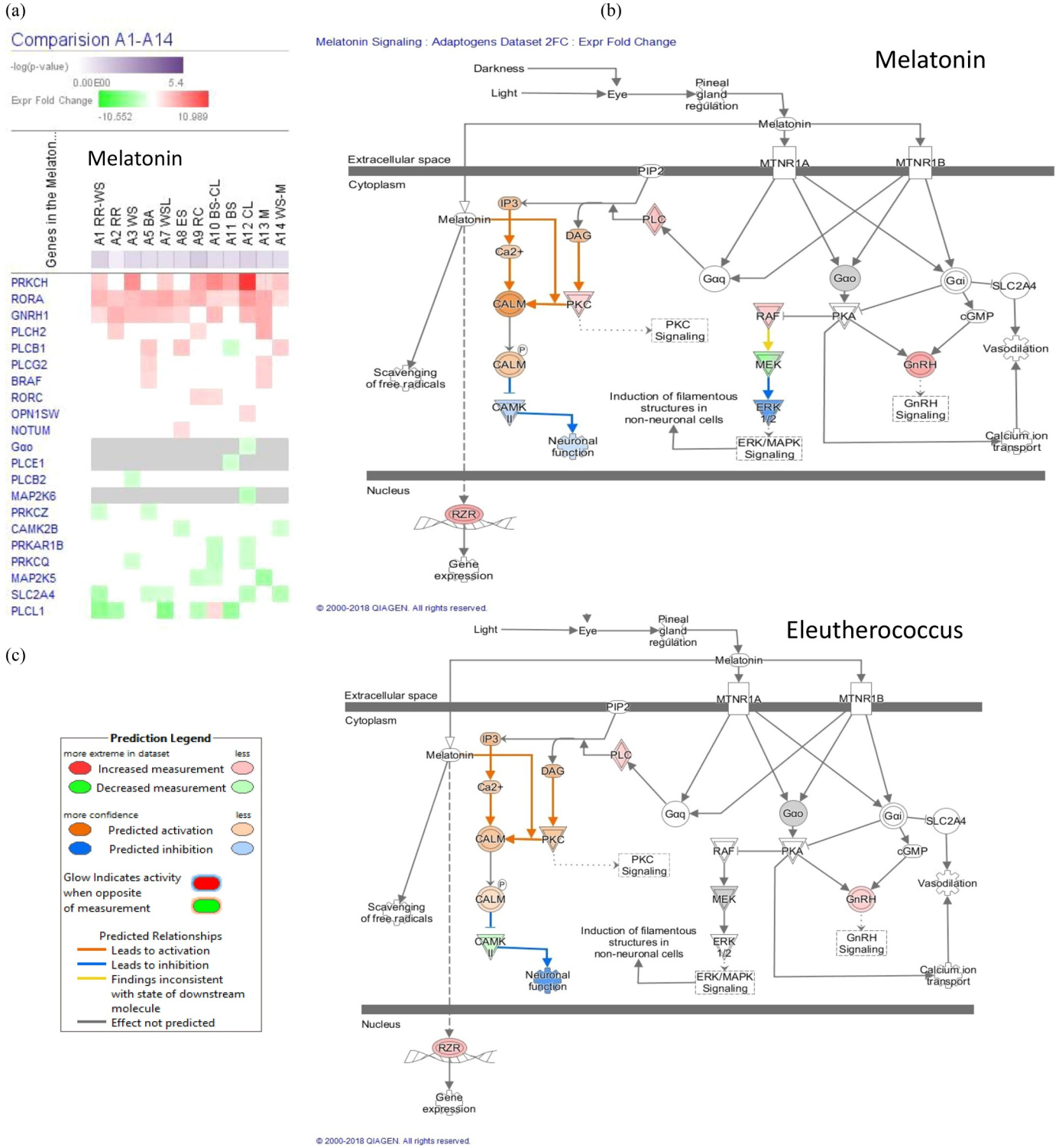

Fig. 2. (a) Effects of test samples on gene expression related to melatonin signaling pathways in human T98G neuroglia. (b) Effect of melatonin, (c) effect of *Eleutherococcus* expression. All adaptogens and melatonin upregulated expression of the gonadotropin-releasing hormone gene *GNRH1* and RAR related orphan receptor A (*RORA*) (RZR).

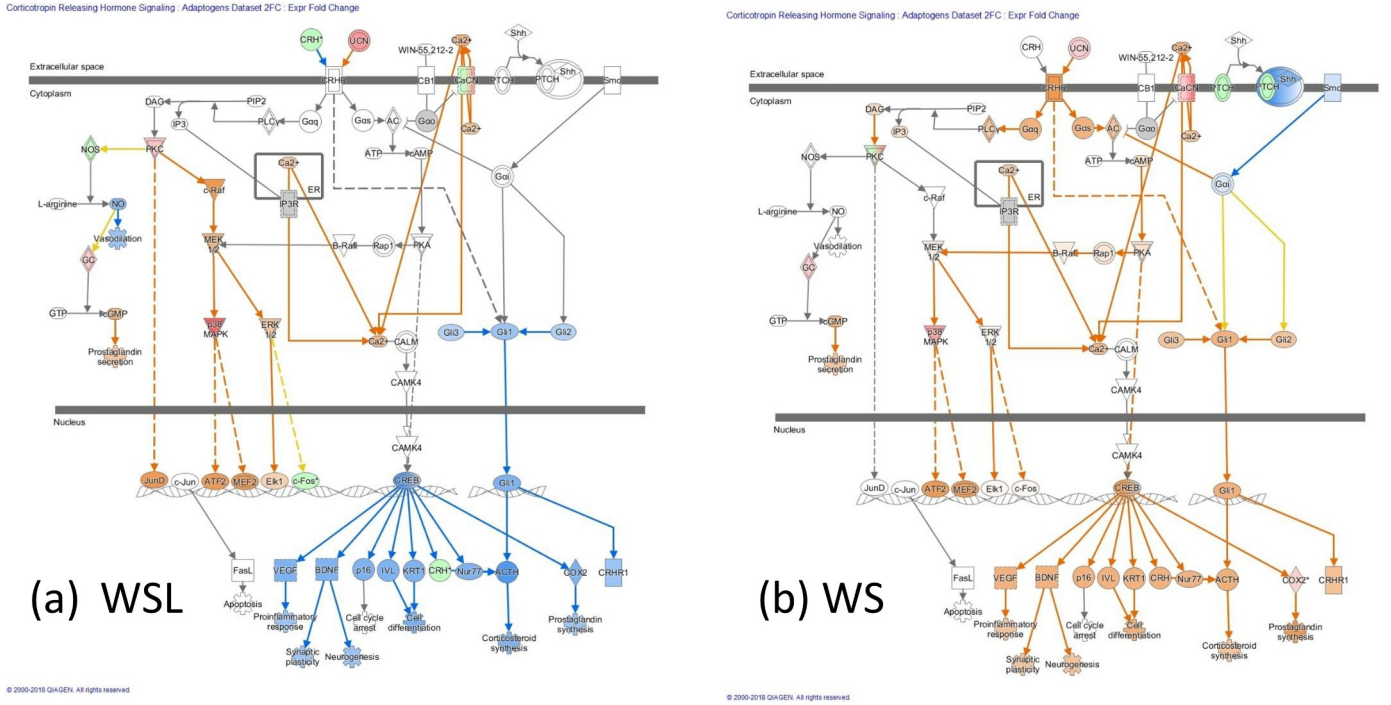

Fig. 3. CRH signaling pathways differently regulated by *Withania somnifera* extract at a concentration of 1.5 mg/l (corresponding to the dose of 90 mg in humans), WSL - (a) and 5 mg/l (corresponding to the dose of 300 mg in humans), WS - (b) in cultivated neuroglial cells. CRH and Urocortin (UCN) receptors are coupled with Gaq and Gas proteins. Downstream signaling transmitters include differently deregulated guanylate cyclase (GC encoded by GCY1A1, GUCY1B1, GUCY1A2), endothelial nitric oxide synthase 3 (encoded by NOS3), COX2 (encoded by PRCS2), stress-activated protein kinase p-38 MAPK (encoded by MAPK13), protein kinase C (encoded by PRKCH and PRKCQ), and AP-1-transcription factor (encoded by FOS) and calcium-voltage gated channel proteins encoded by CACHA1E. Fig. 3a shows inhibition of the CRH receptor–related intracellular signal transduction pathway, while Fig. 3b show s the predicted activation of this pathway. At a concentration of 5 mg/l, corresponding to human daily dose of 300 mg, WS extract had no effect on expression of *CRH*, AP-1 transcription factor subunit (*FOS*), *CACNA1E, CACNG6*, or *CACNA2D3* encoding calcium voltage-gated channel auxiliary subunits as it did at lower concentration of 1.6 mg/l. Protein kinase C eta and zeta encoding genes (PRKCH and PRKCZ) were downregulated, and guanylate cyclase 1 soluble subunits alpha and beta (*GUCY1A3, GUCY1B3*) and prostaglandin-endoperoxide synthase 2/COX-2 (*PTGS2*) genes were upregulated.

innate immunity. TLR initiates signaling to induce expression of cytokines necessary for innate immunity and subsequent adaptive immunity (Kawasaki and Kawai, 2014).

