Relationship between Circadian Clock Gene Expression and Atrial Fibrillation

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COI Disclosure

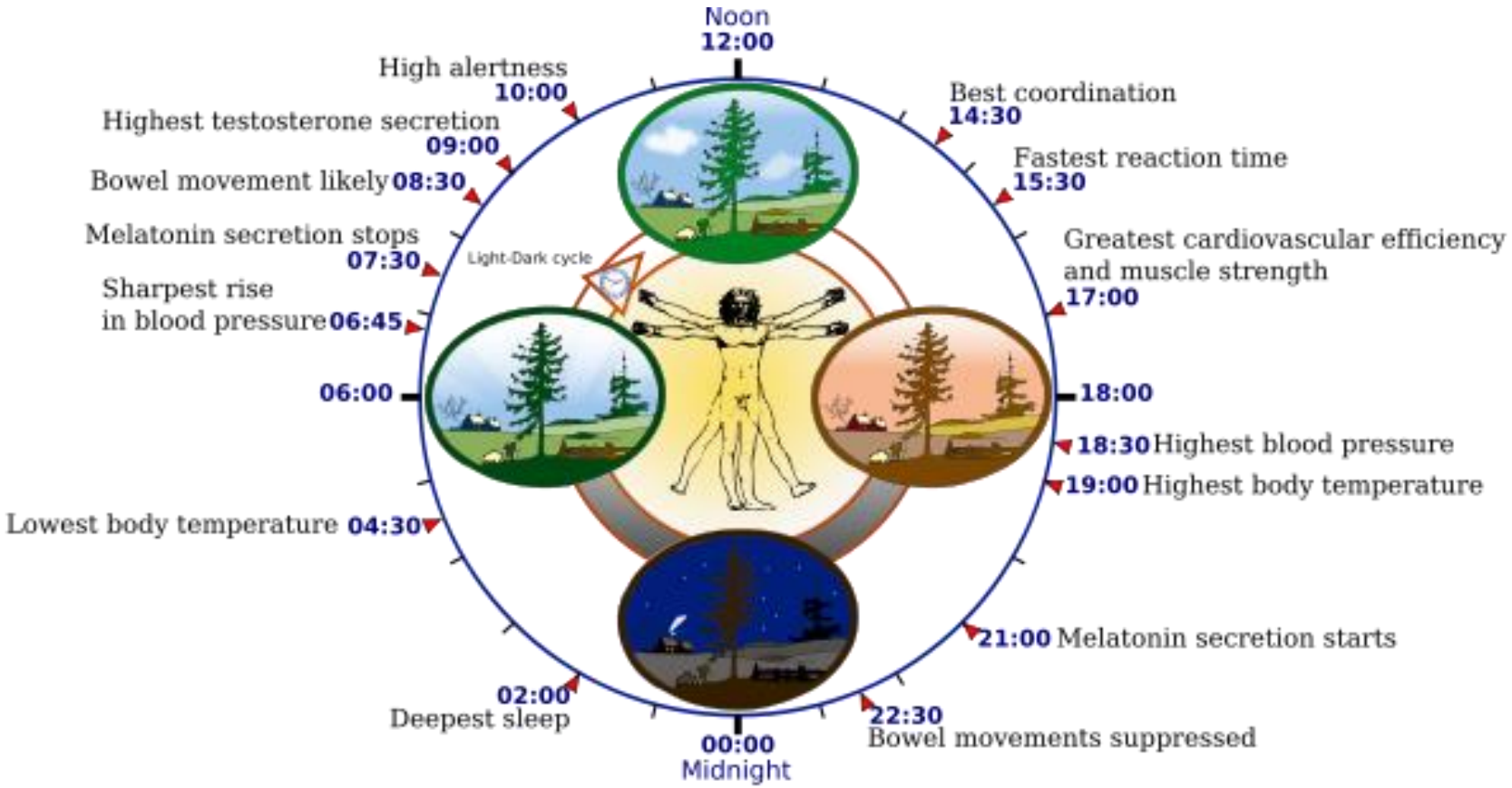
Name of First Author: Yung-Lung Chen

The authors have no financial conflicts of interest
to disclose concerning the presentation

