CrossMark

RESEARCH ARTICLE

Daily chronomics of proteomic profile in aging and rotenone-induced Parkinson's disease model in male Wistar rat and its modulation by melatonin

Anita Jagota · Ushodaya Mattam

Abstract Aging is associated with changes in several basic parameters of circadian timing system (CTS) in mammals leading to circadian dysfunction. We had reported earlier that upon aging and in rotenone induced Parkinson's disease (RIPD) rat model there were significant alterations in the core clock genes expression levels and daily pulses. To identify biomarkers of aging and PD chronomics of proteomic day–night profiles in suprachiasmatic nucleus (SCN), pineal and substantia nigra (SN) in 3 month (m), 12, 24 m and RIPD rat model were studied at two time points i.e. Zeitgeber Time (ZT)-6 (mid-day) and ZT-18 (mid-night). Proteome analysis was done by using two dimensional (2-D) electrophoresis and the spots showing robust day–night variations were identified by using MALDI TOF/TOF analysis. In 3 m rats the number of proteins showing day–night variations were relatively more than 12, 24 m and RIPD rat model in SCN and SN. But in pineal there was increase in number of protein spots showing day–night variations in 24 m. Mass spectroscopy of the protein spots showing robust day night variation in aging and RIPD rats were identified. As melatonin, a multitasking molecule, an endogenous synchronizer of rhythm, an antioxidant and an antiaging drug, declines with aging, the effects of melatonin administration on differential alterations in chronomics of 2-D protein profiles in aging and RIPD male Wistar rats were studied. We report here that the melatonin could be playing an important role in modulating the chronomics of 2-D protein profiles. Additionally, various proteins were identified for the first time in this study showing significant day night variation in SCN, pineal and SN may prove useful towards targeting novel treatments for circadian dysfunction, good health and longevity.

Keywords Suprachiasmatic nucleus · Pineal · Substantia-nigra · Aging · Parkinson's disease · Rotenone · Melatonin · Protein profile · Circadian rhythms

A. Jagota (✉) · U. Mattam
Neurobiology and Molecular Chronobiology Laboratory, Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India
e-mail: ajsl@uohyd.ernet.in

Introduction

Biological rhythm that persists with 24 h periodicity is called as circadian rhythm. In mammals the circadian rhythms are controlled by Suprachiasmatic nucleus (SCN) which is located in hypothalamus (Jagota et al. 2000). SCN with its molecular components synchronizes the physiological and behavioral activities of mammals to the daily light/dark cycles. Such a molecular frame work is also present in every other

peripheral cell that is synchronized by SCN. The SCN mediates its circadian function by various neurotransmitters such as serotonin, glutamate and neuropeptides such as pituitary adenylate cyclase activating polypeptide (PACAP), vasoactive intestinal peptide (VIP), arginine vasopressin (AVP) (Jagota 2006). Melatonin is one of the major clock output and its primary function is to transduce light and dark information to whole body physiology. Melatonin regulates various physiological processes such as sleep (Cardinali et al. 2012), free radical scavenging, tumor growth inhibition, seasonal reproduction as well as immune function (reviewed in Pandi-Perumal et al. 2013). Melatonin being a potent antioxidant shows receptor independent actions due to its direct scavenging ability of reactive oxygen species (Pandi-Perumal et al. 2013, Reiter et al. 2014). Melatonin can directly neutralize many free radicals such as $OH^.$, $ONOO^-$, O_2^-, H_2O_2, $1O_2$, $NO^.$, $LOO^.$ and $HClO$ (Reiter et al. 2014) and stimulate antioxidant enzymes. Melatonin shows two kinds of effects on SCN. One is neuronal firing suppression through MT1 and the other one is circadian phase resetting. This readjustment in circadian phase of SCN is mediated through MT2 (Pandi-Perumal et al. 2013). Rhythmic melatonin secretion also has an important role in dopamine circadian rhythms and thus SCN indirectly regulate dopamine rhythms in the mid brain region substantia nigra (SN) (Khaldy et al. 2002).

Aging is a complex process (Rattan 2012) characterized by the slowing or alteration of cellular and bodily processes over time, reduced functionality of cells, increased susceptibility to disease, alterations in circadian rhythms and ultimately the death of the organism. Age-related changes in the SCN have been linked to age-related circadian dysfunction, decline in circadian neural activity (Nakamura et al. 2011), alteration in serotonin rhythms (Jagota and Kalyani 2008, 2010), neuropeptide content and GABAergic network of the SCN (Palomba et al. 2008). Melatonin production, amplitude and its pulsatile release from pineal gland decreases upon aging (Aujard et al. 2001). With profound effects on the health and well-being of the elderly subjects (Poeggeler 2005). Upon aging the clock gene expression gets altered (Mattam and Jagota 2014). Increased oxidative stress results in most of the age-related neurological diseases like Parkinson's disease (PD), Alzheimer's disease (AD) etc. (Kondratov et al. 2006).

PD is the second most common age-related neurodegenerative disorder (Beal 2001), which is

estimated to affect approximately 1% of the population older than 65 years of age (Jagota 2005). PD patients exhibit cardinal motor symptoms such as resting tremor, bradykinesia, rigidity and abnormal postural reflexes (Uversky 2004). Dopaminergic neurons present in the SN of mid brain region undergo degeneration resulting in the deterioration of the neuronal connection between the SN and the striatum (Dauer and Przedborski 2003) thus leading to decrease in dopamine level in PD as well as with aging. Oxidative damage linked to mitochondrial dysfunction, energy depletion and activation of programmed cell death are the major causes for death of the dopaminergic neurons in PD (Dauer and Przedborski 2003). Many circadian disorders observed in aging are also observed in PD which is more pronounced in case of latter. One of the possible causes of circadian disorders observed in PD is degeneration in circadian clock regulating circuits in the SCN. In aging and PD many of the circadian dysfunction had been related to metabolism, antioxidant defense, melatonin, DNA repair and autophagy (Kondratova and Kondratov 2012). Though there is accumulating data on PD; the biomarkers of aging and PD need to be identified. We have recently reported alteration in various clock gene mRNA expression and serotonin metabolomics in the RIPD male Wistar rat model (Mattam and Jagota 2015). To identify such biomarkers for aging and PD, we studied the day–night variations in protein profile of SCN (pacemaker), pineal (relay centre) and SN (the region undergoing degeneration in PD) in rotenone induced PD (RIPD) rat model. In aging and PD, as melatonin has antioxidant properties and helps in circadian phase resetting, we further studied the effects of melatonin on day–night variations of protein profile in SCN, pineal and SN of aging and RIPD rat model to identify melatonin sensitive protein markers. Additionally protective effects of melatonin against rotenone by administrating simultaneously were also studied.

Materials and methods

Animals

Male Wistar rats in three age groups each with n = 144 Group I: 3 months (m), Group II: 12 m, and Group III: 24 m were used. Each group I, II and

III was further divided into three groups of 48 animals each i.e., (A) Control (B) Melatonin treated (MT) and (C) Vehicle control. For PD studies Group IV (n = 384) male Wistar rats of 60 day old were separated into three groups-IVA, IVB and IVC. To get 400 µg of protein for each gel we have pooled for SCN and Pineal tissue from four animals. Briefly Group IVA (n = 192) was further divided into four groups with n = 48 each as control (age matched control), Sham, Vehicle and rotenone-treated (2.5 mg/ml/kg body weight) at ZT-5 to develop RIPD model (Alam and Schmidt 2002). Group IVB (n = 96) was further divided into two groups with n = 48 each as RIPD, Melatonin vehicle (RIPD, MEL-V) (Pazo et al. 2002; Jagota and Kalyani 2010) and RIPD, Melatonin (RIPD, MEL) (Pazo et al. 2002; Jagota and Kalyani 2010). Group IVC (n = 96) also was further divided into two groups with n = 48 each as rotenone and melatonin vehicle (RT+MEL-V); and rotenone and melatonin (RT+MEL) (Supplementary Fig. 1).

All rats were kept individually in polypropylene cages contained within well ventilated light proof environmental cabinets isolated in animal facility. They were maintained at room temperature at 23 ± 1 °C and relative humidity 55 ± 6% LD 12:12 [lights on: 06:30 A.M. Zeitgeber time (ZT-0) and lights off: 6:30 P.M. (ZT-12)] for 2 weeks prior to experiment. Food and water were provided ad libitum. During handling of animals in dark dim red light was used. Cage changing was done at random intervals. All experiments were performed as per Institutional Animal Ethics (Reddy and Jagota 2014).

Animal treatments were done as explained earlier (Mattam and Jagota 2015).