One hypothesis is that plants protect themselves against microorganisms, insects, pests, fungi, viruses, and hazardous environmental changes by biosynthesis of secondary metabolites in their most vulnerable parts (leaves, flowers, roots) (Mattson, 2008a; Mattson et al., 2007; Murakami, 2018). Plant secondary metabolites play a role in defense and adaptive response against various environmental stressors. Herbivorous and omnivorous animals that rely on plants as a primary source of nutrients have evolved complex mechanisms to neutralize the potentially harmful effects of phytochemicals. At relatively small doses, these natural compounds are not toxic in people but still induce mild cellular stress responses (Dhabhar, 2018). One basic mechanism of action of plant secondary metabolites is that they activate the adaptive cellular stress response pathways in humans (Mattson, 2008a; Murakami, 2018). This phenomenon has been commonly observed and has been described as an adaptive stress response, pre-conditioning, or 'hormesis' (Calabrese et al., 2007; Mattson 2008d). Major components of the hormetic response include various stress resistance proteins, such as heat-shock proteins (HSPs), antioxidants, and growth factors (Mattson, 2008b; Mattson and Cheng, 2006).

One of the most important defensive signaling molecules in animals is the TLR family protein TLR9, which occurs upstream in 152 signaling pathways (see Table 1 and Supplementary data 1a). Excessive activation of TLR signaling could be harmful and lead to tissue injury, including chronic inflammation and autoimmune diseases (Kawasaki and Kawai, 2014). However, it is now obvious that mammalian TLRs play a prominent role in the direct activation of host defense mechanisms. Activation of TLRs stimulates an innate immune response, which involves the production of direct antimicrobial effector molecules, including NO, and increases an adaptive immune response by inducing the production of IL-1h, IL-6, TNFα, and IL-12, which augments both cell-mediated and humoral immune responses. TLRs play crucial roles in the innate immune system by recognizing pathogen-associated molecular patterns derived from various microbes (Kawasaki and Kawai, 2014).

Perhaps most common for adaptogens are their effects on genes encoding protein kinases and phosphatases, which control the level of active forms of signaling molecules. Among these were the mitogen-activated protein kinase 10 (*JNK-3*), mitogen-activated protein kinase 13 (related to p-38 MAP kinase), protein kinase C eta, tyrosine kinases *FLT1, MERTK*, and *ROS1*, and tyrosine phosphatases *PTRRD* and *PTPRR*.

Among transcription regulators significantly modulated by adaptogens were signal transducer and activator of transcription 5A (*STAT5A*), Fos proto-oncogene, AP-1 transcription factor subunit (*FOS*), forkhead box O6 (*FOXO6*), scleraxis bHLH transcription factor (*SCX*), and zinc finger proteins.

The stress-activated protein kinase MAPK13 is responsive to various stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and is involved in cell differentiation, apoptosis, and autophagy (Roux and Blenis, 2004; Supplementary data 1 and 1b). Persistent aging-related activation of the p38 MAPK pathway in muscle satellite cells (muscle stem cells) impairs muscle regeneration (Segalés et al., 2016).

Among the genes encoding metabolic enzymes and chaperone proteins were several involved in many signaling pathways and molecular

Table 5
Effect of test samples on genes involved in regulation of Corticotropin Releasing Hormone Signaling canonical pathway. Fold change values vs. control values.

Symbol	Entrez Gene Name	RR-WS	RR	WS	BA	WSL	ES	RC	C-BS	BS	C	M	M-WS
ADCY1	adenylate cyclase 1		**2.11**										
ADCY10	adenylate cyclase 10								2.14		3.55		
ADCY5	adenylate cyclase 5		**-2.37**				**-2.03**		-2.40			**-2.22**	
CACNA1E	**calcium voltage-gated channel subunit alpha1 E**					**-2.00**		**-2.08**	-3.93		-9.60		-2.54
CACNA1I	**calcium voltage-gated channel subunit alpha1 I**	2.26		**2.31**	**2.59**	**4.90**	**3.23**	**2.34**	3.22		3.55	3.38	
CACNA2D1	**calcium voltage-gated channel auxiliary subunit alpha2delta 1**	2.06					**2.76**	**4.09**		3.41		2.82	2.48
CACNA2D2	**calcium voltage-gated channel auxiliary subunit alpha2delta 2**	3.76		**6.93**	**2.59**	**2.80**	**4.85**	**2.34**	2.14	2.47	3.55		2.48
CACNA2D3	**calcium voltage-gated channel auxiliary subunit alpha2delta 3**		**2.86**		**3.88**	**2.10**		**5.86**		2.47			
CACNG6	calcium voltage-gated channel auxiliary subunit gamma 6					**-2.33**		**-2.09**		-2.22	-6.72		
CRH	corticotropin releasing hormone		**-2.09**			**-2.83**	**-2.47**			-2.41			
FOS	Fos proto-oncogene, AP-1 transcription factor subunit				**-2.08**	**-2.65**	**-3.15**	**-2.83**	-3.75	-2.06	-5.02		
GLI1	GLI family zinc finger 1	-2.37							-2.33		-5.51		
GUCY1A2	guanylate cyclase 1 soluble subunit alpha 2		**2.09**		**2.59**	**2.52**		**2.10**	2.36	2.14		4.51	
GUCY1A3	guanylate cyclase 1 soluble subunit alpha			**2.49**							-5.44	2.22	
GUCY1B3	guanylate cyclase 1 soluble subunit beta			**2.31**									
MAPK13	**mitogen-activated protein kinase 13**	2.31	**12.35**	**4.73**	**10.34**	**7.70**	**7.27**	**3.51**	6.43	2.53	9.75		3.39
NR4A1	nuclear receptor subfamily 4 group A member 1	-2.21							2.15		2.49		
OPN1SW	opsin 1, short wave sensitive										2.23		
PRKCH	protein kinase C eta	3.02		**6.16**		**2.81**		**4.69**	6.43	4.94	11.53	2.25	3.31
PRKCQ	protein kinase C theta			**-2.27**					-3.06		-2.63		
PRKCZ	protein kinase C zeta	-2.23			**-2.19**								
PTCH2	patched 2		**-2.29**	**-2.83**	**2.07**					2.08	-2.46		
PTGS2	prostaglandin-endoperoxide synthase 2			**2.14**						2.07			
UCN	**urocortin**	2.35		**2.38**	**8.94**	**5.04**	**2.44**		3.29	5.93	3.61	2.20	4.23

networks with key roles in metabolic regulation and cellular repair functions, such as guanylate cyclase 1 soluble subunit α 2 (*GUCY1A*), heat shock protein family A (Hsp70) member 6 HSPA6, lactate dehydrogenase D (*LDHD*), lipase E, hormone sensitive type (*LIPE*), and phosphodiesterase 3B (*PDE3B*).

Of note, many genes modulated by adaptogens encode key signaling proteins involved in canonical stress response signaling, such as TLR. As mentioned, TLR9 is involved in signal transduction of 152 pathways (Supplementary data 1a), and the protein kinases PKC, MAPK10, and MAPK13 are involved in 72, 77, and 59 signaling pathways. Their numerous molecular, cellular, and physiological functions, as well as associated biological processes and diseases, are shown in Supplementary data 1b.