Outline

- Background
- Methods
- Results
- Conclusion

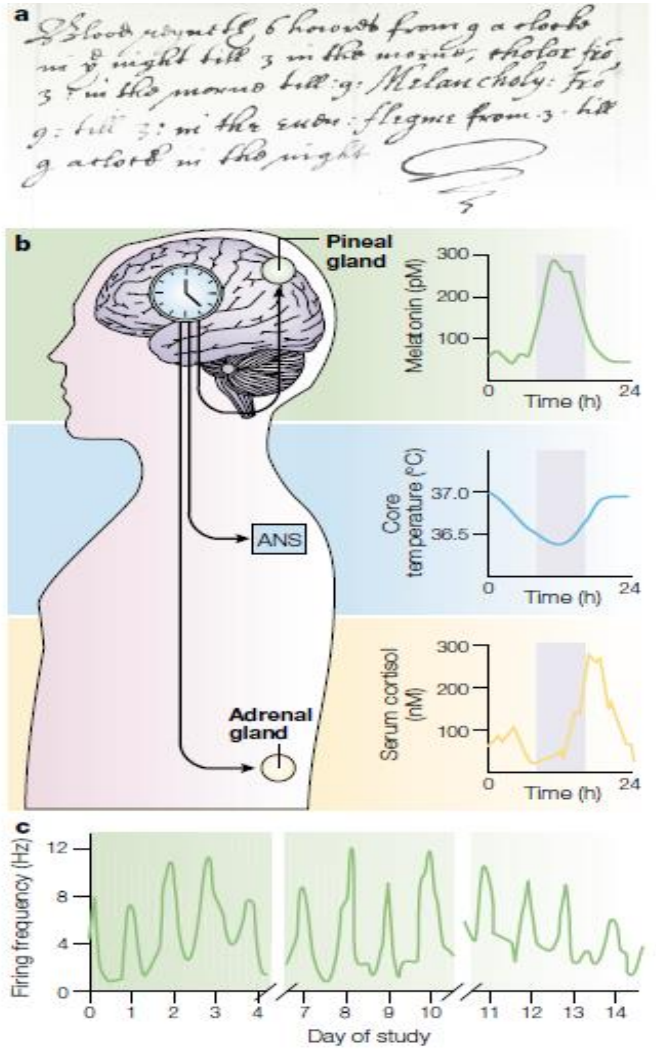
Human circadian biologic clock



Circadian timing in our brain and periphery

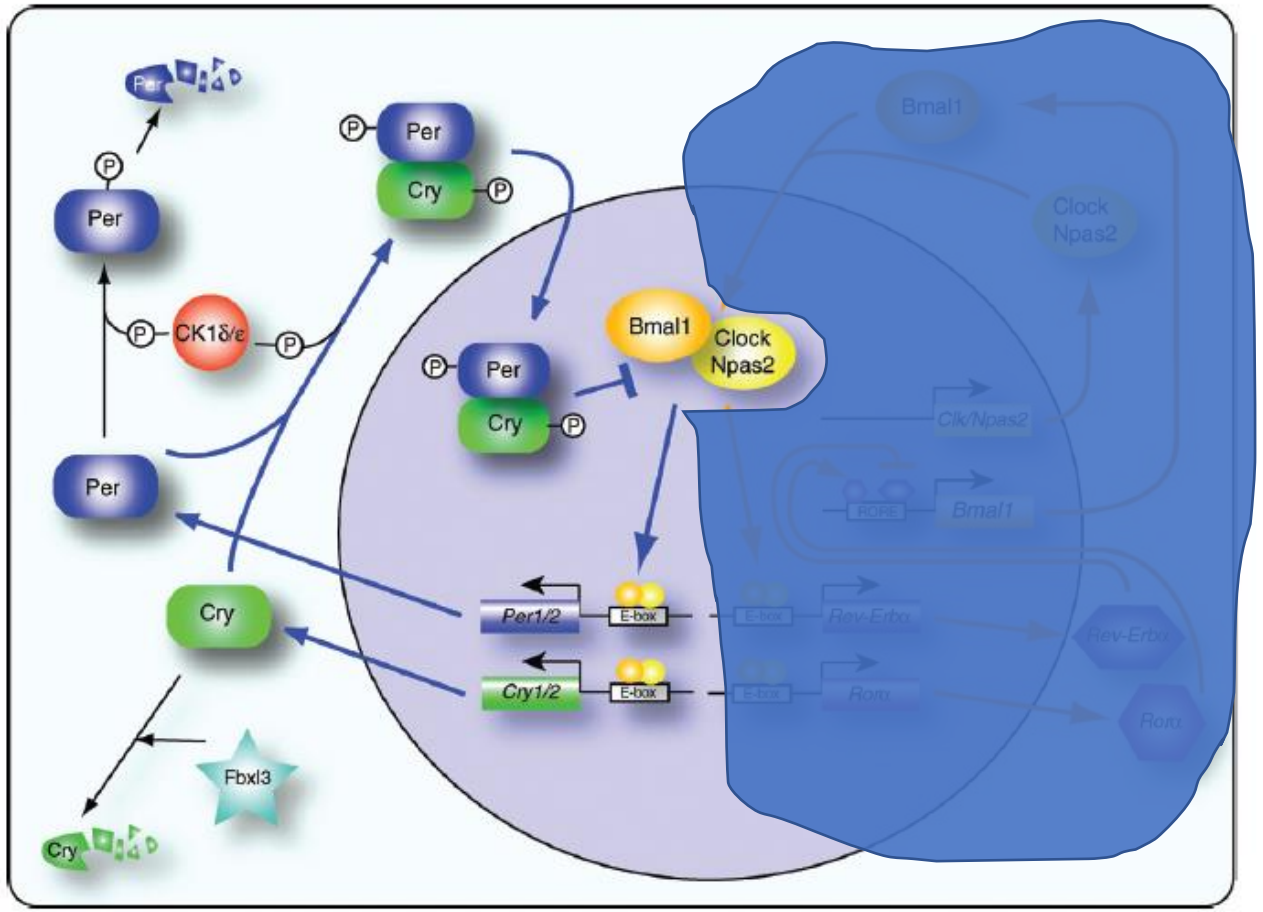
Suprachiasmatic nuclei (SCN)
: principal circadian oscillator

