Objective methods for estimating chronotype in the field

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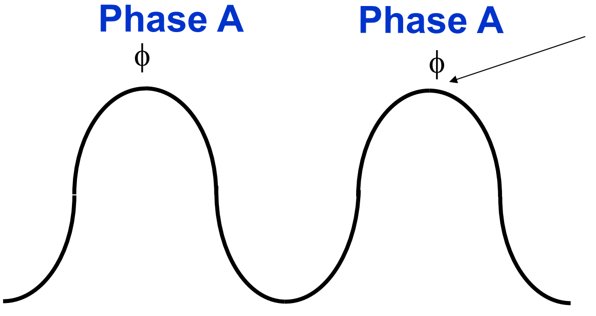
Chronotype

Circadian phase (ϕ) (phi)

Phase of entrainment (Ψ) (psi)

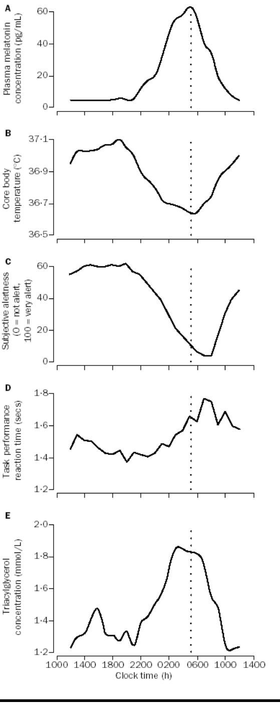
Diurnal preference
(morningness/eveningness)

Biological rhythms: circadian phase



Phase: a defined point in the cycle

Circadian rhythms



melatonin

core body temp

subjective alertness

task performance

triacylglycerol

Rajaratnam &Arendt 2001

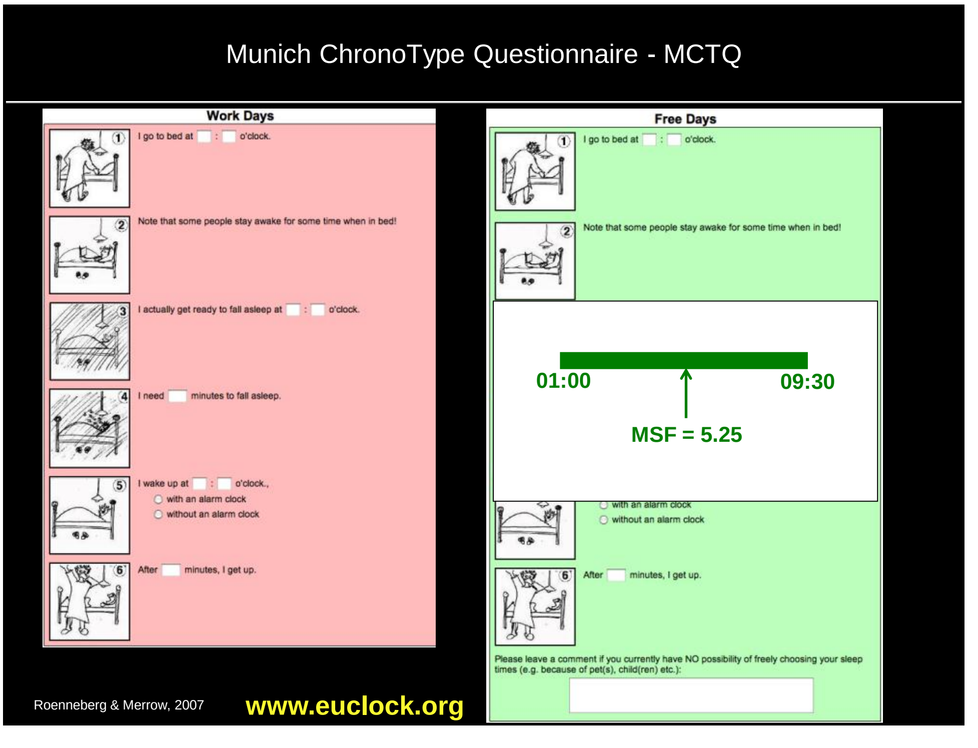
Phase of entrainment (Ψ)

Difference between
a given phase of circadian rhythm

e.g., trough of core body temperature, melatonin onset or midpoint of sleep)

and that of the zeitgeber (time cue)

e.g., dawn or mid-dark.