Rotenone administration to develop RIPD model

Rotenone emulsified in sunflower oil (2.5 mg/ml/kg body weight) was administered intraperitoneally at ZT-5 for 48 days to develop as RIPD model. Sham control group received 48 days of intraperitoneal administration of phosphate buffered saline (PBS; 1 ml/kg body weight) at ZT-5. Vehicle control received 48 days of intraperitoneal administration of sunflower oil (1 ml/kg body weight) at ZT-5 (Alam and Schmidt 2002; Mattam and Jagota 2015).

Melatonin administration

3, 12, 24 m age groups were administered 30 µg/kg body weight of melatonin in 10% ethanol in physiological saline subcutaneously at 1 h before the onset of darkness (ZT-11) for 11 days (Pazo et al. 2002; Jagota and Kalyani 2010). On 12th day rats of variable age groups were sacrificed at ZT-6 and 18.

RIPD, MEL group received 11 days of subcutaneous administration of melatonin (30 µg/kg body weight in 10% ethanol in physiological saline) similarly after development of RIPD. However, RT+MEL group received intraperitoneal administration of rotenone emulsified in sunflower oil (2.5 mg/mL/kg body weight) at ZT-5 and subcutaneous administration of melatonin (30 µg/kg body weight in 10% ethanol in physiological saline) at ZT-11 simultaneously for 48 days (Mattam and Jagota 2015).

SCN, pineal and SN tissue separation

Animals were sacrificed by decapitation and the brains were dissected out carefully at two time points such as ZT-6 and 18. Pineal glands were separated (Jagota et al. 1999). 500 µm brain slices were made using rat brain slicer (Zivic Instruments; Pittsburg USA) and the SCN was carefully punched out with the help of a sharp scalpel (Jagota and Reddy 2007). SN was removed by the method of Heffner et al. (1980) (Mattam and Jagota 2015).

Proteomic studies by two-dimensional (2-D) electrophoresis

Preparation of protein samples for 2-D electrophoresis

20% homogenate of SCN, pineal and SN was prepared with lysis buffer (7 M urea, 2 M thiourea, 2% CHAPS, 2% ampholines, 20 mM DTT and 1 mM PMSF). SCN and pineal samples were prepared by pooling the tissues from four male wistar rats for each sample. Average weight of each SCN and pineal was approximately 1–2 mg, SN was approximately 7–10 mg. Cells were disrupted by sonication for 2 min and centrifuged at 10,000 rpm for 5 min at 4 °C. Clear supernatant as transferred to fresh tubes. 750 µL of ice cold acetone was added to clear supernatant to precipitate proteins and kept at −20 °C for 24 h.

Acetone precipitated samples were dissolved in rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 2% ampholines, 20 mM DTT). Protein estimation was done by Amido Black method (Schaffner and Weissmann 1973).

2-D electrophoresis

400 µg of protein was dissolved in 350 µL of rehydration buffer. This solution was used to rehydrate IPG strip (18 cm strip, pH 3–10 NL; GE Healthcare) in an Immobiline drystrip re-swelling tray (GE Healthcare) for 20 h at room temperature. First dimension Iso Electro Focusing (IEF) was performed with the Ettan IPG Phor3 system (GE Healthcare) for 24 h for 60,000 Vh. After IEF, strips were equilibrated in 25 mM DTT and 50 mM Iodoacetamide. Strips were subjected to second dimension 12% SDS PAGE gel electrophoresis followed by Silver Staining (Mortz et al. 2001). Details of various buffers and solutions used is provided in supplementary material.

Analysis of 2-D gels and statistics

Gels (Fig. 1) were scanned on high resolution scanner and saved as TIFF-files and transferred to the Image Master 2D Platinum software version 7 (GE Healthcare) on which the analysis was performed. All the gels (n = 6) (Supplementary Figs. 2, 3, 4) were matched and spots on these gels with the same match identity number (id). Spots were quantified and the volume percentage was obtained for all spots. Changes in expression between ZT-6 and ZT-18 were considered significant at p values less than 0.05 (ANOVA) given by software.

Proteomic analyses: in-gel digestion and mass spectrometry (MS)

In-gel digestion and matrix-assisted laser desorption/ionization time of flight mass spectrometric (MALDI-TOF MS) analysis was performed with a MALDI-TOF/TOF mass spectrometer (Bruker Autoflex III smartbeam, Bruker Daltonics, Bremen, Germany) according to the method described by Shevchenko et al. (1996) with slight modifications. Silver-stained protein spots were manually excised from two reproducible gels. The excised gel pieces were destained with 30 µL of Potassium ferricyanide and $Na_2S_2O_3$ (1:1) for 30 min and then with 50% acetonitrile (ACN)

in 25 mM ammonium bicarbonate NH_4HCO_3 for 15 min. Thereafter, the gel pieces were treated with 10 mM DTT in 25 mM NH_4HCO_3 and incubated at 56 °C for 1 h. This was followed by treatment with 55 mM iodoacetamide in 25 mM NH_4HCO_3 for 45 min at room temperature (25 ± 2 °C), washed with 25 mM NH_4HCO_3 and ACN (1:1), dried in speed vac and rehydrated in 20 µL of 25 mM NH_4HCO_3 solution containing 12.5 ng/µL trypsin (sequencing grade, Promega, Wisconsin, USA). The above mixture was incubated on ice for 10 min and kept overnight for digestion at 37 °C. After digestion, a short spin for 10 min was given and the supernatant was collected in a fresh eppendorf tube. The gel pieces were re-extracted with 50 µL of 1% trifluoroacetic acid (TFA) and ACN (1:1) for 15 min with frequent vortexing. The supernatants were pooled together and dried using speed vac and were reconstituted in 5 µL of 1:1 ACN and 1% TFA. 2 µL of the above sample was mixed with 2 µL of freshly prepared a-cyano-4-hydroxycinnamic acid (CHCA) matrix in 50% ACN and 1% TFA (1:1) and 1 µL was spotted on target plate.

Protein identification: peptide mass fingerprinting and MS/MS analysis

Protein identification was performed by database searches (PMF and MS/MS) using MASCOT program (http://www.matrixscience.com) employing Biotools software (Bruker Daltonics).The similarity search for mass values was done with existing digests and sequence information from NCBInr and Swiss Prot database. The taxonomic category was set to *Rattus norvegicus*. The other search parameters were: fixed modification of carbamidomethyl (C), variable modification of oxidation (M), enzyme trypsin, peptide charge of 1^+ and monoisotropic. According to the MASCOT probability analysis ($p \leq 0.05$), only significant hits were accepted for protein identification.

Results

Day night variation in protein profile in SCN, pineal and SN upon aging and RIPD rat model: effect of melatonin administration

Representative 2-D gel images with protein profiles of spots of proteins in the 3–10 pH range in SCN, pineal
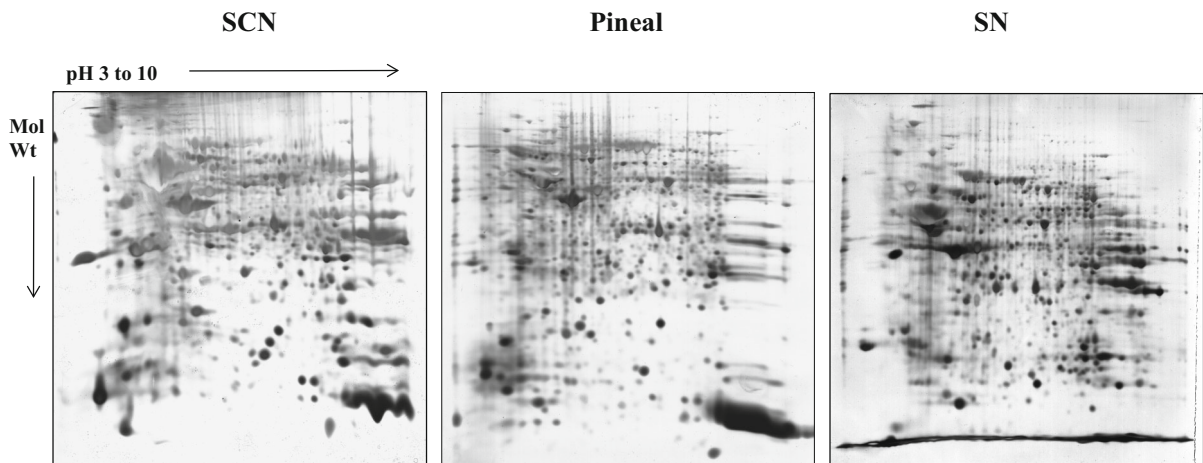
Fig. 1 Representative 2D gel images with protein profiles of spots of proteins in the 3–10 pH range in SCN, pineal and SN protein profiles (listed in Table 1, Supplementary Table)

and SN protein profiles have been shown here as Fig. 1. Analysis of 2-D gels, gave the count of protein spots in the 12 month (m), 24, 12 m melatonin treated (12 m MT), 24 m MT and rotenone induced PD (RIPD) rat model, RIPD given melatonin for 11 days (RIPD, MEL); and rotenone and melatonin treatment together for 48 days (RT+MEL). The common spots between two groups control and melatonin treated were then counted followed by picking from these common protein spots, the ones that showed significant variation ($p < 0.05$) (Fig. 2; Supplementary Table 1). Further, among the oscillating protein spots, the number of spots with up regulation at ZT6 and ZT18 in various experimental groups were identified (Table 1).