Local versions of the SCN clockwork are also active **in peripheral**, non-neural tissues, driving the tissue-specific cycles of gene expression that underpin circadian organization.



NATURE REVIEWS 2003(4) 649-661

Hypothetical clock mechanism in mammals



Per: Period (1-3)
Clock
Cry: Cryptochrome(1-2)
BMAL1: Brain and muscle ARNT-like protein 1
CK1E: Casein kinase 1E
TIM: Timless
Rev-Erb- α (or NR1D1)
ROR- α: retinoic acid–related orphan receptor

Eur Heart J 2010; 31, 896.

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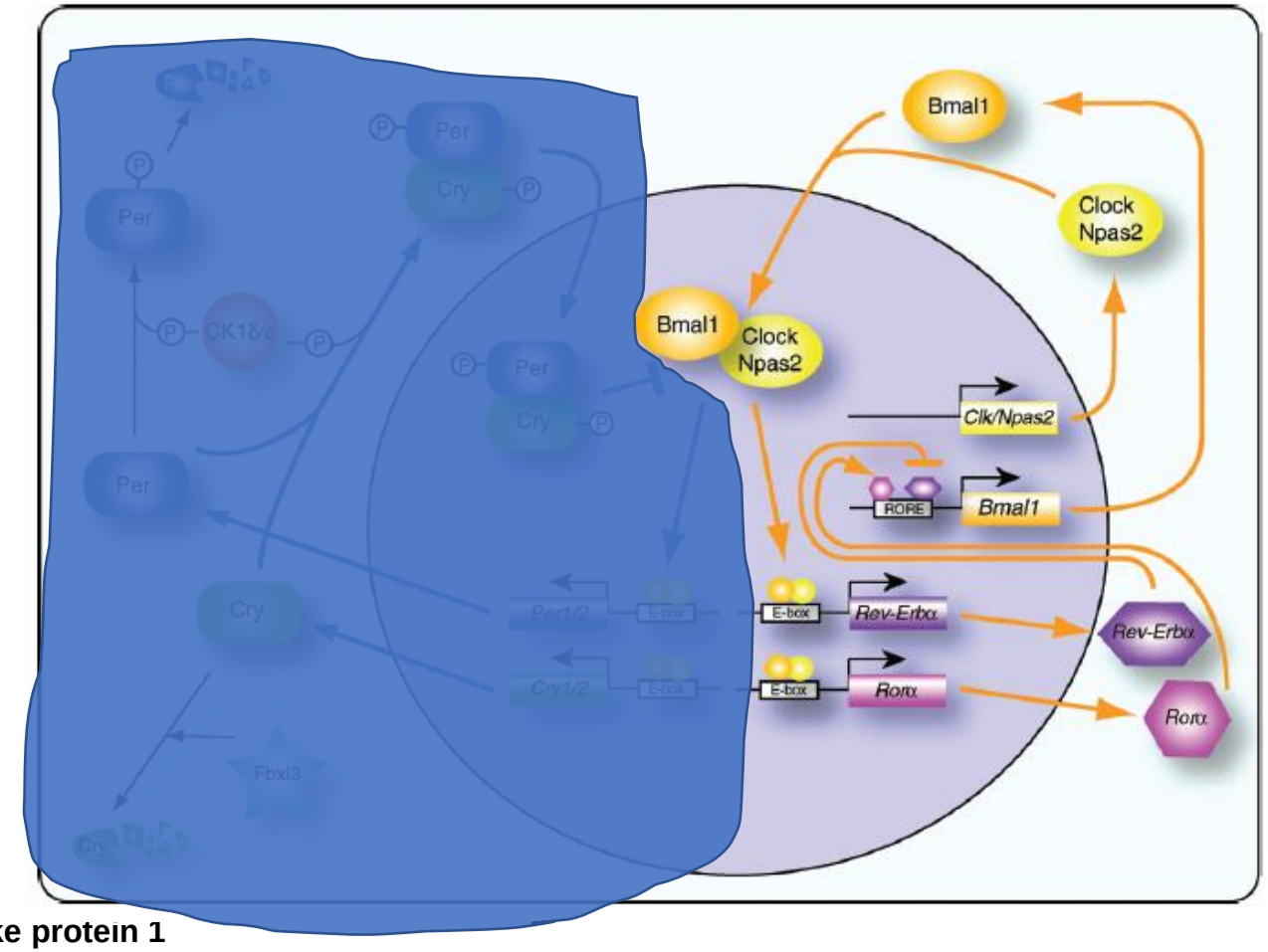