Diurnal preference

Chronotype as a personality trait

Separate morningness from eveningness

Horne and Östberg, 1976

HORNE-OSTBERG QUESTIONNAIRE

Identification Code: ……………………….. DATE:………..……..

This questionnaire will used to assess whether you are a "morning" type (i.e. a lark) or an "evening" type (i.e. an owl) person.

QUESTION 1

Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?

AM 5 6 7 8 9 10 11 12

QUESTION 2

Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?

PM 8 9 10 11 12 AM 1 2 3

Non-invasive measurement tools

- Diurnal preference

Horne-Östberg questionnaire (MEQ)

- Phase of entrainment

Munich Chronotype questionnaire (MCTQ; MCTQshift)

Challenges - Objective measure

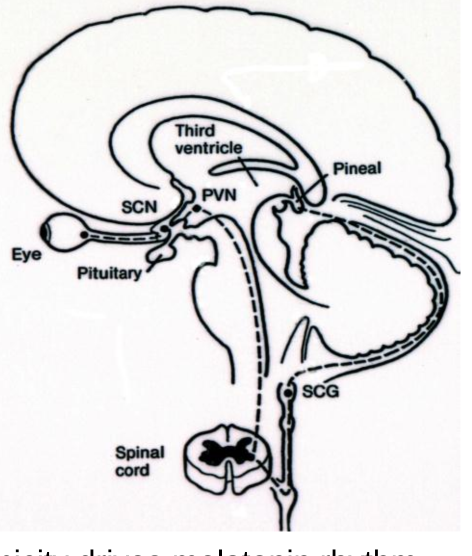
Need a reliable marker of circadian phase of worker

- assess circadian clock timing
- optimise timing of chronotherapy

Melatonin as a reliable marker of circadian phase

Pineal gland - primary site of melatonin synthesis

Retina-SCN-PVN-SCG-pineal pathway



SCN rhythmicity drives melatonin rhythm
Entrained to 24 h by light/dark via the retina-RHT pathway

Melatonin as a reliable marker of circadian phase

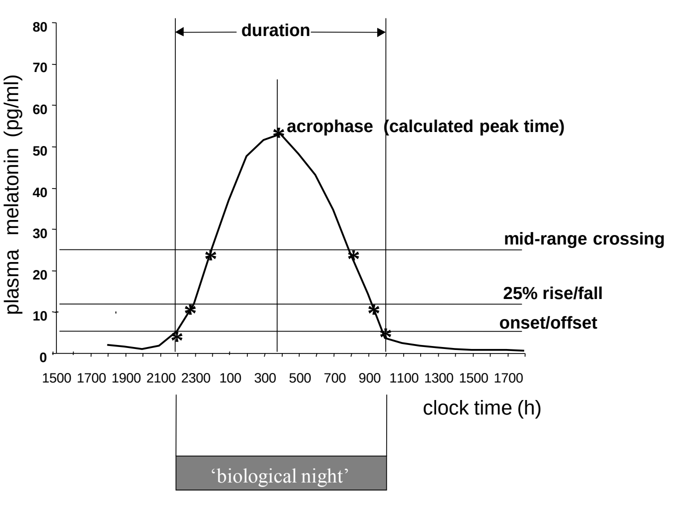
- unaffected by:

  meals, stress, bathing, sleep

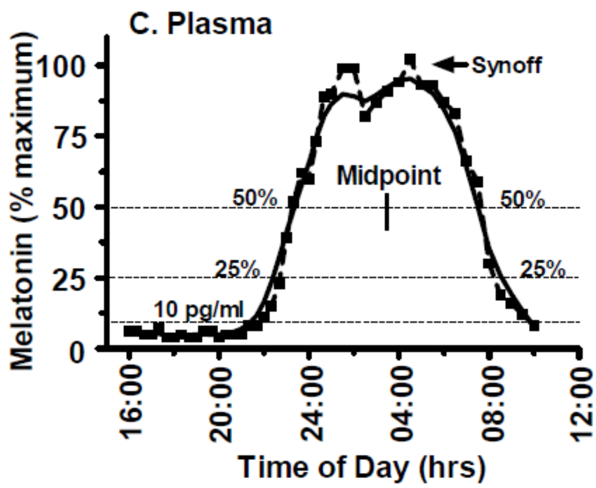
- dim light conditions (< 8 lux)
- exclude drugs
- control posture, exercise