The number of protein spots showing day night variation in RIPD were found comparable to 24 m age. Upon melatonin administration the number of proteins showing day night variation were differentially restored in various experimental groups. No significant difference was found between the control and the vehicle control. The spots in each group with robust day–night variation were selected for peptide identification. MS/MS analysis revealed identification of proteins with robust day–night variation in SCN, pineal and SN in various experimental groups studied (Table 2).

SCN

Semaphorin-4F, Sodium driven chloride bicarbonate exchanger, Retinol binding protein 1, syntaphilin,

Malate dehydrogenase, Solute carrier family 25 member 39 and Merlin were identified with MS/MS analysis in SCN. In 3 m "Semaphorin-4F" showed robust day–night variation which persisted in 12 m and abolished in 24 m and RIPD rat model. Upon melatonin administration its day–night variationwas not restored in both aging (12 and 24 m) and RIPD rat model (Fig. 3a). In 3 and 12 m "Sodium driven chloride bicarbonate exchanger" showed robust day–night variation. Upon aging and in RIPD rat model its day–night variation has been abolished. Upon melatonin administration its day–night variation could not be restored in neither aging (12 and 24 m) nor RIPD rat model (Fig. 3b). In 12 and 24 m "Retinol binding protein 1" showed robust day–night variation though neither in 3 m nor RIPD rat model. Upon melatonin administration its day–night variation were lost in 12, 24 m and RT+MEL but established in RIPD MEL group (Fig. 3c). In 12 and 24 m MT "syntaphilin" showed robust day–night variation though no day–night variation was observed in 3, 12 and 24 m age groups and RIPD rat model. Upon melatonin administration its day–night variation was not observed in RT+MEL and RIPD MEL group (Fig. 3d). "Malate dehydrogenase" displayed day–night variation in 24 m and RIPD rat model though not in 3 and 12 m. However, day–night variation was observed in 24 m, RT+MEL and RIPD MEL group upon melatonin administration (Fig. 3e). "Solute carrier family 25 member 39" showed day–night variation in 3 m and RIPD rat model but not in 12 and 24 m. Upon

Table 1 Effect of melatonin on daily rhythms of protein profiles in aging and RIPD rat model

Tissue	No. of protein spots shown day–night variation							
	Age in months (m)					RIPD	RIPD MEL	RT+MEL
	3 m	12 m	24 m	12 m MT	24 m MT			
SCN	32	20	9	30	20	8	22	15
	ZT6:19	ZT6:18	ZT6:7	ZT6:23	ZT6:8	ZT6:4	ZT6:19	ZT6:9
	ZT18:13	ZT18:2	ZT18:2	ZT18:7	ZT18:12	ZT18:4	ZT18:3	ZT18:6
Pineal	51	18	62	52	71	36	58	31
	ZT6:23	ZT6:11	ZT6:25	ZT6:22	ZT6:30	ZT6:15	ZT6:34	ZT6:23
	ZT18:28	ZT18:7	ZT18:37	ZT18:30	ZT18:41	ZT18:21	ZT18:24	ZT18:8
SN	59	30	9	36	23	12	12	9
	ZT6:9	ZT6:23	ZT6:4	ZT6:28	ZT6:10	ZT6:5	ZT6:9	ZT6:7
	ZT18:50	ZT18:7	ZT18:5	ZT18:8	ZT18:13	ZT18:7	ZT18:3	ZT18:2

Day-night variation in protein spots in aging (3 month (m), 12, 24, 12 m melatonin treated (12 m MT), 24 m MT and rotenone induced PD (RIPD) rat model, RIPD given melatonin for 11 days (RIPD, MEL) and rotenone treatment and melatonin treatment for 48 days (RT+MEL). Among the protein spots showing day night variation, the spot numbers showing upregulation at ZT 6 and ZT18 have been tabulated

melatonin administration there was restoration in 12 m (Fig. 3f). "Merlin" showed day–night variation only in 12 m but not in 3, 24 m and RIPD but with melatonin administration RT+MEL and RIPD MEL groups showed day night variation (Fig. 3g).

Pineal

The proteins identified in pineal using MS/MS analysis were PHD finger protein-II, AP-1 complex subunit beta-1, isovaleryl CoA dehydrogenase, Protein FAM131B and Small G protein signaling modulator 3. It was found that "PHD finger protein-II" showed a day night variation in 3 m but not in 12 and 24 m and RIPD rat model. With melatonin administration restoration in day–night variation was observed in both 12 and 24 m groups and RIPD MEL (Fig. 4a). "AP-1 complex subunit beta-1" showed a robust day–night variation in 12 and 24 m group but not in 3 m and RIPD rat model. With melatonin administration its day–night variation persisted in 12 and 24 m (Fig. 4b). In 24 m and RIPD group "isovaleryl CoA dehydrogenase" showed robust day–night variation but not in 3 and 12 m. Upon melatonin administration the day–night variation of this protein was observed in 12 m and RT+MEL (Fig. 4c). "Protein FAM131B" showed robust day–night variation in 12 m MT and RIPD group (Fig. 4d). "Small G protein signaling

modulator 3" showed robust day–night variation in 3 m and RIPD but not in 12 and 24 m. With melatonin administration restoration in day–night variation was observed in 12, 24 m, RT+MEL and RIPD MEL (Fig. 4e).

SN

Upon MS/MS analysis, Neuropilin-1, RalGTPase-activating protein subunit beta, SH2B adaptor protein 3, SH2B adaptor protein 3, Amyloid beta A4 precursor protein-binding family A member 3, Pro-neuregulin, Protein RUFY3 and Eno1 protein were identified in SN. It was found in 3 m group "Neuropilin-1" showed robust day–night variation which was lost by 12 m and abolished in 24 m and RIPD groups. Melatonin administration could not restore its day–night variation in any of the groups studied (Fig. 5a). The protein "RalGTPase-activating protein subunit beta" displayed robust day–night variation in 3, 12 and 24 m and RIPD. With melatonin treatment its day–night variation persisted only in RIPD MEL group but not in 12, 24 m and RT+MEL (Fig. 5b). In 12, 24 m and RIPD group, SH2B adaptor protein 3 showed a robust day–night variation but not in 3 m group. The melatonin administration did not show any effect in restoring this protein (Fig. 5c). In 12 and 24 m, "Guanine nucleotide binding protein subunit alpha-
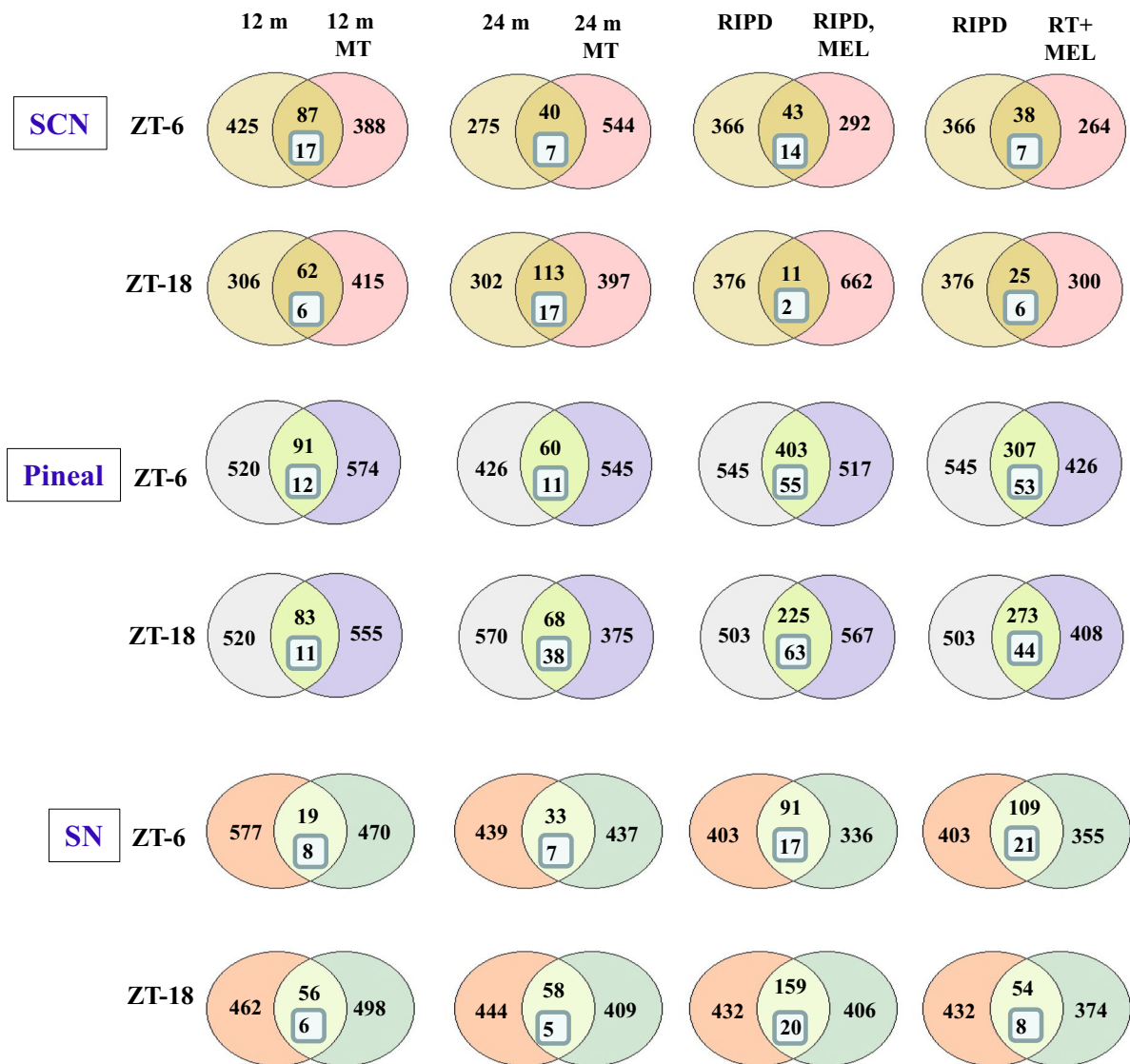
Fig. 2 Effect of melatonin on number of protein spots in SCN, pineal and SN in aging and RIPD rat model. Various animal groups: (12 month (m), 24, 12 m melatonin treated (12 m MT), 24 m MT and rotenone-induced PD (RIPD) rat model, RIPD given melatonin for 11 days (RIPD, MEL) and rotenone treatment and melatonin treatment for 48 days (RT+MEL).