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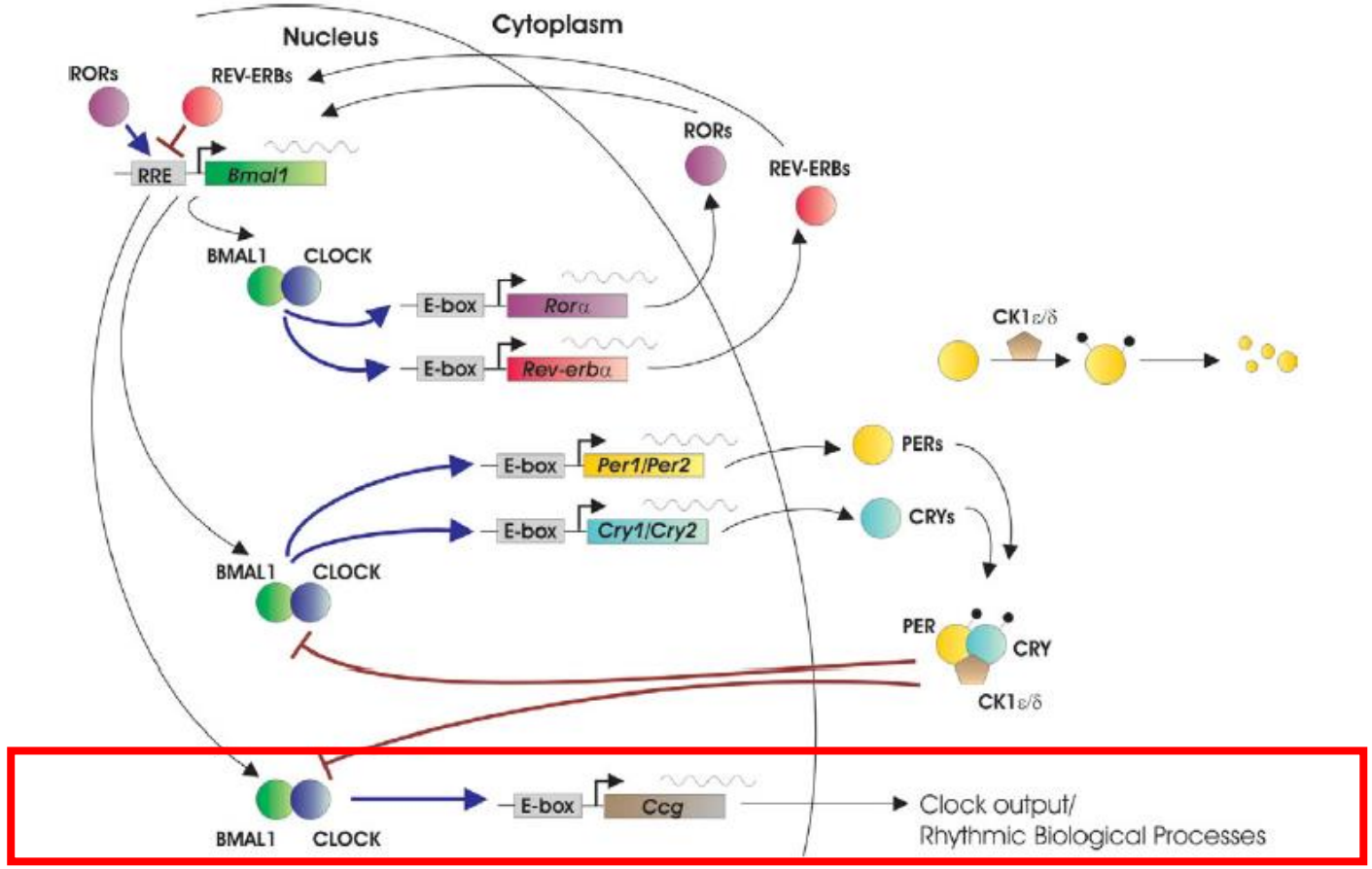