Markers of the melatonin rhythm

used to characterise the timing of the circadian clock

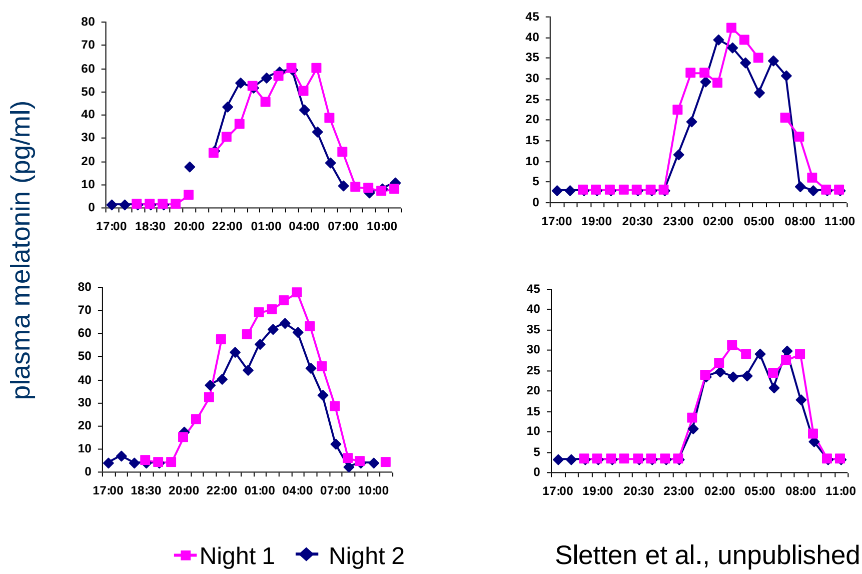


Arendt & Skene, Sleep Medicine Reviews (2005) 9:25-39

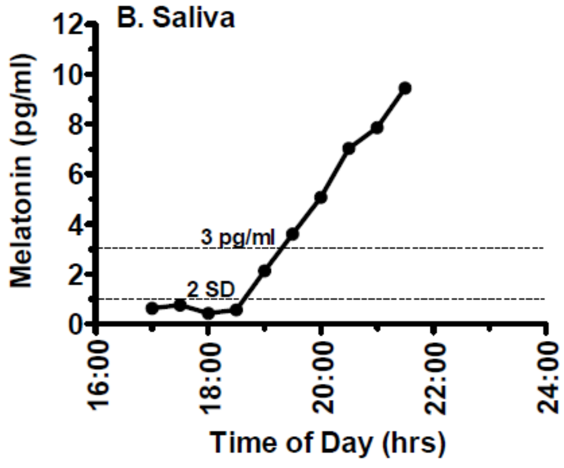


Benloucif et al., 2007

Melatonin rhythms - Inter-individual variation
- Within individual consistency



Sletten et al., unpublished



Benloucif et al., 2007

RIA and ELISA technology

- Easy to measure plasma, saliva
- Sensitive, specific
- High throughput
- Relatively inexpensive

BÜHLMANN

STOCKGRAND LTD

DRG International, Inc.