Left and *Right circle* show total number of proteins detected in the respective group. *Two circles* at their overlapping areas represent number of matched spots between two groups. The *central square* represents number of protein spots showing significant variation between the groups ($p \leq 0.05$)

12" showed a robust day–night variation but not in 3 m and RIPD group. With melatonin treatment its day–night variation persisted only in 12 m and RT+MEL group (Fig. 5d). "Amyloid beta A4 precursor protein-binding family A member 3" showed day–night variation in 3, 24 m and RIPD but not in 12 m. Upon melatonin administration its day–night variation was observed in 12, 24 m and RIPD MEL

groups (Fig. 5e). "Pro-neuregulin 2", membrane bound isoform showed day–night variation in 3, 12 and 24 m and RIPD rat model. Upon melatonin administration its day–night variation persisted in 12 and 24 m, abolished in RIPD MEL and RT+MEL (Fig. 5f). Protein RUFY3 showed day–night variation in 12, 24 m and RIPD but not in 3 m. Upon melatonin administration its day–night variation persisted in

Table 2 Identified proteins showing robust daily pulse in SCN, pineal and SN in aging and RIPD and upon melatonin administration

S. No.	Group	Protein identified	Peptide sequence matched	Observed (Mwt in kD/pI)	SC (%)	MS/MS score
SCN						
1	3 m	Semaphorin-4F	LQSCSECILA QDPVCAWSFR	85/6.59	2	28
2	12 m	Sodium-driven chloride bicarbonate exchanger	AAIVFPM MVLALVFVR	126/6.07	2	13
3	12 m MT	Syntaphilin	MAMSLQGSR	55/5.8	1	24
4	24 m	Retinol-binding protein 1	ALDVNVALR	15.9/5.1	6	16
5	24 m MT	Malate dehydrogenase, cytoplasmic	DLDVAVLVGSMPR SAPSIPKENFSCLTR GEFITTVQQR VEGLPINDFSR	36.6/6.16	14	116
6	RIPD	Solute carrier family 25 member 39	QMSLGAV EAMRVKPPR	39.6/9.45	4	23
7	RT+MEL	Merlin (fragment)	IASRMSFSSLKR VLHMKEEATMANEAL MR	68.9/6.2	4	36
Pineal						
1	3 m	PHD finger protein 11	DAAVK APFLKK	38.2/6.1	3	32
2	12 m	AP-1 complex subunit beta-1	LSH ANSAVVLSAVK	105/5	1	17
3	12 m MT	Protein FAM131B	SSFSGISRSMK DPEMSTALSR	36/4.3	6	37
4	24 m	Isovaleryl-CoA dehydrogenase, mitochondrial	QYVYNVAR YEIGGGTSEVRR	46.8/8.03	4	122
5	24 m MT	Small G protein signaling modulator 3	EGSEPGCSQ MAGTPLVEDPPQR	86.1/5.33	2	21
SN						
1	3 m	Neuropilin-1	AKSF EGNNNYDTPE LRAFTPLSTR FIR TFCHWEHDSHAQLR	104/5.71	4	33
2	12 m	Ral GTPase-activating protein subunit beta	LSM PQSAAVNTTP PHNR ATMKTST VTTAHTSK	166/6.3	2	25
3	12 m MT	Guanine nucleotide binding protein subunit alpha-12	RRNRGKPLFHHFTT AIDTENIR	44.2/9.84	5	25
4	24 m	SH2B adapter protein 3	LSGYVVVV SQAPGR	40/9.64	3	13

Table 2 continued

S. No.	Group	Protein identified	Peptide sequence matched	Observed (Mwt in kD/pI)	SC (%)	MS/MS score
5	24 m MT	Amyloid beta A4 precursor protein-binding family A member 3	GPEVPSED	61.5/5.16	11	40
			HPSNTQWALGPR			
			LLQPPEDPGGDPG			
			WMEGTEPADNRSSSSS PELW LETAPLVTHR			
6	RIPD	Pro-neuregulin 2, membrane bound isoform	AAMPPY	95.2/9.52	1	27
			HDSIDSLR			
7	RIPD, MEL	Protein RUFY3	TNQMAATIK	53.1/5.36	4	31
			QAKTLNSAANK			
8	RT+MEL	Eno1 protein	AAVPSGASTGIYEALELR	51.7/6.7	23	426
			HIADLAGNPEVILPVPAFNV INGGSHAGNKLAMQEFMILPVGASSFR			
			AGYTDQVVIGMDVAASEFYR			
			YITPDQLADLYK			
			LAQSNGWGVMVSHR			

Robust daily pulse shown proteins in aging (3 month (m), 12, 24, 12 m melatonin treated (12 m MT), 24 m MT and rotenone induced PD (RIPD) rat model, RIPD given melatonin for 11 days (RIPD, MEL) and rotenone treatment and melatonin treatment for 48 days (RT+MEL). MW: Molecular weight in kD; SC: sequence coverage; p*I*: isoelectric point

In SCN (RIPD, MEL) and pineal (RIPD; RIPD, MEL; RT+MEL) selected protein spots failed to qualify MASCOT probability analysis (p ≤ 0.05), as only significant hits can be accepted for protein identification

24 m and RIPD MEL (Fig. 5g). Eno1 protein showed day–night variation only in RIPD and RT+MEL but not in other groups (Fig. 5h).

Discussion

We have reported recently the alterations of clock genes upon aging and in RIPD and the differential effect of melatonin on such changes (Mattam and Jagota 2014, 2015). In this study, as a step towards understanding the proteomic profiles, we studied the complete proteome analysis of 3, 12, 24 m old rats and RIPD rats. We report here significant robust alterations in the protein expression with day–night variations in SCN, pineal and SN in aging and RIPD rats.