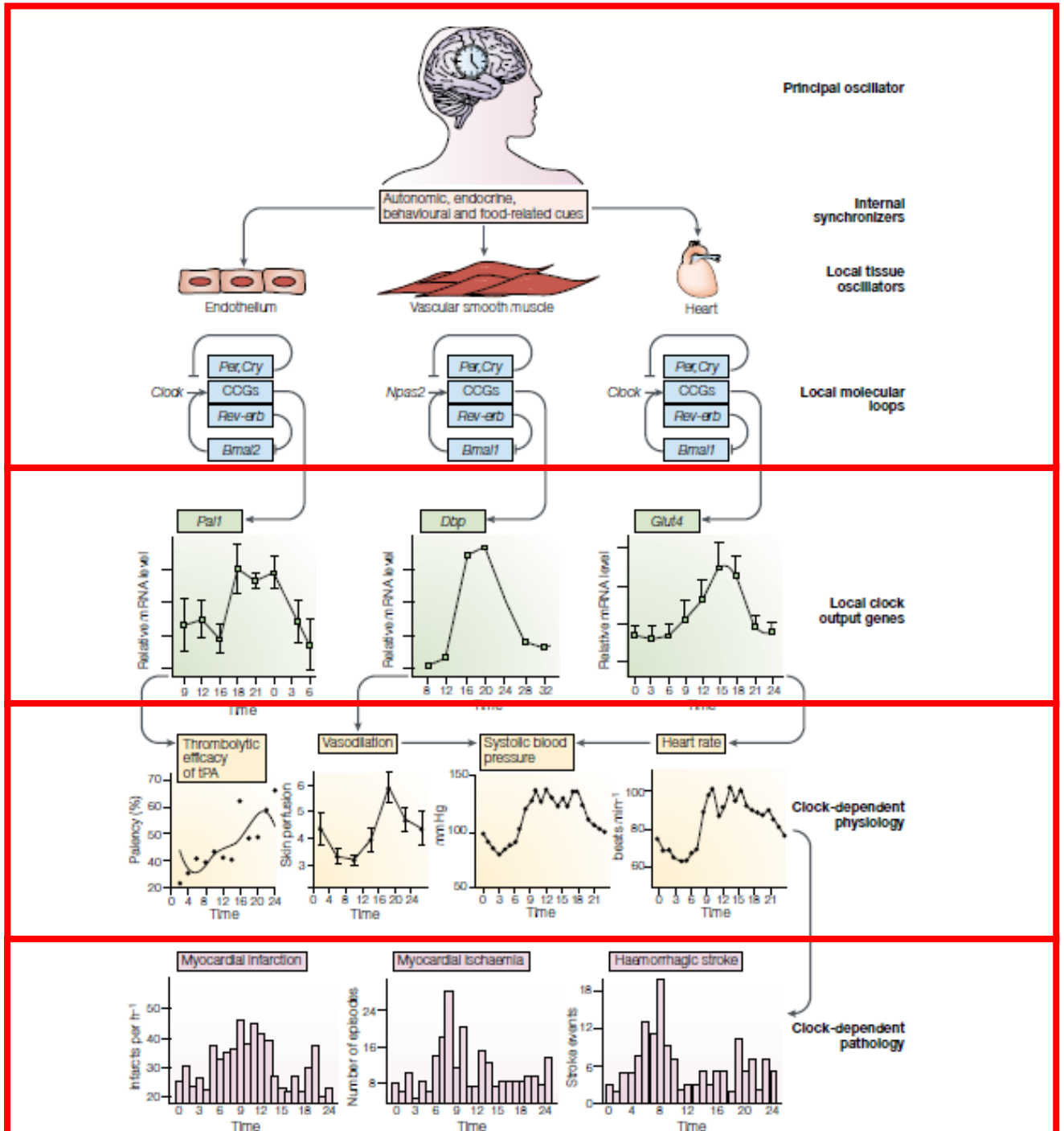
clock-controlled genes expression is also exhibiting daily day–night fluctuation cycles