Melatonin as a reliable marker of circadian phase

aMT6s as a reliable marker of melatonin rhythms

- non invasive
- convenient for field studies

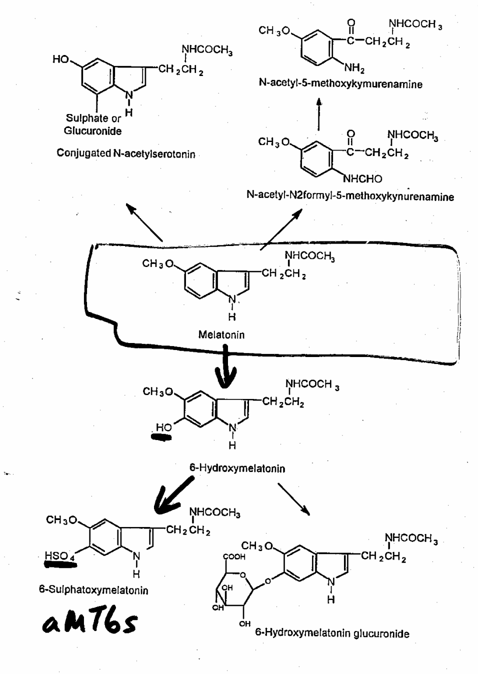
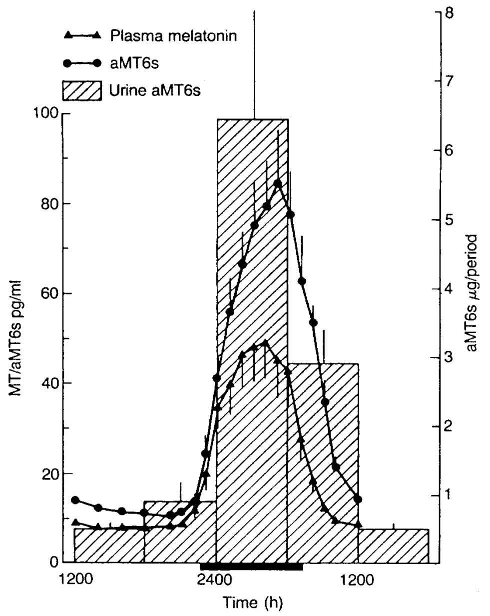


Fig 3. Diagram showing the major routes involved in the metabolism of melatonin

Melatonin and aMT6s rhythms

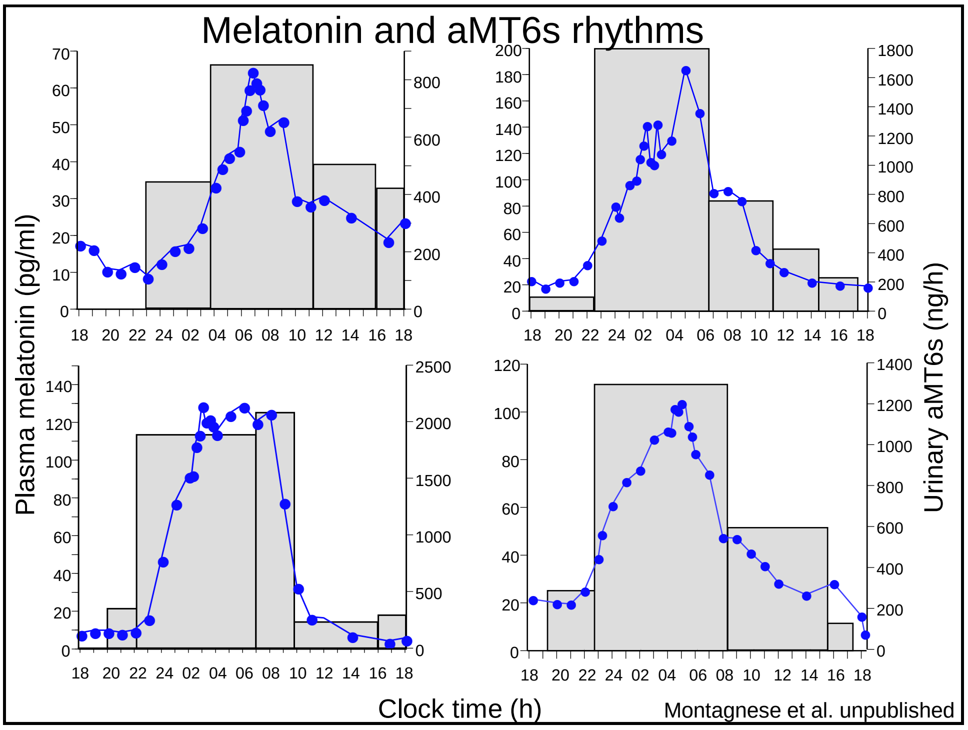


Timed urine sampling

4 h (+ overnight) for 48 h

Calculate aMT6s peak (6-sulphatoxymelatonin)