It therefore is not surprising that adaptogenic plants exhibit

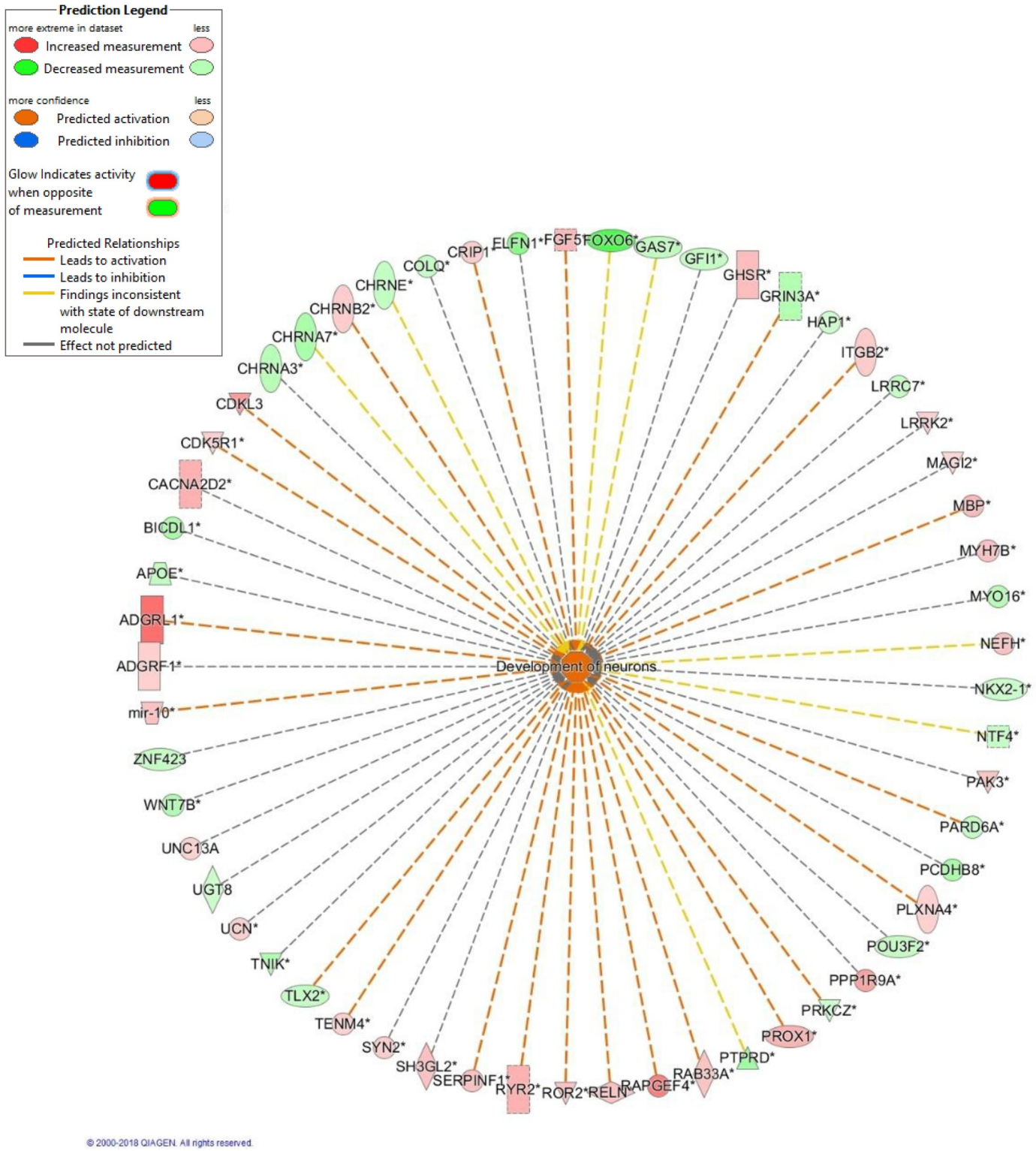

Fig. 4. Effects of RR-WS (Adaptra) on gene expression in human T98G neuroglia cells, which support the predicted activation of the development of neurons.

polyvalent pharmacological activity and have been used in traditional medicinal systems for many diseases. Nevertheless, the most characteristic features for adaptogens are those associated with adaptive stress response, which declines with aging (Cornelius et al., 2013a; Cornelius et al., 2013b; Mattson, 2008a; Raefsky and Mattson, 2017; Walther et al., 2016).

Effects of adaptogens on adaptive stress signaling pathways

Of 177 signaling pathways, 23 that adaptogens significantly affected (Table 3, Fig. 1) were associated with the cellular adaptive stress response. Normally, adaptive signaling pathways are activated by exercise, dietary energy restriction, chemicals, medicines, cognitive

stimulation/emotions, toxins, radiation, or temperature and involve kinases and transcription factors that induce expression of genes that encode antioxidant enzymes, protein chaperones, phase-2 enzymes, neurotrophic factors, and other cytoprotective proteins (Fig. 8) (Mattson, 2008a).

In this study, all of these pathways were mainly activated by adaptogens, except for CRH signaling, CREB signaling in neurons, and CXCR4 signaling pathways (Table 4), in which the direction of the effect depended on the concentration. Most characteristic for the adaptive stress response, the CRH signaling pathway (Table 5, Fig. 3, and Supplementary data 2d) was associated with proinflammatory response, apoptosis, synaptic plasticity, neurogenesis, cell cycle arrest, cell differentiation, glucocorticoid and prostaglandin synthesis, vasodilatation, and prostaglandin secretion.

The effects of various adaptogens on expression of CRH and UCN have been addressed above (Table 2, Supplementary data 2d). CRH and UCN actions were mediated through the activation of two types of GPCR, which are widely distributed in the CNS and in peripheral tissues, including adrenal, lung, liver, stomach, pancreas, small intestine, skin, and the immune, cardiovascular, and reproductive systems. In brain, the stimulation of CRH receptors leads to activation of adenylate cyclase (AC) and the consequent increase in cAMP levels. CRHRs modulate many protein kinases including PKA, PKC, Akt, ERKs and p38 MAPKs, and CaM kinases, which stimulate various transcription factors (FOXO, FOS, AP-1, STAT5, SCX, NFkB, Nrf2), which in turn stimulate the expression of other genes and ACTH (Grammatopoulos and Ourailidou, 2017; Lawrence et al., 2015).

FOS and *MAPK10/JNK* are early response genes induced by a variety of external stresses and that encode protein subunits comprising the AP-1 transcription factor (Fig. 7). The AP-1 transcription factor is important in regulating genes involved in cell cycle progression, inflammation, and apoptosis.

CRH stimulates adrenal steroidogenesis and prostaglandin synthesis via the PKC pathway by activation of NOS, GC, and cGMP. Production of NO by CRH brings about vasodilation. The PKC pathway mediates several of the immune effects of CRH. In addition, CRHR induces the transcription of the COX2 gene through the involvement of the PKC pathway, resulting in the release of arachidonic acid and prostaglandins. The effects of CRH on hippocampal cells also involve activation of the PKC pathway by stimulating the MAPKs. The CRHR–MAPK cascade mediates the neuroprotective effects of CRH and CRH-like peptides. The CRHR-activated PKA pathway activates BDNF expression, which leads to synaptic plasticity and neurogenesis. FasL activation by CRHR-activated p38 MAPK culminates in apoptosis. In immune cells, CRH both inhibits and stimulates the production of the proinflammatory cytokines (IL-1 and IL-6) by peripheral blood mononuclear cells. CRH has proinflammatory effects in mast cells. Overexpression of CRH causes anxiety, sleep disruption, and adverse changes in cardiovascular, metabolic, and immune functions (IPA pathway analysis, 2018).

Some of these signaling cascades, such as p38 MAPK, protein kinase C (PKC), extracellular signal-regulated protein kinase (ERK), c-jun N-terminal kinase (JNK), and phosphatidylinositol-3-kinase (PI3K)-mediated cascades may either individually, or in a combined manner, activate nuclear factor E2, Nrf2, which is one of the most important defensive mediators of the oxidative stress response and activates the expression of phase II detoxifying enzymes and antioxidants in response to noxious stimuli (Chen et al., 2004; Farombi et al., 2008; Gopalakrishnan and Tony Kong, 2008). Nrf2 is phosphorylated in response to the PKC, phosphatidylinositol 3-kinase, and MAP kinase pathways. After phosphorylation, Nrf2 translocates to the nucleus and transactivates detoxifying enzymes and antioxidant enzymes, such as glutathione S-transferase, cytochrome P450, NAD(P)H quinone oxidoreductase, heme oxygenase, and superoxide dismutase. Because oxidative stress is associated with inflammatory diseases, particularly with aging-related atherosclerosis, Parkinson's disease, and Alzheimer's disease, the ability of adaptogens to activate PKC, PI3K, and MAPK signaling pathways followed by activation of Nrf2 (see Supplementary data 3f) might be beneficial in these diseases. In experiments relevant to the pathogenesis of stroke and Alzheimer's and Parkinson's diseases, adaptogens have broad neuroprotective actions against oxidative stress (Fang et al., 2012; Fujikawa et al., 2005; González-Burgos et al., 2015; Jansen et al., 2014; Jin et al., 2013; Kuboyama et al., 2014; Lee et al., 2012; Li et al., 2016a,b; Li et al., 2013; Li et al., 2014a; Liu et al., 2012; Mao et al., 2015; Morgan and Grundmann, 2017; Nabavi et al., 2016; Prakash et al., 2014; Sa et al., 2015; Singh and Ramassamy, 2017; Sun et al., 2016; Tan et al., 2015; Wang et al., 2017, 2015; Wu et al., 2013; Zhang et al., 2016; Zhou et al., 2016). Studies of cultured neural cells suggest that the underlying mechanisms involve activation of the PI3 kinase–Akt pathways (Li et al., 2016a; Sa et al., 2015; Zhang et al., 2016). Two other pathways that play important roles in neuronal stress adaptation are those involving the NF-κB and FOXO transcription factors (Brunet et al., 2004; Camandola and Mattson, 2007; Mattson and Meffert, 2006; van der Horst and Burgering, 2007).