Melatonin, a neurohormone plays an important role in regulating circadian rhythms through its rhythmic release from pineal gland (Jagota 2012; Pandi-Perumal et al. 2013). The melatonin levels have been reported to decrease upon aging leading to the

circadian system disruption (Karasek 2004). The exogenous administration of melatonin proved to be beneficial in restoring the abolished rhythms in several aspects of physiology and behavior as reported by several researchers (Pandi-Perumal et al. 2013). We reported earlier the exogenous effects of melatonin on restoration of daily rhythms of serotonin, serotonin metabolism (Jagota and Kalyani 2010; Reddy and Jagota 2015), antioxidant enzymes and lipid peroxidation (Manikonda and Jagota 2012), clock genes *rPer1, rPer2, rCry1, rCry2* and *rBmal1* (Mattam and Jagota 2014) as well as in NO levels and *Socs1* expression (Vinod and Jagota 2016, 2017) in aging and restoration of clock genes and serotonin metabolism in RIPD (Mattam and Jagota 2015). We therefore studied effects of administration of melatonin on altered protein profiles in aging and RIPD in present study.

In SCN we found that "Semaphorin-4F" and "Sodium driven chloride bicarbonate exchanger"(NCBE), a member of SLC4 family of bicarbonate transporters known to regulate intracellular pH and
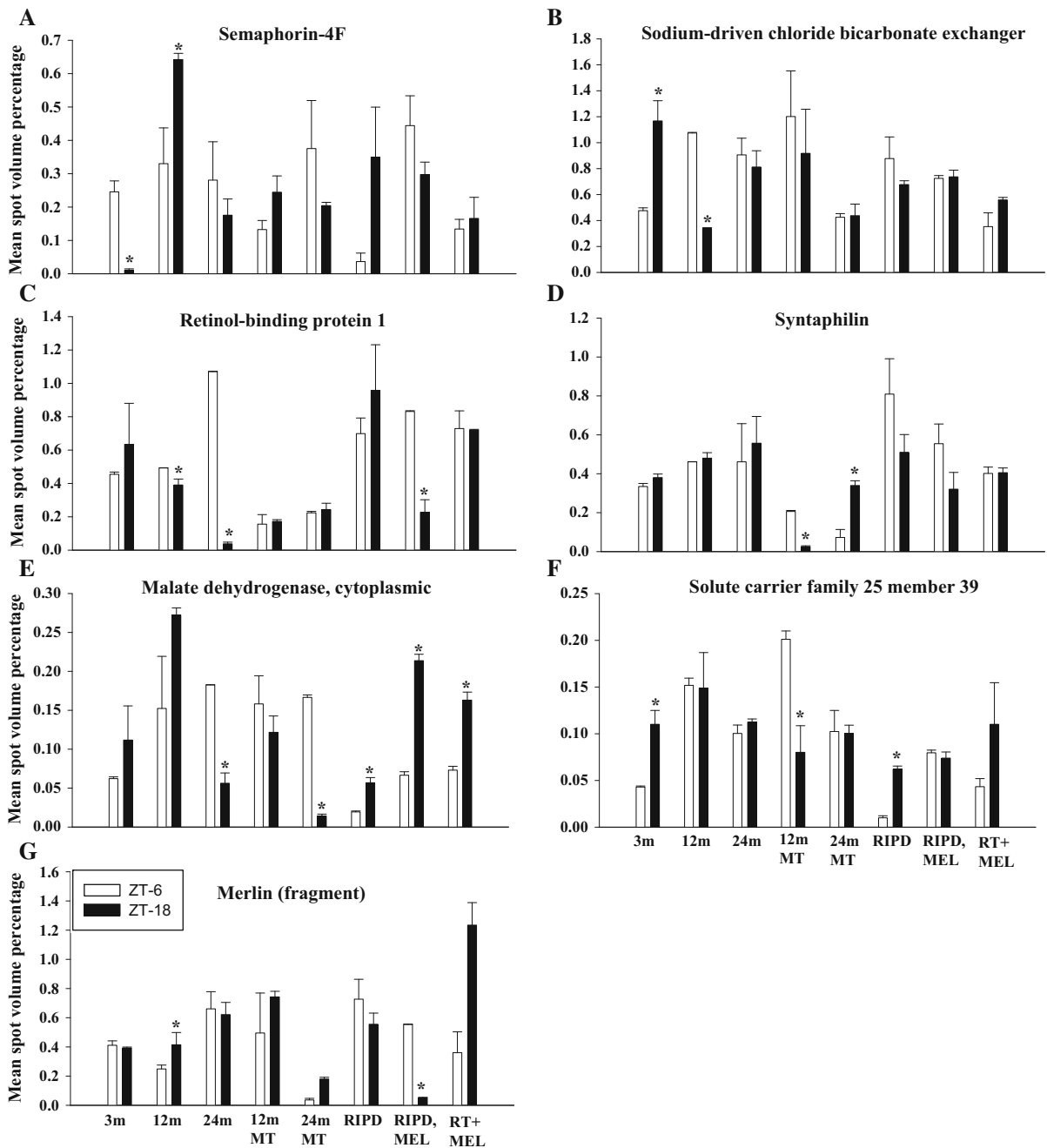
Fig. 3 The proteins identified in SCN showing significant daily pulse ($p \leq 0.05$), **a** semaphorin-4F; **b** sodium-driven chloride bicarbonate exchanger; **c** retinol-binding protein 1; **d** syntaphilin; **e** malate dehydrogenase, cytoplasmic; **f** solute carrier family 25 member 39; **g** merlin (fragment). *Asterisk* represents comparison with ZT-6 within the group

chloride ion concentration (Wang et al. 2000) showed robust day–night variation in 3 and 12 m but in 24 m and RIPD rats such day–night variation was abolished. In the SCN, diurnal changes in intracellular chloride concentration modulate the GABA equilibrium

potentials altering its excitatory and inhibitory status between day and night, respectively (Wagner et al. 2001). We show for the first time that NCBE is expressed in the SCN, its rhythmic expression profile may be indicative of its role in changes in the electrical
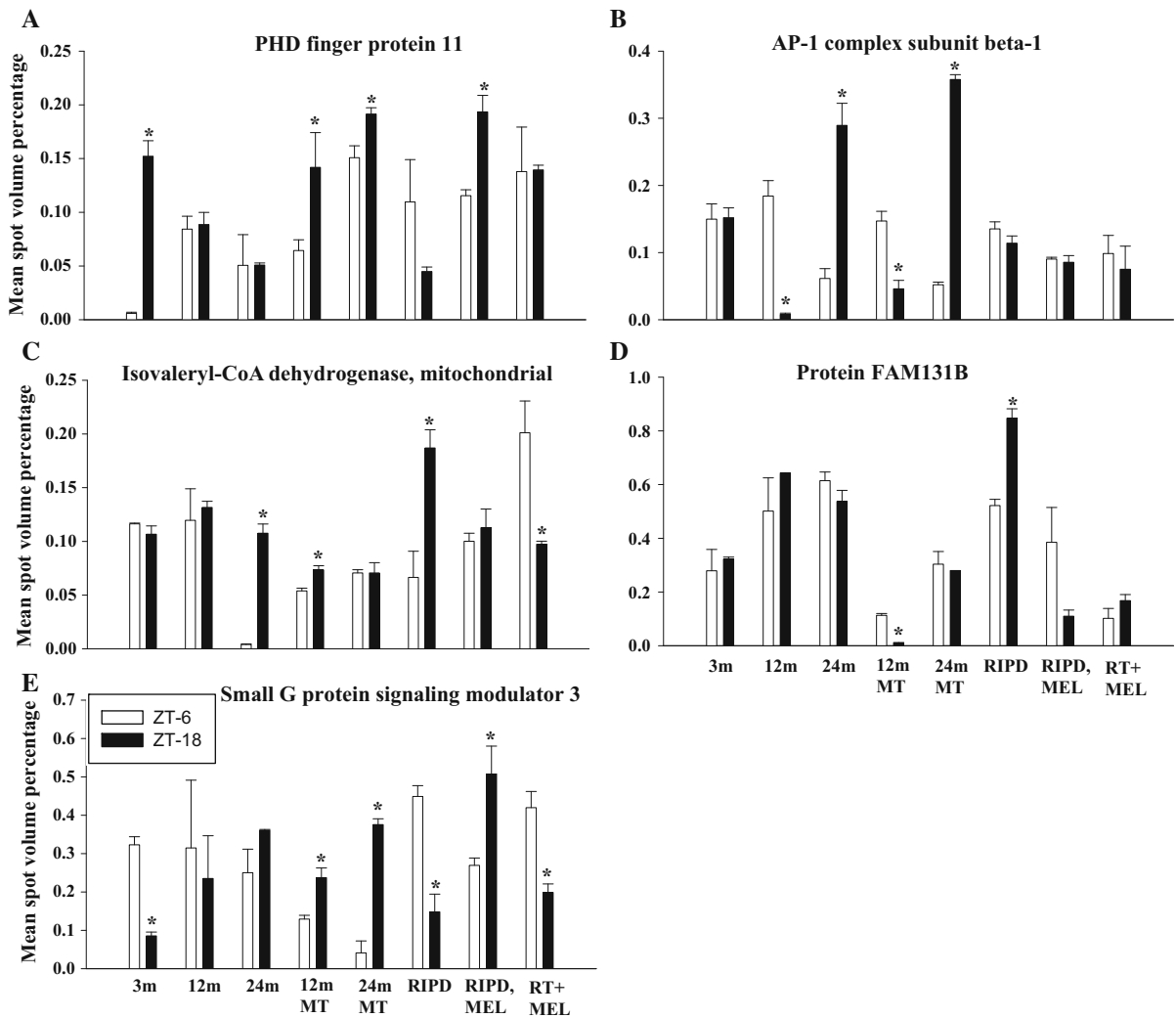
Fig. 4 The proteins identified in pineal showing significant daily pulse ($p \leq 0.05$), **a** PHD finger protein 11; **b** AP-1 complex subunit beta-1; **c** isovaleryl-CoA dehydrogenase, mitochondrial; **d** protein FAM131B; **e** small G protein signaling modulator 3. *Asterisk* represents comparison with ZT-6 within the group