Arendt et al., 1985;
Bojkowski et al., 1987



Sampling details for measurement of melatonin and aMT6s

Biological fluid	Analyte	Sampling procedure	Precautions
Blood	Melatonin	Collect blood into heparinised tubes. Centrifuge within 15 mins. Store plasma at -20°C.	Haemolysed plasma and plasma left in plastic pipettes for more than 2-3 minutes may give falsely elevated melatonin levels.
Saliva	Melatonin	The best method is to ask subjects to spit into polypropylene tubes. Do not stimulate saliva production. Store at -20°C. Salivettes with an untreated cotton plug can be used. Centrifuge for 15 mins at 3000 rpm. Store at -20°C.	Do not eat within 30 mins of sampling. Rinse mouth with tap water before spitting. Saliva left in plastic pipettes for more than 2-3 minutes may give falsely elevated melatonin levels. Use of salivettes may give falsely elevated melatonin levels, standards should be run through salivettes to correct for this.
Urine	aMT6s	Ask subjects to collect all urine passed over a preset period into a standard urine bottle. Measure and record the volume, store circa 5 ml at -20°C. Urine should be collected at least every 3-4 h (longer during sleep period) for at least 24 h, preferably for 48 h or longer.	Do not wash urine bottles with bleach or another oxidant. No preservative is required aMT6s is stable in urine for 1 day at room temperature, 2 days at 4°C and for at least 2 years at -20°C.

For further details see www.stockgrand.co.uk

Melatonin and aMT6s measurement in shift work

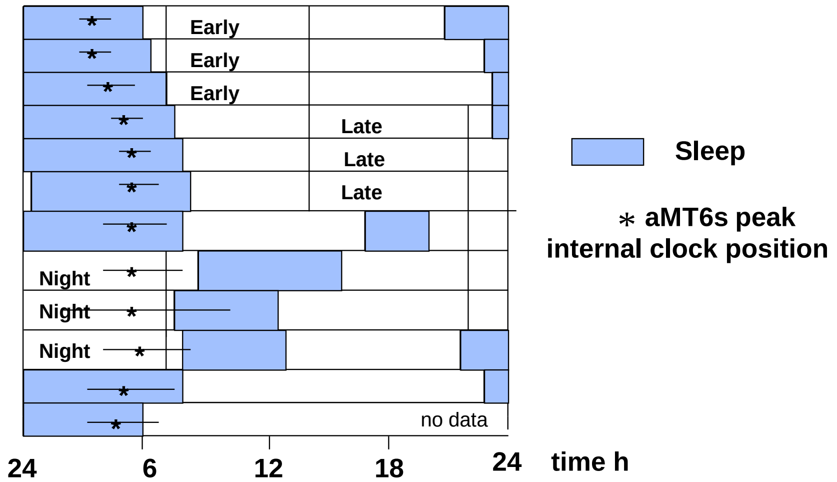
Field studies

Different shift schedules

Professor Josephine Arendt

Rotating shifts
No or little circadian adaptation (aMT6s)