Overall, adaptogens initiate the adaptive stress response by stimulating cellular and organismal defense systems, activating intracellular

Table 6
Effect of RR, WS and their combination RR-WS (Adaptra) on genes involved in regulation of neuronal development.

Gene Symbol	Entrez Gene Name	Literature findings	Prediction	Gene expression, fold change		
				RR-WS	RR	WS
ADGRF1	adhesion G protein-coupled receptor F1	Affects (4)	Affected	2.29	2.28	
ADGRL1	adhesion G protein-coupled receptor L1	Increases (2)	Increased	6.93		
APOE	apolipoprotein E	Affects (13)	Affected	-2.84		
BICDL1	BICD family like cargo adaptor 1	Affects (2)	Affected	-3.98		-2.34
CACNA2D2	calcium voltage-gated channel auxiliary subunit α2 δ2	Affects (2)	Affected	3.76		6.93

(*continued on next page*)

Table 6 (*continued*)

CDK5R1	cyclin dependent kinase 5 regulatory subunit 1	Increases (4)	Increased	2.33		
CDKL3	cyclin dependent kinase like 3	Increases (3)	Increased	4.82		
CHRNA3	cholinergic receptor nicotinic alpha 3 subunit	Affects (2)	Affected	-3.09	-2.45	
CHRNA7	cholinergic receptor nicotinic alpha 7 subunit	Increases (1)	Decreased	-3.74		
CHRNB2	cholinergic receptor nicotinic beta 2 subunit	Increases (8)	Increased	2.45		-5.20
CHRNE	cholinergic receptor nicotinic epsilon subunit	Increases (1)	Decreased	-2.65		-2.59
COLQ	collagen like tail subunit of acetylcholinesterase	Affects (2)	Affected	-2.65	-6.30	-2.69
CRIP1	cysteine rich protein 1	Increases (1)	Increased	2.41	3.01	
ELFN1	extracellular leucine rich repeat and fibronectin type III domain containing 1	Affects (1)	Affected	-5.31		
FGF5	fibroblast growth factor 5	Increases (1)	Increased	3.52		4.23
FOXO6	forkhead box O6	Increases (3)	Decreased	-7.93	-2.10	-3.89
GAS7	growth arrest specific 7	Increases (3)	Decreased	-2.85	-2.26	
GFI1	growth factor independent 1 transcriptional repressor	Affects (1)	Affected	-2.65		-2.59
GHSR	growth hormone secretagogue receptor	Affects (3)	Affected	3.15		
GRIN3A	glutamate ionotropic receptor NMDA type subunit 3A	Decreases (4)	Increased	-3.33		
HAP1	huntingtin associated protein 1	Affects (1)	Affected	-2.21		
ITGB2	integrin subunit beta 2	Increases (1)	Increased	2.45	3.05	2.66
LRRC7	leucine rich repeat containing 7	Affects (1)	Affected	-2.66	-2.11	
LRRK2	leucine rich repeat kinase 2	Affects (4)	Affected	2.26		
MAGI2	membrane associated guanylate kinase,	Affects (10)	Affected	2.01	3.01	2.18
MBP	myelin basic protein	Increases (1)	Increased	3.48		
mir-10	microRNA 100	Increases (1)	Increased	2.89		3.53
MYH7B	myosin heavy chain 7B	Affects (1)	Affected	3.01	5.70	3.08
MYO16	myosin XVI	Affects (1)	Affected	-3.32		
NEFH	neurofilament heavy	Decreases (18)	Decreased	3.02		
NKX2-1	NK2 homeobox 1	Affects (4)	Affected	-2.38		
NTF4	neurotrophin 4	Increases (5)	Decreased	-2.61		

(*continued on next page*)

Table 6 (*continued*)

PAK3	p21 (RAC1) activated kinase 3	Affects (4)	Affected	2.86		2.36
PARD6A	par-6 family cell polarity regulator alpha	Decreases (2)	Increased	-2.84		-2.39
PCDHB8	protocadherin beta 8	Affects (1)	Affected	-3.97		-3.89
PLXNA4	plexin A4	Increases (5)	Increased	2.25	9.49	10.97
POU3F2	POU class 3 homeobox 2	Affects (4)	Affected	-2.65		
PPP1R9A	protein phosphatase 1 regulatory subunit 9A	Affects (6)	Affected	4.51	2.85	5.38
PRKCZ	protein kinase C zeta	Decreases (2)	Increased	-2.23		
PROX1	prospero homeobox 1	Increases (1)	Increased	3.76	2.85	
PTPRD	protein tyrosine phosphatase, receptor type D	Increases (3)	Decreased	-4.32	-3.42	-2.11
RAB33A	RAB33A, member RAS oncogene family	Increases (1)	Increased	2.81	-3.11	
RAPGEF4	Rap guanine nucleotide exchange factor 4	Increases (2)	Increased	6.31	11.27	3.92
RELN	reelin	Increases (9)	Increased	3.01		
ROR2	receptor tyrosine kinase like orphan receptor 2	Increases (5)	Increased	3.01		
RYR2	ryanodine receptor 2	Increases (2)	Increased	3.75	2.85	3.07
SERPINF1	serpin family F member 1	Increases (1)	Increased	3.04	2.88	
SH3GL2	SH3 domain containing GRB2 like 2, endophilin A1	Affects (2)	Affected	3.02		2.16
SYN2	synapsin II	Affects (3)	Affected	2.41		2.56
TENM4	teneurin transmembrane protein 4	Increases (3)	Increased	2.25		
TLX2	T cell leukemia homeobox 2	Decreases (2)	Increased	-2.64	2.39	-2.59
TNIK	TRAF2 and NCK interacting kinase	Affects (1)	Affected	-3.53	-3.60	
UCN	urocortin	Affects (1)	Affected	2.35		2.38
UGT8	UDP glycosyltransferase 8	Affects (2)	Affected	-2.21		
UNC13A	unc-13 homolog A	Affects (2)	Affected	2.25		
WNT7B	Wnt family member 7B	Affects (2)	Affected	-3.54		
ZNF423	zinc finger protein 423	Affects (4)	Affected	-2.66		

*Development of neurons predicted to be increased (z-score −2,87). Overlap p-value 7.29E-03.
**Prediction is based on measurement direction and literature data: 22 of 57 genes deregulated by RR-WS have measurement direction consistent with increase in development of neurons
***25 Genes deregulated due to synergistic interactions of RR and WS in the fixed combination Adaptra are in red color text.

and extracellular adaptive signaling pathways and expression of stress-activated proteins, resulting in transient changes in protection or repair capacity and increased non-specific resistance and adaptation to stress (**Figs. 6–8**). In the context of responses of cells and organisms to stress (Dhabhar, 2018), adaptogens act as “mild stress vaccines” or “smooth” pro-stressors that reduce the reactivity of host defense systems and decrease damaging effects of various stressors due to increased basal level of mediators involved in the stress response (Panossian, 2017; Panossian et al., 1999a,b; Wiegant et al., 2008), suggesting a beneficial effect in stress-induced and aging-related disorders (Panossian and Gerbarg, 2016).

Dose-response reversal effects of adaptogens, hormesis, and adaptive homeostasis

As a rule, the dose–effect relationship curve is not linear for adaptogens but has a biphasic (bell) shape (Kurkin et al., 2003; Panossian et al., 2010; Perfumi and Mattioli, 2007; Schriner et al., 2009; Wiegant et al., 2009). Therefore, one of the plant extracts, WS, was tested in two concentrations: one, corresponding to a normal single dose in humans (300 mg), assuming that the maximal concentration in blood is 5 mg/l; and the other, 1.5 mg/l, corresponding to a dose of 90 mg. We

Table 7
Effect of M, WS and their combination M-WS (Adaptra PM) on genes involved in regulation of quantity of glucagon.