properties of the SCN across a circadian day. Importantly the protein rhythms were abolished in the 24 m and RIPD group studied. The mechanisms involved in the rhythmic expression of ion channels and its reciprocal regulation by core clock oscillators with significance to physiological rhythms is less understood (Ko et al. 2009), however age related alteration in SCN membrane properties have been well studied (Banks et al. 2016). Recently, lack of NCBE was shown to affect visual acuity and temporal changes in light-evoked ganglion cell responses in retina (Hilgen et al. 2012). We observed an abolition of day–night variation of "Semaphorin-4F" in aged and RIPD rats.

Semaphorin-4F has been reported as a retinal axon guiding molecule that helps in guiding the axons from retina to the target region (Parrinello et al. 2008). The decrease in day night variation of "Semaphorin-4F" can be linked to the impairment of circadian rhythms because of diminished signaling from retina. This retinol binding protein 1a, has been reported as a cytoplasmic protein involved in intracellular transport of retinol (Ghyselinck et al. 1999). Interestingly we found "retinol binding protein 1", showing significant day–night variation in 12 and 24 m rats were lost in RIPD. This was in corroboration with the previous study (Van Dycke et al. 2015) showing "retinol
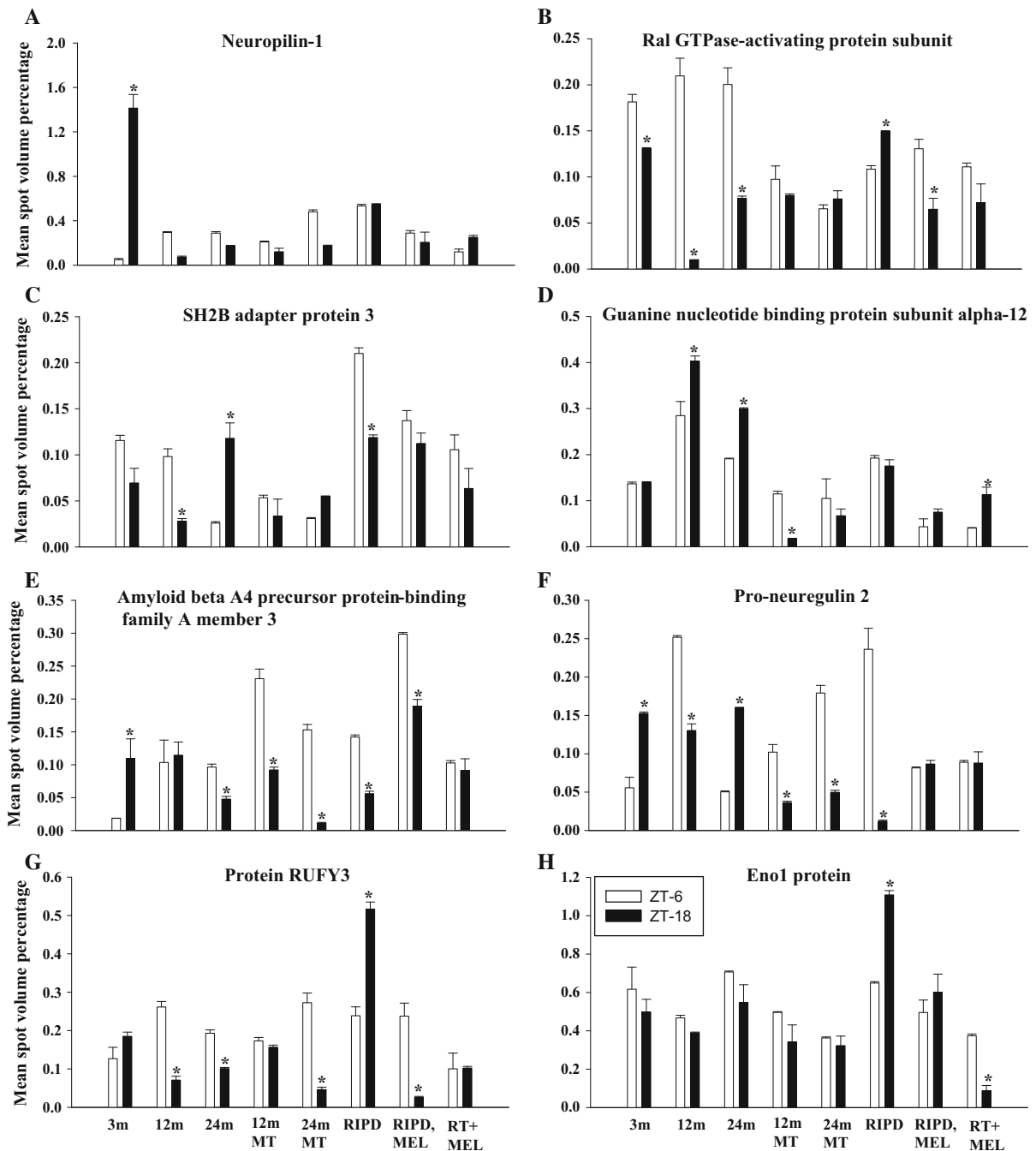
Fig. 5 The proteins identified in SN showing significant daily pulse ($p \leq 0.05$), **a** neuropilin-1; **b** Ral GTPase-activating protein subunit; **c** SH2B adapter protein 3; **d** guanine nucleotide binding protein subunit alpha-12; **e** Amyloid beta A4 precursor protein-binding family A member 3; **f** pro-neuregulin 2, membrane bound isoform; **g** protein RUFY3; **h** Eno1 protein. *Asterisk* represents comparison with ZT-6 within the group

binding protein 1"as marker for circadian disruption though it did not show day–night variation as was observed in 3 m rats. Syntaphilin was found to show

day night variation in 12, 24 m melatonin treated rats though not observed in 3, 12 and 24 m rats. This can be linked to capability of externally administered

melatonin in gene regulation (Koyama et al. 2014). Another protein "Solute carrier family 25 member 39" show a day–night variation in 3 m rats but not in 12 and 24 m rats could be a possible target for regulation by clock genes. Further, another protein "Merlin" showed the day–night variation in 12 m rats but not in 3 and 24 m rats.

In pineal we observed that PHD finger protein II, a nuclear protein involved in positive regulation of Th-1 type cytokine gene expression, showed significant day–night variation in 3 m but not in 12 and 24 m and RIPD rats. Till date there is no study showing "PHD finger protein II" with day–night oscillations in its protein expression. We for the first time showed the day–night expression of this protein in rats. Further, melatonin administration restored PHD finger protein II day–night variation not only in 12 and 24 m rats but also in RIPD rats. AP-1 complex subunit beta-1 has been reported to play an important role in protein sorting in the late-Golgi/trans-Golgi network (TGN) and/or endosomes (Gallusser and Kirchhausen 1993), and has been believed to be the target of miR-219 a target for clock and bmal1 (Cheng et al. 2007). Interestingly we found AP-1 complex subunit beta-1 showing day–night variation in 12 and 24 m rats.