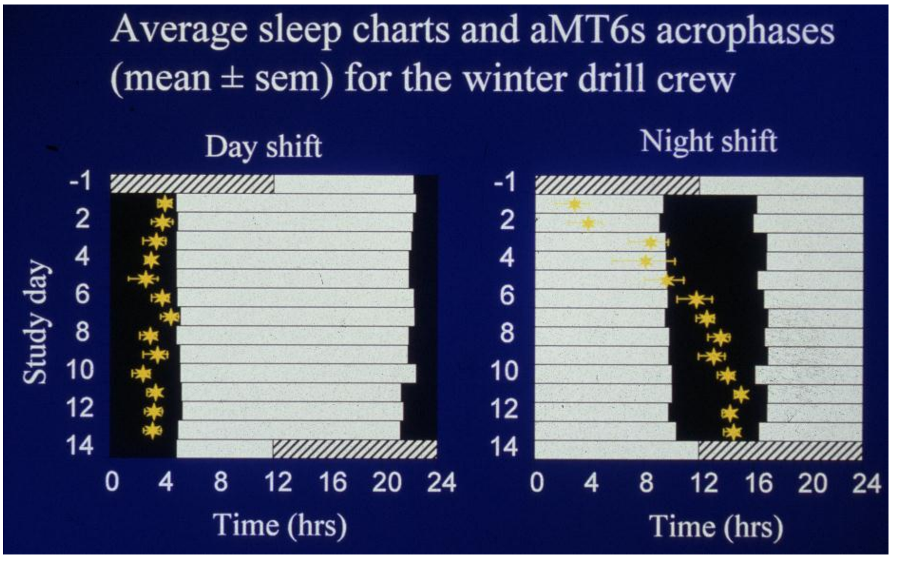
Three day fast rotation shift



Hall, English, Arendt., unpublished

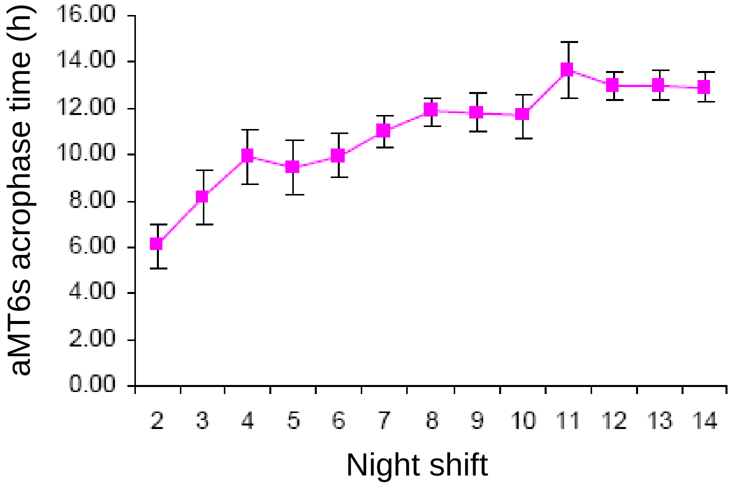
People who do adapt to night shift

Stable shift – adapt to night shift



Barnes et al., 2000

aMT6s acrophase during a night shift



14 day night shift: 18.00-06.00 h offshore

Gibbs et al., 2005

Adaptation depends on:

- Shift schedule (type and timing)
- Light/dark environment
- Season

Barnes et al, 1998;2000; Gibbs et al, 2002; 2005

Adaptation depends on:

- Shift schedule (type and timing)
- Light/dark environment
- Season

Problem if adapt to shift, then have to re-adapt to new shift/rest day

Permanent night and day workers

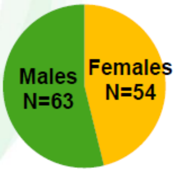
Methods

Design
Cross-sectional

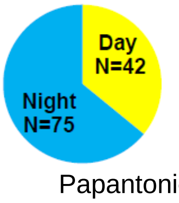
Recruitment
March 2011-June 2011

Subjects
117 volunteers, 22-64 yrs, working in 4 companies in BCN

Sex (N)



Shift schedule (N)



Papantoniou et al., 2014

Urinary 6-sulphatoxymelatonin rhythms in permanent night workers

Urine samples

Repeated urine samples from **ALL** voids during 24h on a working day (48h for 13 subjects +day off)

Providing information on time and date of collection of each sample

Papantoniou et al., 2014

Urinary 6-sulphatoxymelatonin rhythms in permanent night workers

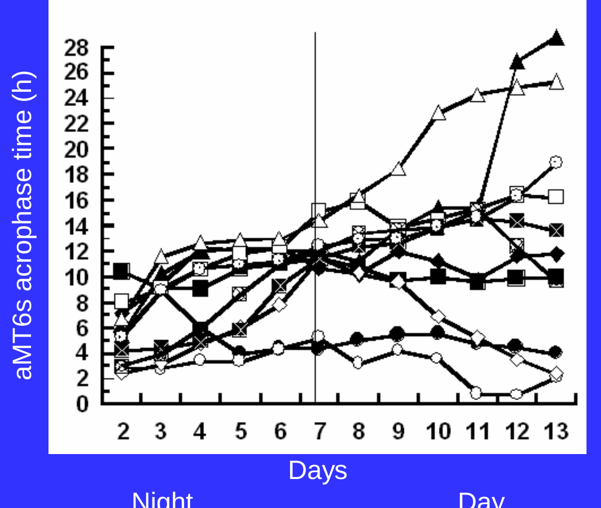
aMT6s	Day workers (n = 41)	Night workers (n = 72)
aMT6s levels (ng/mg creatinine/h)	15.4 (95% CI 12.3-19.3)	10.9* (95% CI 9.5-12.6)
aMT6s peak time (h:min)	05:36 h 05:06-06:12)	08:42 h* (95% CI 07:48-09:42)