Gene Symbol	Entrez Gene Name	Literature findings	Prediction**	Gene expression, fold change		
				M-WS*	M	WS
CACNA1E	calcium voltage-gated channel subunit alpha1 E	increases	Decreased	-2.54		
CD36	CD36 molecule	increases	Decreased	-2.12	-3.98	-2.99
UCN	urocortin	decreases	Decreased	4.23	2.20	2.38
PCSK2	proprotein convertase subtilisin/kexin type 2	increases	Decreased	-2.42		

Fx7

*Quantity of glucagon predicted to be decreased (z-score −2). Overlap *p*-value 1.14E-02; -log p = 1.94
**Prediction is based on measurement direction and literature data; 4 of 4 genes deregulated by M-WS have measurement direction consistent with decrease in quantity of glucagon.
***Genes deregulated due to synergistic interactions of M and WS in the fixed combination Adaptra PM are in red color text.

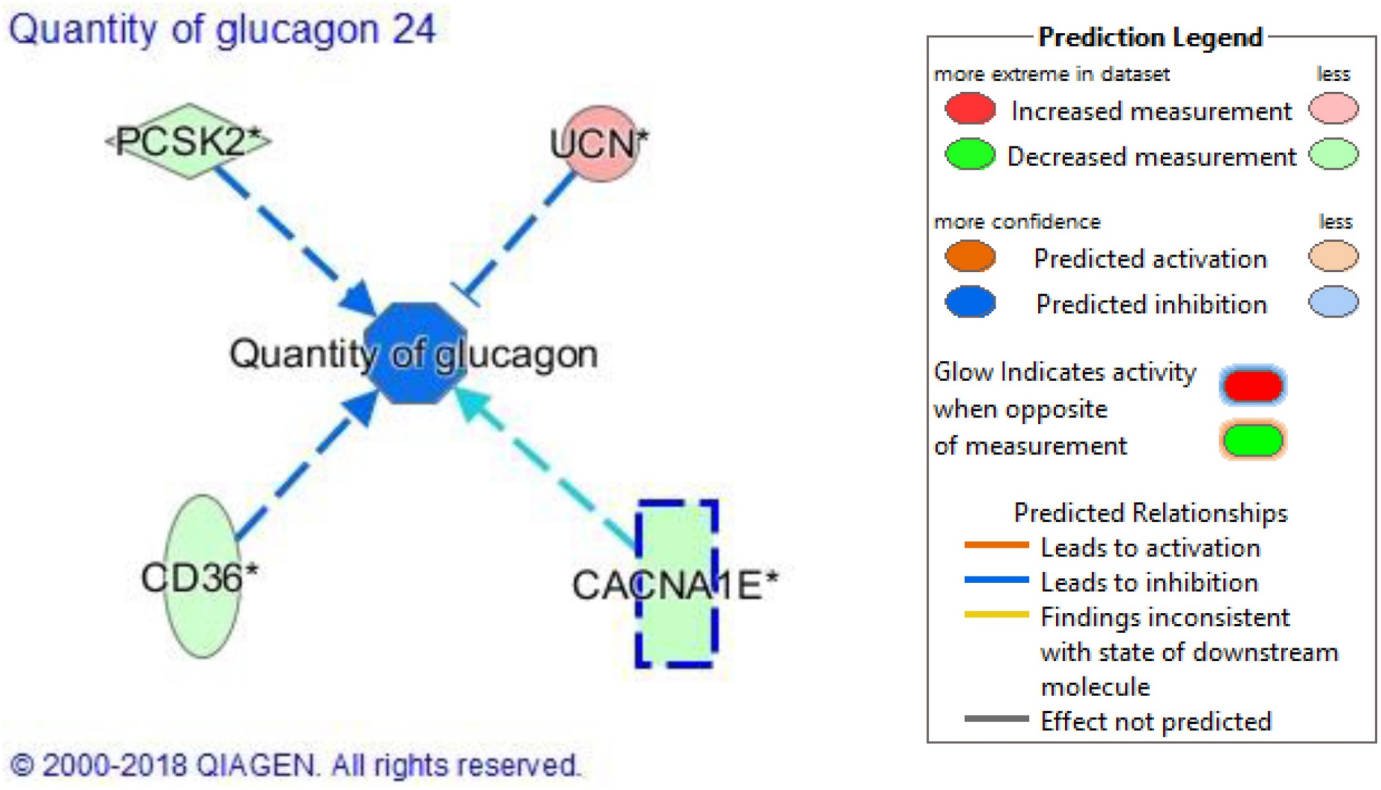

Fig. 5. Effects of M-WS (Adaptra PM) on gene expression in human T98G neuroglia cells, which support the predicted inhibition of glucagon levels.

observed many dose-dependent reversal effects. For example, the predicted inhibition of the proinflammatory response, corticosteroid synthesis, prostaglandin synthesis, cell differentiation, synaptic plasticity, and the overall signaling pathway was seen at the concentration of 1.5 mg/l, and the reversal effect occurred at the higher concentration of 5 mg/l. Activation of all of these processes at higher concentration was presumably the result of a lack of downregulation of CRH expression (**Fig. 2**). One explanation is that at low concentrations, the CRH receptor responds both to UCN, which is upregulated, and to CRH, which is downregulated by *Withania* at a concentration of 1.5 mg/l. As a result, there was an overall integrative effect regarding an inhibition of inflammation and CRH pathway signaling. However, at higher concentrations, *Withania* did not inhibit expression of pro-inflammatory CRH, and the overall effect was activation of inflammation. We also suggest that the concentration-dependent reversal effects of WS on CRH gene expression were likely due to withanolides concentration-dependent allosteric modulation via various allosteric binding sites of glucocorticoid receptors (Keenan et al., 2016). This was followed by biased signaling, which is common for GPCR (Kenakin, 2012), including glucocorticoid (Keenan et al., 2016) melanocortin-4 (Breit et al., 2011); c-AMP/adenosine (Billington and Hall, 2012; Vecchio et al., 2018); melatonin (Cecon et al., 2017); opioid (Kelly, 2013; Fujita et al., 2015; Pradhan et al., 2012); and other chemokine receptor signaling (Zweemer et al., 2014). Biased signaling from GR is also likely to be a consequence of interactions among over 150 different proteins (Keenan et al., 2016).