Human Molecular Genetics, 2006, Vol. 15, Review Issue No. 2 R271–R277

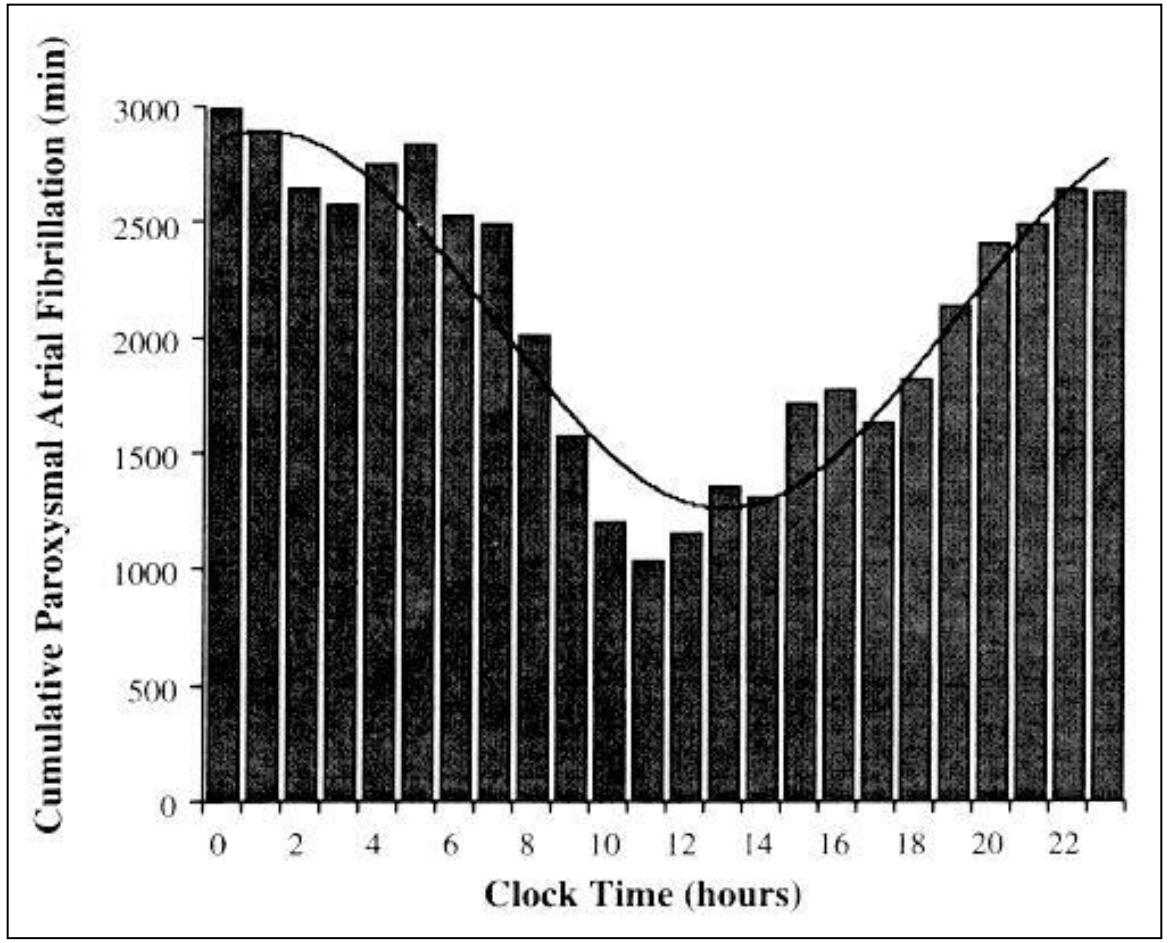


NATURE REVIEWS 2003(4) 649-661

Circadian Clock Genes and CV disease

- Diurnal variation in cardiovascular events (MI, SCD) → high incidence in morning – mechanism unclear
- *Bmal1* SNP associated with hypertension and T2D (Woon *et al* 2007 PNAS) (Rat and human)
- Clock gene mutants/KO mice display an array of cardiovascular disease e.g. metabolic syndrome, atherosclerosis, BP dysfunction (e.g. Turek *et al* 2005 Science)
- Time-of-day dependence in myocardial I/R tolerance in mice, mediated by the cardiomyocyte-specific clock → in phase with rhythm in MI onset in human (Durgan *et al* 2010 Circ Res)
- RORα KO mouse models display overt cardiovascular phenotypes (e.g. atherosclerosis, vascular tone dysfunction)

Circardian variation of paroxysmal AF



Circadian Variation of Paroxysmal Atrial Fibrillation.
Yamashita, Takeshi; Murakawa, Yuji; Sezaki, Kazunori; Inoue, Masashi; Hayami, Noriyuki; Shuzui, Yutaka; Omata, Masao

Circulation. 96(5):1537-1541, September 2, 1997.

Figure 1 . Hourly total duration of paroxysmal atrial fibrillation accumulated in the total of 150 patients. A single-harmonic fit is represented by a curved line. A prominent circadian rhythm is present, with a peak at midnight and a nadir at 11 AM.