Lower aMT6s levels and later peak times in permanent night workers

Papantoniou et al., 2014

Individual differences in adaptation to shift work

Individual differences in adaptation to shift work



16 h to adapt by delay

8 h to adapt by advance

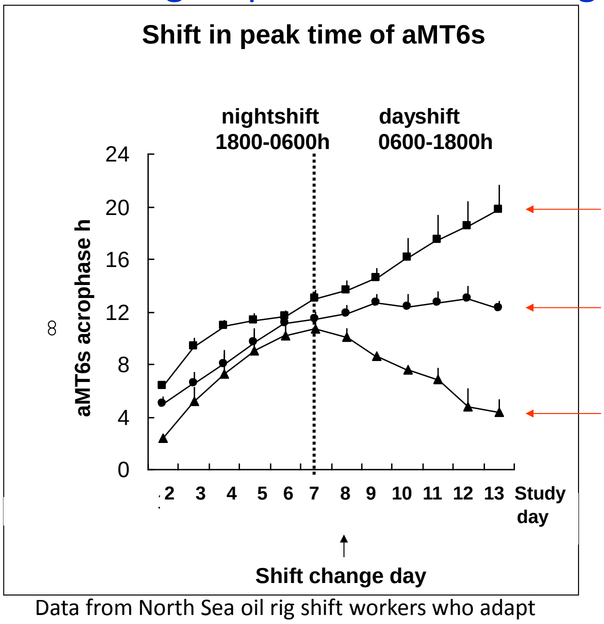
Night

Day

7 Nights (18.00-06.00 h) **7 Days (06.00-18.00 h)**

Gibbs et al., 2002

Predicting response to shift change: aMT6s phase



Data from North Sea oil rig shift workers who adapt rapidly to night shift work from 1800-0600h

Barnes et al, 1998a & b; Gibbs et al, 2001; 2007

Individual differences predicting adaptation to shift work

- Genotype eg clock gene polymorphisms?
- Circadian period (τ)
- Circadian phase, chronotype

- Light sensitivity - retinal processing
- Age
- Sleep/wake patterns
- Light/dark exposure

Challenges

Need an **immediate** marker of circadian phase

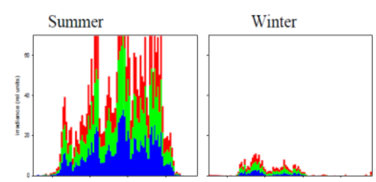
- online
- ambulatory conditions

- melatonin biosensor

- "ClockWatcher"
- "LightWatcher"

EUCLOCK

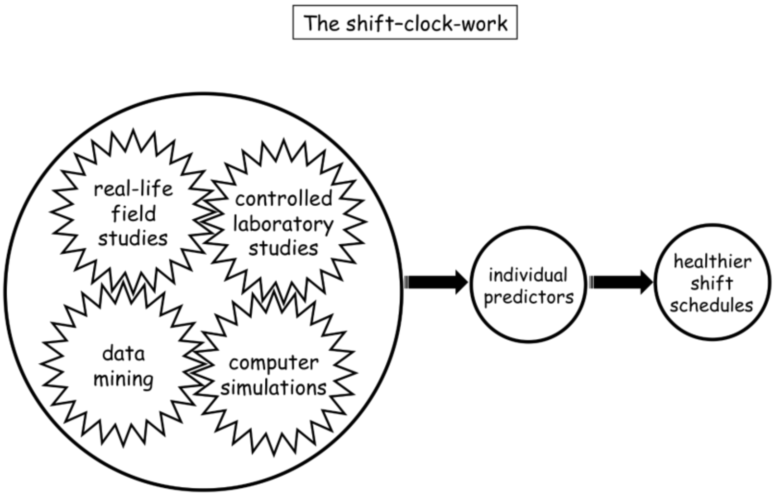
red, green, blue, uv, infrared



Ambulatory Circadian Monitoring

Correlation with melatonin onset (DLMO)