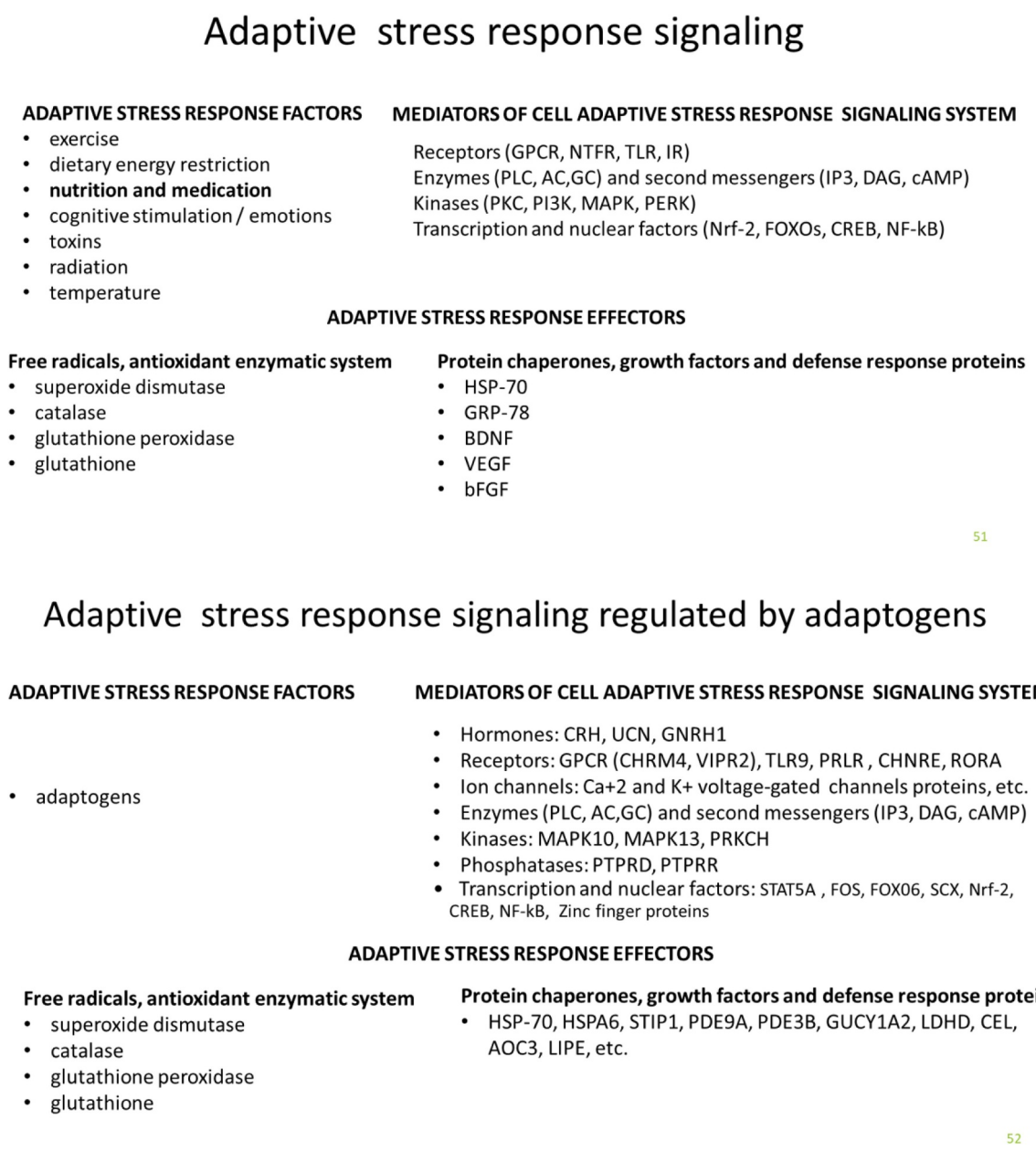

Fig. 6. Adaptive stress trsponce signaling regulated by adaptogens. updated and adapted from Ref. Mattson, 2007.

Similar dose-dependent reversal effects were observed in other stress response signaling pathways, *e.g.*, glucocorticoid receptor signaling, where an inhibition of inflammatory symptoms was predicted at a low concentration of WSL, and an activation of inflammatory symptoms at higher concentration was predicted by IPA (Supplementary data 2e). These predictions were in line with the results of an unpublished study by Dimpfel (personal communication), in which the electrical activity of rat hippocampus slices was measured. Biphasic concentration effects were observed with the same extract activation at a concentration of 1.5 mg/l and inhibition at a concentration of 5 mg/l (Supplementary data 4). However, they are not in line with the hormesis concept, which suggests a stimulatory effect at low doses but toxicity at high doses, because inhibition of inflammation at low dose cannot be considered as a stimulation of defensive immune responses.

Furthermore, the results of our study showed that low doses were not always superior to higher doses in terms of pharmacological efficacy. For instance, WS extract downregulated *PRLR* gene expression (PRL receptor) at a concentration of 1.5 mg/l but upregulated that gene at a higher concentration of 5.0 mg/l, which was positively associated with the anxiolytic effect of *Withania* (Andrade et al., 2000; Dey et al., 2016; Gupta and Kaur, 2018; Gupta and Rana, 2007, 2008; Kaur and Kaur, 2017; Kaur et al., 2017) and PRL (Torner, 2016). At the same time, the *Withania* extract downregulated *PDE9A* gene expression (phosphodiesterase 9A) at a concentration of 5 mg/l but upregulated that gene at a lower concentration of 1.5 mg/l. This result was in line with the hormesis concept from a mechanistic point of view: Low doses of *Withania* (WSL) upregulated *PDE9S* gene expression, while higher doses (WS) downregulated it. However, the pharmaco-toxicological approach associated with the risk–benefit relationship was not in line with the hormesis concept: The low dose is harmful for cardiac muscle, while higher doses are beneficial in cardiac muscle hypertrophy because *PDE9A* selectively regulates the cGMP catabolic process and positively regulates cardiac muscle hypertrophy (Lee et al., 2015).

The concentration-dependent reversal (hormetic) actions of withanolides (the active constituents of *Withania*) could be mediated by allosteric modulation of various allosteric binding sites, inducing a shift

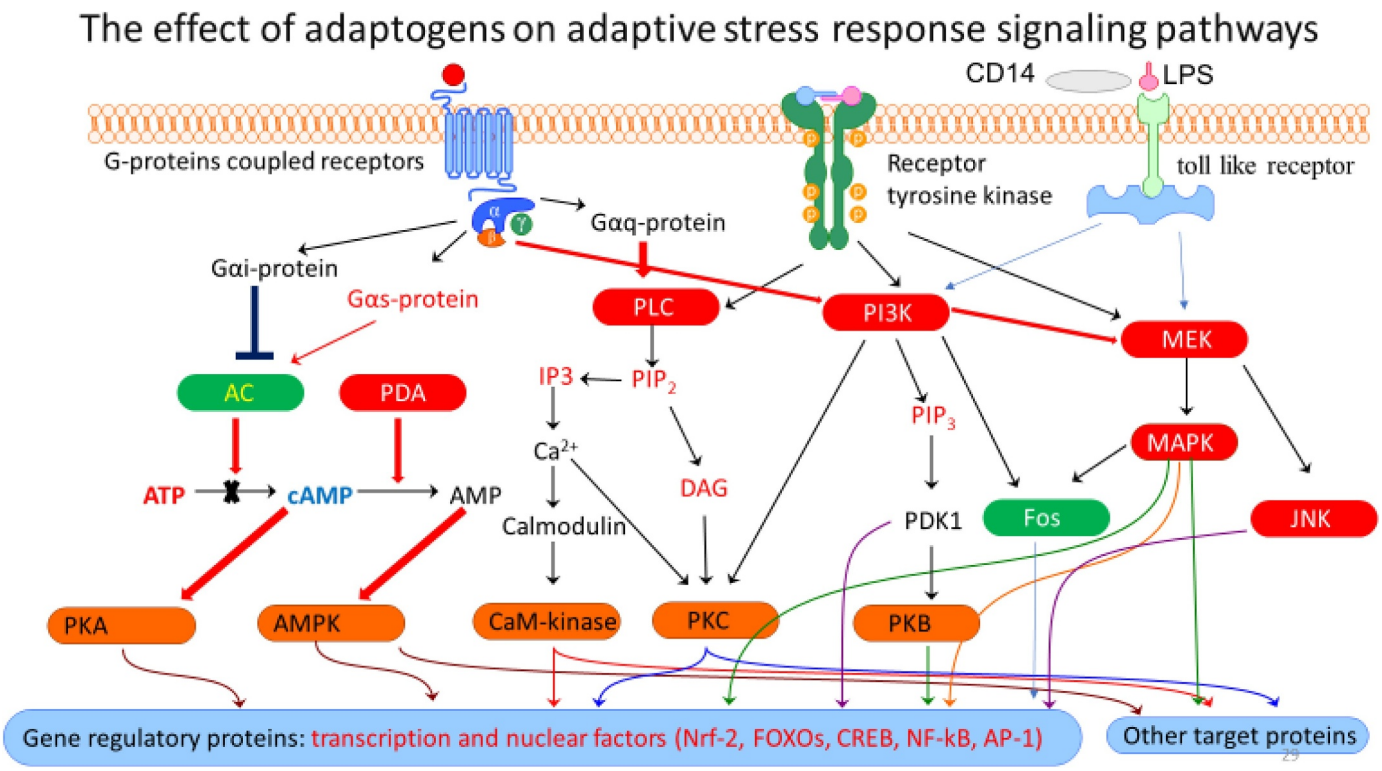

Fig. 7. The effect of adaptogens on adaptive stress response signaling pathwyas.