In SN "Neuropilin-1", showed robust daily rhythms in 3 m, decreased in 12 m and abolished in 24 m and RIPD rats. "Neuropilin-1", a single pass type-1 membrane receptor has been reported crucial for the assembly of fore brain neuronal circuits (Chauvet et al. 2007). Interestingly, the neuroprotective effects of Vascular Endothelial growth factor (VEGF) elicited upon rotenone administration is mediated through dopaminergic (DA) neuron expressed Neuropilin receptor signalling (Cabezas et al. 2013). The altered Neuropilin profile with aging and RIPD can thus be linked to progression of DA neuron degeneration with aging or PD. We observed RalGTPase-activating protein subunit beta a showing robust day–night variation in 3, 12 and 24 m rats revealing its possible link with the circadian rhythms. The non-catalytic subunit of the heterodimer RagGap-1 and RalGap-2 complexes were reported by some researchers to act as GTPase activator for Ras like small GTPases (Shirakawa et al. 2009). We also found that SH2B adaptor protein 3 showed alteration in day–night variation with aging and RIPD. Amyloid beta A4 precursor protein-binding family A member 3, may modulate processing of the beta-amyloid precursor

protein (APP) and hence formation of beta-APP. This protein is extensively studied in Alzheimer's disease (Tanahashi and Tabira 1999). We found it showing robust day–night variations in 3 and 24 m rats and RIPD rats. Our results show that pro-neuregulin and RUFY3 also showed day–night oscillations. The current knowledge on these proteins is very limited.

We observed day night variation in the expression profile of some proteins in the SCN, pineal and SN only in the 24 m and in RIPD model. This finding can be linked to the fact that PD is an age related disorder with the possibility of common pathological changes. This may be associated with decline in ubiquitin/proteasome pathway (UPP) functions upon aging (Vernace et al. 2007) or disease condition. Due to the accumulation of 'uncleared' protein products the expression profile probably appears rhythmic. In the SCN, Malate dehydrogenase' (MDH) showed day–night variation in 24 m rats but not in 3 and 12 m rats. MDH is also reported to form aggregates in liver mitochondria disrupting 'mitochondrial homeostasis' upon senescence (Bezawork-Geleta et al. 2015). As compared to Alzheimer's disease (Qureshi and Parvez 2007) decreased expression of MDH in RIPD rats in the present study also demonstrates its differential effects in various neurological disorders. In the pineal 'isovaleryl CoA dehydrogenase" a mitochondrial protein involved in synthesis of (S)-3-hydroxy-3-methylglutaryl-CoA from 3-isovaleryl-CoA, a sub pathway of leucine degradation (Ikeda and Tanaka 1983), Protein FAM131B and Small G protein signaling modulator 3 exhibited robust day–night variation in RIPD rats. The information on these proteins in relation to the circadian rhythms and Parkinson's disease is obscure and the present data demonstrated the immediate necessity to study these proteins further to understand their role in disease progression. Similarly in the SN, Enolase1(eno1) exhibited day–night variation in RIPD but not in 3, 12 and 24 m rats. However, eno 1 is reported to be a circadian protein in peripheral clocks (Podobed et al. 2012).

Deery et al. (2009) reported that the master clock SCN involves 13% of its intracellular proteome that is under circadian regulation out of which 65% peaks during day and 35% peaks during night. The pineal and retina showed a lesser extent of circadian regulation with 3.4 and 2.7% respectively. The proteins that showed robust circadian pattern include heat

shock proteins which belong to ATPases, Immunoglobulins, RNA/protein/DNA metabolism and energy production and transduction. Our results are in agreement with findings from Deery et al. (2009) and Lee et al. (2013) as well as with a study on day–night proteome analysis in pineal by Moller et al. (2010) which revealed that the proteins involved in glucose metabolism, Krebs cycle, structural formations, energy transduction, signaling cascades, protein/DNA/RNA metabolism, calcium binding and intracellular transport showed day night variations.

The alterations in the male rat can be related to changes in middle age female rats such as serotonin uptake, serotonin transporter (SERT) binding sites, maintenance of normal cyclic release of luteinizing hormone (LH), alpha-1 adrenergic receptors, decreased sensitivity of Gonadotropin-releasing hormone (GnRH) neurons to vasoactive intestinal polypeptide (VIP) input (i.e. decreased *c-fos* expression) and changes in glucose utilization (Cohen and Wise 1988; Krajnak et al. 2003). These changes have been related to mood, memory and sleep (Krajnak et al. 2003). Onset of menopause has been related with the alterations in the biological rhythms and CNS function due to reduced exposure to estradiol (Smith et al. 2005). Epidemiologic studies by some workers suggested that females are relatively protected from PD compared with males and that exposure to endogenous and exogenous estrogen contributes to the sex differences. The estrogen could be protecting dopaminergic neuron depletion induced by neurotoxins in PD animal models and therefore is neuroprotective. The sexual dimorphisms at the molecular levels in dopaminergic neurons in the basal ganglia had been linked to such differences (Gillies et al. 2014). The studies for such sexual differences in PD may prove useful in understanding underlying mechanisms.

Proteomics methods based on mass spectrometry hold special promise for the discovery of novel biomarkers. Some of the proteins identified in present study may prove useful towards targeting novel treatments for age induced circadian dysfunction and neurological disorders such as PD.

References

Alam M, Schmidt WJ (2002) Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav Brain Res 136:317–324

Aujard F, Herzog ED, Block GD (2001) Circadian rhythms in firing rate of individual suprachiasmatic nucleus neurons from adult and middle aged mice. Neuroscience 106:255–261

Banks G, Nolan PM, Peirson SN (2016) Reciprocal interactions between circadian clocks and aging. Mamm Genome 27:332–340

Beal MF (2001) Experimental models of Parkinson's disease. Nat Rev Neurosci 2:325–334

Bezawork-Geleta A, Brodie EJ, Dougan DA, Truscott KN (2015) LON is the master protease that protects against protein aggregation in human mitochondria through direct degradation of misfolded proteins. Sci Rep. doi:10.1038/srep17397

Cabezas R, Avila MF, Torrente D, El-Bachá RS, Morales L, Gonzalez J, Barreto GE (2013) Astrocytes role in Parkinson: a double-edged sword. In: Kishore U (ed) Neurodegenerative disease. INTECH Open Access Publisher, Rijeka, pp 491–517

Cardinali DP, Srinivasan V, Brzezinski A, Brown GM (2012) Melatonin and its analogs in insomnia and depression. J Pineal Res 52:365–375

Chauvet S, Cohen S, Yoshida Y, Fekrane L, Livet J, Gayet O, Segu L, Buhot MC, Jessell TM, Henderson CE, Mann F (2007) Gating of Sema3E/PlexinD1 signaling by neuropilin-1 switches axonal repulsion to attraction during brain development. Neuron 56:807–822

Cheng HY, Papp JW, Varlamova O, Dziema H, Russell B, Curfman JP, Nakazawa T, Shimizu K, Okamura H, Impey S, Obrietan K (2007) microRNA modulation of circadian-clock period and entrainment. Neuron 54:813–829

Cohen IR, Wise PM (1988) Age-related changes in the diurnal rhythm of serotonin turnover in microdissected brain areas of estradiol-treated ovariectomized rats. Endocrinology 122:2626–2633

Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. Neuron 39:889–909

Deery MJ, Maywood ES, Chesham JE, Sládek M, Karp NA, Green EW, Charles PD, Reddy AB, Kyriacou CP, Lilley KS, Hastings MH (2009) Proteomic analysis reveals the role of synaptic vesicle cycling in sustaining the suprachiasmatic circadian clock. Curr Biol 19:2031–2036

Gallusser A, Kirchhausen T (1993) The beta 1 and beta 2 subunits of the AP complexes are the clathrin coat assembly components. EMBO J 12:5237–6244

Ghyselinck NB, Båvik C, Sapin V, Mark M, Bonnier D, Hindelang C, Dierich A, Nilsson CB, Håkansson H, Sauvant P, Azaïs-Braesco V, Frasson M, Picaud S, Chambon P (1999) Cellular retinol-binding protein I is essential for vitamin A homeostasis. EMBO J 18:4903–4914

Gillies GE, Pienaar IS, Vohra S, Qamhawi Z (2014) Sex differences in Parkinson's disease. Front Neuroendocrinol 35:370–384

Heffner TG, Hartman JA, Seiden LS (1980) A rapid method for the regional dissection of the rat brain. Pharmacol Biochem Behav 13:453-456

Hilgen G, Huebner AK, Tanimoto N, Sothilingam V, Seide C, Garrido MG, Schmidt KF, Seeliger MW, Löwel S, Weiler R, Hübner CA, Dedek K (2012) Lack of the sodium-driven chloride bicarbonate exchanger NCBE impairs visual function in the mouse retina. PLoS ONE 7(10):e46155

Ikeda Y, Tanaka K (1983) Purification and characterization of 2-methyl-branched chain acyl coenzyme A dehydrogenase, an enzyme involved in the isoleucine and valine metabolism, from rat liver mitochondria. J Biol Chem 258:9477–9487