Bonmati-Carrion et al., 2014



mechanisms underpin inter-individual differences
in response to shift work?
→ design better and healthier shift-work schedules

Kantermann, T…..Skene, D.J., Prog. Brain Res., 2012

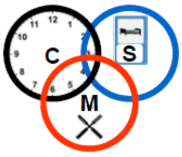
Biomarker challenge – shift work

- Global systems "-omics" approach

  Transcriptomics

  Proteomics

  Metabolomics



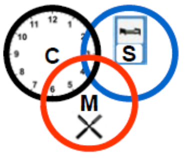
Biomarker challenge – shift work

- Shift work

  Sleep restriction/sleep deprivation

  Circadian misalignment

  Metabolic disturbance



- Elucidate underlying mechanisms

Targeted metabolomics

- **MetaDis*IDQ*** kit (Biocrates)

LC/MS: Waters Xevo TQ-S mass spectrometer coupled to an Acquity H LC system

- Absolute concentrations for 183 metabolites

Acylcarnitines (40)
Amino Acids and Biogenic Amines (40)
Hexoses (1)
Sphingolipids (14)
Glycerophospholipids (88)

- **10 µl plasma**

Davies, S.K. et al., PNAS, 2014

Sleep vs sleep deprivation metabolomics

- Effect of total sleep deprivation on metabolite rhythms

PNAS

Effect of sleep deprivation on the human metabolome

Sarah K. Davies[a], Joo Ern Ang[b], Victoria L. Revell[a], Ben Holmes[a], Anuska Mann[a], Francesca P. Robertson[a], Nanyi Cui[a], Benita Middleton[a], Katrin Ackermann[c,1], Manfred Kayser[c], Alfred E. Thumser[a], Florence I. Raynaud[b,2], and Debra J. Skene[a,2,3]

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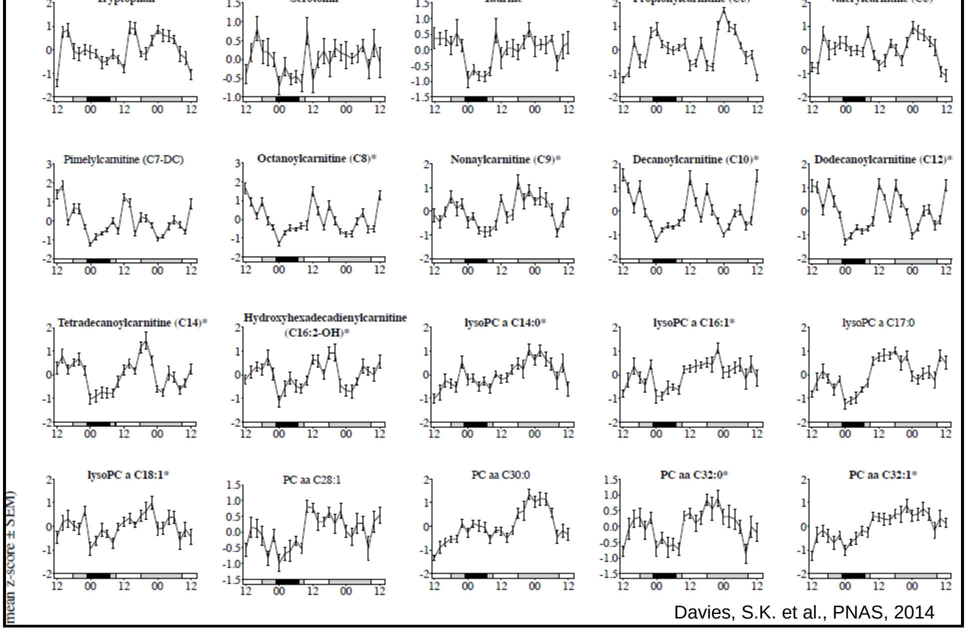
PNAS | July 22, 2014 | vol. 111 | no. 29 | 10761–10766

Davies et al., PNAS, 2014

Metabolite rhythms



Davies, S.K. et al., PNAS, 2014

Thank you

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