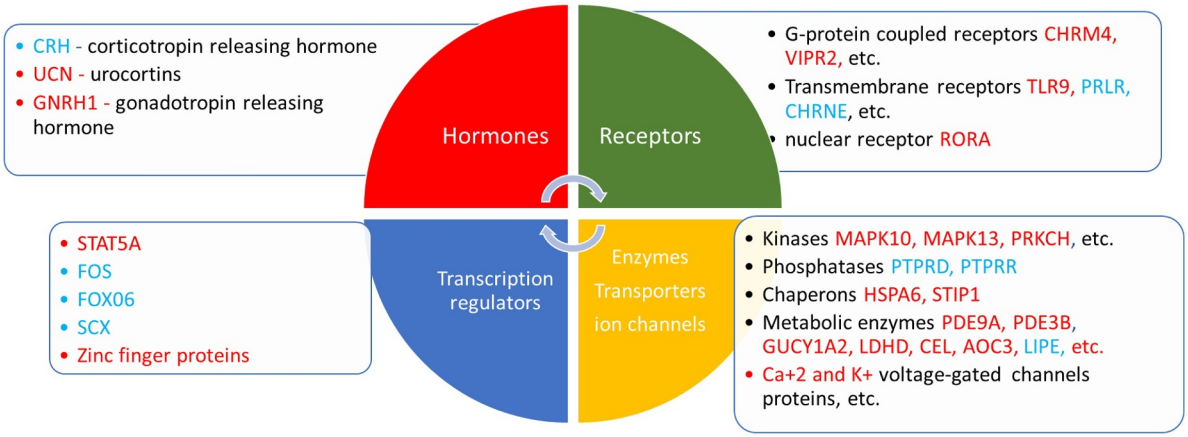

Fig. 8. Effects adaptogens genes involved in regulation of adaptive stress response.

in receptor-ligand stoichiometric homeostasis. We hypothetize also that an allosteric inhibition of *PDE9A* gene expression at low concentration of withanolides (WSL) could lead to the decrease in PDE efficacy irrespective of the amount of available ligand. This would thereby prompt the cell to upregulate PDE to facilitate the desired response, leading to an increased PDE expression leaving the cell at either an advantage or a disadvantage once the allosteric inhibitor is removed. Allosteric activation of *PDE9A* gene expression at higher concentrations of withanolides (WS) or could possibly lead to increased PDE efficacy irrespective of the amount of available ligand. This would thereby swift the cell to downregulate PDE to enable the anticipated response, leading to decreased PDE expression leaving the cell at either an advantage or a disadvantage once the allosteric inhibitor is removed.

The term 'hormesis' was initially used to describe a process by which sublethal damage caused by small doses of a poison produces a repair response upon which the organism becomes stronger than before (Southam and Ehrlich, 1943). The term hormesis is commonly used by toxicologists to describe biphasic dose-response curves in which a chemical has a stimulatory effect at low doses but is toxic at high doses (Calabrese et al., 2010b; Calabrese et al., 2010bc; Calabrese and Mattson, 2017; Fang et al., 2017; Mattson, 2008a,b,c, 2014, 2015). The concept of hormesis has been adopted in the fields of biology and medicine to depict the adaptive response of cells and organisms to moderate stress (Mattson, 2008). Major components of the hormetic response include various stress-resistance proteins such as HSPs, antioxidants, and growth factors (Mattson, 2008a; Mattson and Cheng, 2006). Classical examples of hormetic stress are exercise and calorie restriction (CR). (Fontana and Klein, 2007; Radak et al., 2008) (Fig. 8). The hormesis concept is important for understanding biphasic dose responses to damaging levels of various environmental and industrial toxins. However, the critical point is the association of the biphasic dose response with repair or restoration of damage to cope with stress.

In many observations, the homeostatic range for multiple functions is increased without any damaging initiating stimulus and, therefore, without repair processes (Davies, 2016). That is true for plant secondary metabolites playing important roles in the defense response to microorganisms and insects (Dinan et al., 1997; Metcalf et al., 1980) and obviously characteristic for adaptogenic plants, which specifically interact with host-adaptive stress-response systems (Panossian et al., 1997, 1999a,b). Indeed, in the present study, we have identified positive interactions of plant adaptogens with gene expression playing key role in the adaptive stress signaling response and adaptive homeostasis.

Research on signal transduction pathways that regulate gene expression in response to external and internal stimuli clarifies that biological systems are continuously adapting in the short-term both to set points and to the range of 'normal' capacity. These transient adaptations typically occur in response to relatively mild changes in conditions, to programs of exercise training, or to sub-toxic, non-damaging levels of chemical agents (Dhabhar, 2018). In this context, we believe that the term 'adaptive homeostasis' (Devies, 2016) is more appropriate than hormesis to describe the cellular capability to adjust the homeostatic range in response to exposure to sub-toxic, non-damaging, signaling molecules such as herbal adaptogens.

Toxic hormetins exhibit a narrow dose range ($\leq$ 20-fold) before there is a significant decrease in biological response, while some adaptogens exhibit a wide response ($>$1000-fold) before the zero-equivalence point is reached (Panossian et al., 2010). In our opinion, the amounts of adaptogenic extracts normally consumed by humans fall into the low dose/stimulatory range of concentrations.

Downstream effects analysis and predicted effects of adaptogens and melatonin on physiological functions and aging-related disorders

Further IPA core analyses of the gene expression dataset using the curated information from the QIAGEN Knowledge Base predicted significant effects of adaptogenic plant extracts on metabolic, cardiovascular, inflammatory, immune, neurological, and behavioral diseases; cancer; skeletal, muscular, and connective tissue and endocrine system disorders; and organismal injuries and abnormalities (Fig. 1 in Supplementary data 3). Epidemiological studies have shown that advancing age is associated with an increased prevalence of all of these conditions (Walther et al., 2016). A correlation between aging and the accumulation of oxidatively damaged proteins, lipids, and nucleic acids has been reported (Stadtman, 2001). In fact, age-related disorders and diseases are complications arising from senescence because of decreased adaptability to cope with stress, which induces cellular degeneration and chronic inflammation. Senescence is a cellular response to damage and stress. In living organisms, macromolecules, cells, and tissues are continuously damaged and repaired. Consequently, adaptive mechanisms may have evolved under selective pressure to optimize tissue maintenance and repair. Among these adaptive mechanisms is inflammation. Inflammation can be beneficial as an acute, transient defensive immune response to harmful conditions, such as traumatic tissue injury or an invading pathogen. This response also facilitates repair, turnover, and adaptation of many tissues. However, acute inflammatory responses to pathogen-associated molecular patterns may be impaired during aging, leading to increased susceptibility to infection.

Chronic inflammation has many features of acute inflammation but is usually low grade and persistent, resulting in responses that lead to tissue degeneration. Many aged tissues are probably in a chronically inflamed state, albeit without signs of infection. The term 'inflammaging' describes the low-grade, chronic, systemic inflammation during aging in the absence of overt infection ("sterile" inflammation). It has been coined to indicate the contribution of chronic inflammatory processes to the progression of aging. Inflammaging is a highly significant risk factor for both morbidity and mortality in elderly people. Low-grade chronic inflammation associated with enhanced production of reactive oxygen species (ROS) and insufficient removal by scavenging systems are hallmarks of senescence and contribute to brain inflammaging.

The intrinsic link between gene regulation and aging often relates directly to transcription factors and their regulatory actions. Age-related upregulation of AP-1 (associated with proliferation and migration), NF-κB (associated with inflammatory responses), and Nrf2 (associated with detoxification of ROS) and downregulation of FoxO (associated with antioxidant defense, longevity, and stress) signaling pathways has been demonstrated in vascular smooth muscle cells (Li and Fukagawa, 2010).

In this context, the eustress-mimetic/hormetic effects of adaptogens increasing adaptive suggest potential benefits for healthy aging. In aging cells, significantly reduced expression of Hsp70 and its precursor, heat shock transcription factor HSF1, correlates with a decreased ability to cope with stress (Heydari et al., 1994, 2000). In addition, Hsp70 directly protects cells against entry into apoptic pathways. In brain cells, the inhibition of HSF1 and Hsp70 expression occurs in Alzheimer's disease (Bhat et al., 2004). The age-related decline of hepatic Hsp70 expression contributes to reduced liver detoxification (Gagliano et al., 2007) and protection from toxic substances (Lindquist and Craig, 1988). In most people, a decline in induction of Hsp70 by stress is associated with aging and age-related disease (Singh et al., 2007). Remarkably, Hsp70 does not decrease with age in some individuals who live more than 100 years (Ambra et al., 2004).

The results of this study show that all adaptogens tested upregulated the expression of Hsp70 (Tables 1 and 2). This finding was in line with the results of previous studies, where the adaptogens *R. rosea, S. chinensis*, and *E. senticossus* alone and in combination upregulated the transcription factor HSF1 and initiated increased production of Hsp70 *in vitro* and *in vivo* (Asea et al., 2013; Chiu and Ko, 2004; Hernández-Santana et al., 2014; Lee et al., 2009; Li et al., 2014b; Panossian et al., 2009, 2010), suggesting beneficial effects in aging (Panossian and Gerbarg, 2016).