Jagota A (2005) Aging and sleep disorders. Indian J Gerontol 19:415–424

Jagota A (2006) Suprachiasmatic nucleus: the center for circadian timing system in mammals. Proc Indian Natl Sci Acad 71:275–288

Jagota A (2012) Age induced alterations in biological clock: therapeutic effects of melatonin. In: Thakur MK, Rattan SI (eds) Brain aging and therapeutic interventions. Springer, Dordrecht, pp 111–129

Jagota A, Kalyani D (2008) Daily serotonin rhythms in rat brain during postnatal development and aging. Biogerontology 9:229–234

Jagota A, Kalyani D (2010) Effect of melatonin on age induced changes in daily serotonin rhythms in suprachiasmatic nucleus of male Wistar rat. Biogerontology 11:299–308

Jagota A, Reddy MY (2007) The effect of curcumin on ethanol induced changes in suprachiasmatic nucleus (SCN) and pineal. Cell Mol Neurobiol 27:997–1006

Jagota A, Olcese J, Rao H, Gupta PD (1999) Recognition of light in variable photoperiods by anophthalmic mutant rats. Brain Res 825:95–103

Jagota A, de la Iglesia HO, Schwartz WJ (2000) Morning and evening circadian oscillations in the suprachiasmatic nucleus in vitro. Nat Neurosci 3:372–376

Karasek M (2004) Melatonin, human aging, and age-related diseases. Exp Gerontol 39:1723–1729

Khaldy H, León J, Escames G, Bikjdaouene L, García JJ, Acuña-Castroviejo D (2002) Circadian rhythms of dopamine and dihydroxyphenyl acetic acid in the mouse striatum: effects of pinealectomy and of melatonin treatment. Neuroendocrinology 75:201–208

Ko GYP, Shi L, Ko ML (2009) Circadian regulation of ion channels and their functions. J Neurochem 110:1150–1169

Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadianclock. Genes Dev 20:1868–1873

Kondratova AA, Kondratov RV (2012) The circadian clock and pathology of the ageing brain. Nat Rev Neurosci 13:325–335

Koyama FC, Azevedo MF, Budu A, Cha abarti D, Garcia CR (2014) Melatonin-induced temporal up-regulation of gene expression related to ubiquitin/proteasome system (UPS) in the human malaria parasite Plasmodium falciparum. Int J Mol Sci 15:22320–22330

Krajnak K, Rosewell KL, Duncan MJ, Wise PM (2003) Aging, estradiol and time of day differentially affect serotonin transporter binding in the central nervous system of female rats. Brain Res 990:87–94

Lee JE, Zamdborg L, Bruce R, Southey BR, Atkins N Jr, Mitchell JW, Li M, Gillette MU, Kelleher NL, Sweedler JV (2013) Quantitative peptidomics for discovery of circadian-related peptides from the rat suprachiasmatic nucleus. J Proteom Res 12:585–593

Manikonda PK, Jagota A (2012) Melatonin administration differentially affects age-induced alterations in daily rhythms of lipid peroxidation and antioxidant enzymes in male rat liver. Biogerontology 13:511–524

Mattam U, Jagota A (2014) Differential role of melatonin in restoration of age-induced alterations in daily rhythms of expression of various clock genes in suprachiasmatic nucleus of male Wistar rats. Biogerontology 3:257–268

Mattam U, Jagota A (2015) Daily rhythms of serotonin metabolism and the expression of clock genes in suprachiasmatic nucleus of rotenone-induced Parkinson's disease male Wistar rat model and effect of melatonin administration. Biogerontology 16:109–123

Moller M, Lund-Andersen C, Rovsing L, Sparre T, Bache N, Roepstorff P, Vorum H (2010) Proteomics of the photoneuroendocrine circadian system of the brain. Mass Spectrom Rev 29:313–325

Mortz E, Krogh TN, Vorum H, Görg A (2001) Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. Proteomics 1:1359–1363

Nakamura JT, Nakamura W, Yamazaki S, Kudo T, Cutler T, Colwell CS, Block GD (2011) Age-related decline in circadian output. J Neurosci 31:10201–10205

Palomba M, Nygard M, Florenzano F, Bertini G, Kristensssion K, Bentivoglio M (2008) Decline of the presynaptic network, including GABAergic terminals, in the aging suprachiasmatic nucleus of the mouse. J Biol Rhythms 23:220–231

Pandi-Perumal SR, BaHammam AS, Brown GM, Spence DW, Bharti VK, Kaur C, Hardeland R, Cardinali DP (2013) Melatonin antioxidative defense: therapeutical implications for aging and neurodegenerative processes. Neurotox Res 23:267–300

Parrinello S, Noon LA, Harrisingh MC, Digby PW, Rosenberg LH, Cremona CA, Echave P, Flanagan AM, Parada LF, Lloyd AC (2008) NF1 loss disrupts Schwann cell-axonal interactions: a novel role for semaphorin 4F. Genes Dev 22:3335–3348

Pazo D, Cardinali DP, Cano P, Reyes Toso CA, Esquifino AI (2002) Age-related changes in 24-h rhythms of norepinephrine content and serotonin turnover in rat pineal gland: effect of melatonin treatment. Neurosignals 11:81–87

Podobed PS, Kirby GM, Martino TA (2012) Circadian proteomics and its unique advantage for discovery of biomarkers of heart disease. In: Man TK (ed) Proteomics—human diseases and protein functions. INTECH Open Access Publisher, Rijeka, pp 65–88

Poeggeler B (2005) Melatonin, aging, and age-related diseases: perspectives for prevention, intervention, and therapy. Endocrine 27:201–212

Qureshi GA, Parvez SH (2007) Oxidative stress and neurodegenerative disorders. Elsevier, Oxford

Rattan SI (2012) Rationale and methods of discovering hormetins as drugs for healthy ageing. Expert Opin Drug Discov 7:439–448

Reddy VD, Jagota A (2014) Effect of restricted feeding on nocturnality and daily leptin rhythms in OVLT in aged male Wistar rats. Biogerontology 3:245–256

Reddy MY, Jagota A (2015) Melatonin has differential effects on age-induced stoichiometric changes in daily chronomics of serotonin metabolism in SCN of male Wistar rats. Biogerontology 16:285–302

Reiter RJ, Tan DX, Galano A (2014) Melatonin: exceeding expectations. Physiology 29:325–333

Schaffner W, Weissmann C (1973) A rapid, sensitive, and specific method for the determination of protein in dilute solution. Anal Biochem 56:502–514

Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Shevchenko A, Boucherie H, Mann M (1996) Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad Sci USA 93(25):14440–14445

Shirakawa R, Fukai S, Kawato M, Higashi T, Kondo H, Ikeda T, Nakayama E, Okawa K, Nureki O, Kimura T, Kita T, Horiuchi H (2009) Tuberous sclerosis tumor suppressor complex-like complexes act as GTPase-activating proteins for Ral GTPases. J Biol Chem 284:21580–21588

Smith RG, Betancourt L, Sun Y (2005) Molecular endocrinology and physiology of the aging central nervous system. Endoc Rev 26:203–250

Tanahashi H, Tabira T (1999) X11L2, a new member of the X11 Protein family, interacts with Alzheimer's β-amyloid precursor protein. Biochem Biophys Res Commun 255:663–667

Uversky NV (2004) Neurotoxicant –induced animal modes of Parkinson's disease: understanding the role of rotenone, maneb and paraquat in neurodegeneration. Cell Tissue Res 318:225–241

Van Dycke KC, Pennings JL, van Oostrom CT, van Kerkhof LW, van Steeg H, van der Horst GT, Rodenburg W (2015) Biomarkers for circadian rhythm disruption independent of time of day. PLoS ONE 10:e0127075. doi:10.1371/journal.pone.0127075

Vernace VA, Schmidt-Glenewinkel T, Figueiredo-Pereira ME (2007) Aging and regulated protein degradation: who has the UPPer hand? Aging Cell 6:599–606

Vinod C, Jagota A (2016) Daily NO rhythms in peripheral clocks in aging male Wistar rats: protective effects of exogenous melatonin. Biogerontology 17:859–871

Vinod C, Jagota A (2017) Daily Socs1 rhythms alter with aging differentially in peripheral clocks in male Wistar rats: therapeutic effects of melatonin. Biogerontology. doi:10.1007/s10522-017-9687-7

Wagner S, Sagiv N, Yarom Y (2001) GABA-induced current and circadian regulation of chloride in neurones of the rat suprachiasmatic nucleus. J Physiol 537:853–869

Wang CZ, Yano H, Nagashima K, Seino S (2000) The Na+-driven Cl-/HCO3- exchanger. Cloning, tissue distribution, and functional characterization. J Biol Chem 275:35486–35490