CCGs regulate HRV changes

- Circadian rhythms in heart rate variability are driven by an intrinsic mechanism in humans
- Pts with an extended Per3 tandem repeat exhibit elevated heart rate
- Selective deletion of peroxisome proliferator-activated receptor- γ (PPAR- γ), a putative activator of BMAL1, results in diminished heart rate diurnal variations

CCGs: circadian clock genes
HR: heart rate

Proc Natl Acad Sci U S A. 2004;101:18223–18227.
Am J Physiol Heart Circ Physiol. 2008;295:H2156-H2163.
Cell Metab. 2008;8:482– 491.

Clock-controlled genes and arrhythmia

- Gene expression microarray analysis showed multiple signal transduction cascade components and ion channels as clock-controlled genes
- Potassium channel (Kv1.5,Kv4.2) (rat)
- Gap junction (Connexin 40,43,45) (rodent and mammalian)

*Circulation. 1998;97:*686–691.
Dev Genet. 1999;24:82–90.
*Circ Res.*1994;74:839–851.
*J Interv Card Electrophysiol. 2000;*4:459–467.

Methods

Inclusion: 73 Pts with SSS s/p PPM between 2018/9-2019/12
Exclusion: autoimmune disease, malignancy, and chronic inflammation
Definition of AF and AF type: according to clinical guideline
AHREs (atrial high-rate episodes): atrial rate ≥ 180 BPM more than 5 mins
14 CCGs expression by qRT-PCR