The neurohormone melatonin is considered as an immune buffer acting as a stimulant under basal or immunosuppressive conditions or as an anti-inflammatory compound in the presence of exacerbated immune responses, such as acute and chronic inflammation and inflammaging, respectively. Such a dual immunomodulatory effect is typical for adaptogens. Melatonin participates in many of these mechanisms by acting through two G-protein–coupled membrane receptors (MT1 and MT2). Fig. 2 depicts a major signaling pathway of the melatonin membrane receptors MTNRN1A and MTNR2A and shows mediators of this signaling pathway. Fig. 2 and Table 4 show that these signaling pathways are common targets for adaptogens. All adaptogens tested activated the melatonin signaling pathway, while BS inhibited this pathway (Table 4), presumably because a lack of effect on expression of *GNRH* (Fig. 2). Fig. 2 also shows the simulating effects of melatonin and the "classical" adaptogen ES on canonical melatonin signaling pathways. Furthermore, melatonin activated all adaptive signaling pathways (Table 4) and upregulated expression of *UCN, GNRH1, TLR9, GP1BA, PLXNA4, CHRM4, GPR19, VIPR2, RORA, STAT5A, ZFPM2, ZNF396, FLT1, MAPK10, MERTK, PRKCH*, and *TTN*, which were commonly regulated by all adaptogens tested (Table 2 and Supplementary data 2c). In other words, the molecular mechanism of actions of melatonin and plant adaptogens are alike, particularly regarding the upregulation of the ligand-specific nuclear receptor RORA (PRZ in Fig. 2), which regulates ligand-activated sequence-specific DNA-binding RNA polymerase II transcription factor activity and metal ion, β-catenin binding, and steroid hormone receptor activity. It also plays a role in intellectual disability, neurological disorders, retinopathy, hypertension, dyslipidemia, and cancer, which are common in aging (Supplementary data 2).

Loss of melatonin at older ages may contribute to the incidence or severity of some age-related neurodegenerative diseases. The concentration of melatonin in human serum is in the range from 15–20

(daytime) to 30–180 ng/ml (nighttime). With increasing age, nighttime melatonin levels decrease to 30 pg/ml (Karasek, 2004). The significance of melatonin in aging is obvious from several observations: (i) melatonin increases the lifespan of mice, (ii) decreased melatonin concentrations have been observed in some but not all patients with Alzheimer's disease, (iii) melatonin concentrations in blood significantly decrease in advanced ages accompanied by an increased frequency of sleep disorders, (iv) melatonin is reported to exert sleep-promoting effects (*e.g.*, reduced sleep latency and induction of sleepiness and fatigue), and (v) melatonin (0.3 to 5 mg) improves subjective and/or objective sleep parameters in patients with insomnia (reduced sleep latency, increased total sleep time, and sleep efficacy. Melatonin is effective in treating age-related delayed sleep phase syndrome by short-term usage (Buscemi et al., 2004). At doses of 3–10 mg, the efficacy of melatonin has been demonstrated in numerous clinicals studies in patients with aging-related neurodegenerative and neurocognitive disorders, such as dementia, Alzheimer's disease, Parkinson's disease, mild cognitive impairments, amyotrophic lateral sclerosis, and rapid eye movement–associated sleep behavior disorder (Andersen et al., 2016). All of this evidence suggests a 'rejuvenating' effect of melatonin. Melatonin occurs across taxa, including in people, bacteria, other mammals, birds, amphibians, reptiles, fish, and plants (Chen et al., 2003; Manchester et al., 2000; Nawaz et al., 2015). The hormone has a wide range of functions in plants, such as the promotion of seed germination and seedling growth and influencing plant senescence (Arnao and Hernandez-Ruiz, 2006). The content of melatonin in the plants studied ranged from 0.01 to 4.390 ng/g dry weight.

According to Maestroni and Conti (1994), melatonin is an adaptation hormone that helps to coordinate and synchronize adaptive responses to environmental variables. According to Arushanyan and Bayer (2012), melatonin is a universal adaptogenic agent that plays an important role in regulation of homeostasis. It is a universal chronobiotic, which shows stabilized fluctuations of any physiological functions and protects the brain and peripheral tissues from emotional and oxidative stress. The pleiotropic actions of melatonin in different physiological and pathological conditions indicate that it may play basic role in regulation of homeostasis *per se*.

Synergistic and antagonistic interactions of plant extracts

During this study, several synergistic and antagonistic interactions of combinations RR-WS, M-WS, and CL-BM were observed. Melatonin in combination with *Withania* synergistically downregulated two genes, which were not downregulated with melatonin or *Withania* alone (Table 7). As a result, four genes contributed to the predicted decrease in glucagon by Adaptra PM (Fig. 5), because four of four genes revealed consistent expression with decreased quantities of glucagon (Table 7**)**. Because glucagon is the main catabolic hormone increasing the concentration of glucose and fat in the bloodstream of the body, we suggest that Adaptra PM might be useful for prevention of type 2 diabetes, which has an incidence that increases with age.

Another synergistic interaction of *Withania* with *Rhodiola* (Adaptra) induced deregulation of 20 genes, 10 of which contribute to predicted activation of neuronal development (Fig. 4) in combination (Table 6). Overall 22 of 57 genes revealed expression consistent with the development of neurons (Table 6) that could be inferred to be beneficial against age-related decline in memory and cognitive functions.

We previously described similar synergistic effects *in vitro* (Panossian et al., 2013, 2015, 2018a). However, it seems that a clear definition of synergy is still required (Roell et al., 2017), particularly in systems pharmacology (SP) for predicting *in vivo* drug effects, where biological networks rather than single transduction pathways are considered as the basis of drug action and disease progression (Danhof, 2016). These models consider functional interactions within a biological network, which are relevant if drugs act at multiple targets in the network or if homeostatic feedback mechanisms are operative. SP models are useful to describe complex patterns of drug action, such as synergy.

In 1957, Goldin and Mantel suggested that "Synergy and antagonism imply that the different constituents affect each other's actions" assuming that "if it is synergistic, the drugs will be more effective in combination than separately" (Goldin and Mantel, 1957). Thus, one of two commonly used definitions of synergy in pharmacology and pharmacognosy is related to efficacy, effectiveness, and potency of the combinations of two agents with the same pharmacological activity: "A combination of agents that is more effective than is expected from the effectiveness of its constituents is said to show synergy" (Berenbaum, 1977). Another definition of synergy is formulated as "two or more agents working together to produce a result not obtainable by any of the agents independently" and can be interpreted as generation of new pharmacological activity, which is specific only for the combination of two or more agents (Skirven et.al., 2011).

In this context, it would be reasonable to distinguish among definitions of synergy and implement more relevant definitions for assessing efficacy or effectiveness of the combination (hybrid substance C) of efficacy substances A and B, such as:

- synergy, if efficacy of C is > 0 while efficacies of $A = 0$ and $B = 0$, $0 + 0 > 0$;
- amplification, if $1 + 1 > 2$, instead of synergy;
- potentiation, if $0 + 1 > 1$;
- antagonism, if efficacy of C is 0 while efficacies of $A = 1$ and $B = 1$, $1 + 1 = 0$;
- attenuation, if $1 + 1 < 2$;
- addition, if $1 + 1 = 2$.

The term **synergy** is more suitable for interactions of two or more agents resulting in qualitatively new pharmacological effect, *e.g.*, gene expression that cannot be obtained by any single constituent independently. Similarly, antagonism results from interactions of several constituents in combination, leading to a lack, reduction, or prevention of effects that any individual ingredient in that combination yields.

These definitions are suitable and relevant to pharmacological studies of combinations of plant extracts, where the main focus is the discovery of unexpected potential indications and toxic effects of the combinations from complex intracellular and extracellular interactions of molecular networks with many players at several phases of development of final pharmacological outcomes.

Conclusion

This study has elucidated the genome-wide effects of several adaptogenic herbal extracts in brain cell culture. These data highlight the consistent activation of adaptive stress-response signaling pathways by adaptogens in T98G neuroglia. Adaptogenic herbal extracts affect a large number of genes that play key roles in modulating adaptive homeostasis, indicating their ability to modify genetic expression to prevent stress-induced and aging-related disorders such as chronic inflammation, cardiovascular health, neurodegenerative–cognitive impairment, metabolic disorders, and cancer. Overall, this study provides a comprehensive look at the molecular mechanisms by which adaptogens exerts stress-protective effects.

Conflict of interest

Author AP is currently a consultant to EuroPharma USA, Inc. He is the founder of Phytomed AB (Sweden) and was the former Head of Research & Development at the Swedish Herbal Institute, Gothenburg, Sweden. He is not a member of any pharmaceutical industry-sponsored advisory board or speaker's bureau, and he has no significant financial interest in any pharmaceutical company. The other authors (EJS, TE) declare no competing interests.

Declaration

The authors declare that the results of this study were partially presented in The 22th International Congress Phytopharm 2018. https://www.phytopharm2018.ch/?page_id=2, (Panossian et al., 2018b).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2018.09.204.

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