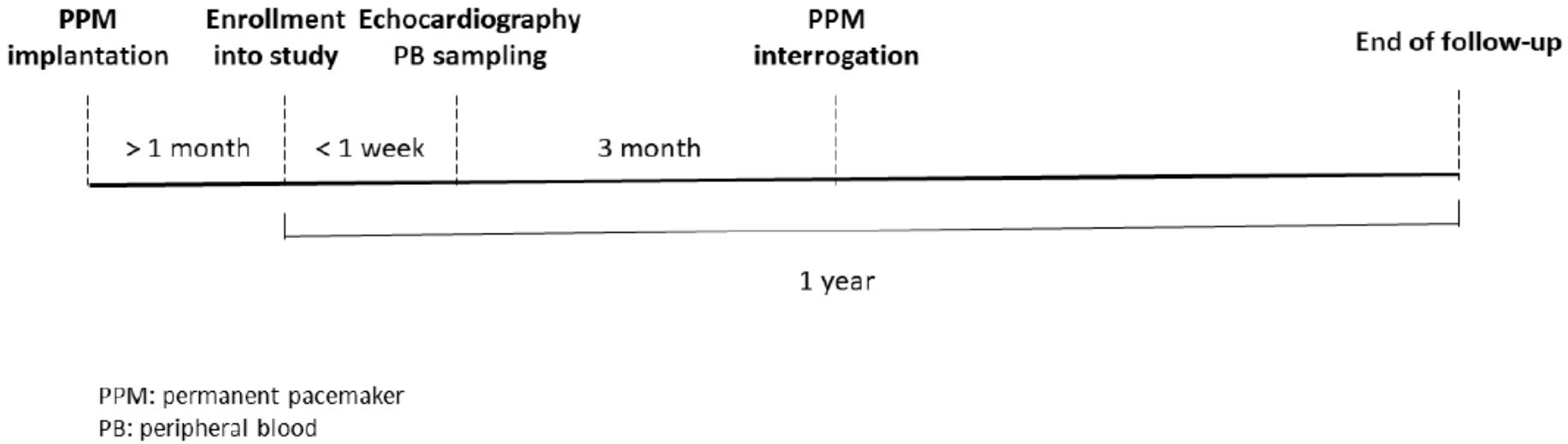


Figure 1. Flow chart of study design.

YL Chen, et al. Diagnostics 2021, 11, 90.

Baseline characteristics of the study population

Variables	Persistent AF (n = 15)	Paroxysmal AF (n = 28)	No AF (n = 30)	p-Value
Age	71.0 ± 8.3	71.0 ± 8.1	72.2 ± 8.7	0.840
Sex (Male/Female)	12/3	12/16 [a]	9/21 [a]	0.006
Hypertension	7 (46.7%)	18 (64.3%)	19 (63.3%)	0.481
Diabetes mellitus	6 (40%)	5 (17.9%)	8 (26.7%)	0.287
Previous stroke	3 (20%)	5 (17.9%)	2 (6.7%)	0.338
Heart failure	1 (6.7%)	5 (17.9%)	2 (6.7%)	0.330
Coronary artery disease	3 (20%)	5 (17.9%)	5 (16.7%)	0.963
Chronic kidney disease	3 (20%)	2 (7.1%)	6 (20%)	0.328
Anxiety	4 (26.7%)	8 (28.6%)	7 (23.3%)	0.900
Benzodiazepine	2 (13.3%)	2 (7.1%)	4 (13.3%)	0.713
Non-benzodiazepine	1 (6.7%)	0 (0%)	2 (6.7%)	0.378
Average heart rate	74.5 ± 5.0	73.4 ± 7.9	71.9 ± 5.3	0.488
AHRE burden (IQR)	100 (100–100)	0.5 (0–3.5) [a]	0 (0–0) [a]	<0.001
Echocardiographic data				
Left atrium diameter(mm)	49.3 ± 9.3	40.8 ± 10.2 [a]	38.9 ± 4.4 [a]	<0.001
Left atrial volume (cm^3)	102.7 ± 37.5	62.4 ± 43.8 [a]	50.7 ± 19.2 [a]	<0.001
Aorta (mm)	32.9 ± 5.1	32.1 ± 4.3	32.7 ± 4.4	0.802
LVEDD (mm)	51.1 ± 8.3	47.4 ± 5.6	48.4 ± 8.3	0.294
LVESD (mm)	35.1 ± 9.4	30.4 ± 4.3	30.8 ± 7.5	0.089
LVEF (%)	60.0 ± 10.9	65.1 ± 7.6	65.9 ± 9.2	0.106
Septal E/e′ ratio	16.3 ± 9.5	13.9 ± 8.9	14.2 ± 9.3	0.740
DT (ms)	181.2 ± 64.6	224.6 ± 72.7	196.7 ± 44.2	0.097
PAP (mmHg)	25.2 ± 10.8	24.9 ± 9.2	24.7 ± 8.4	0.984

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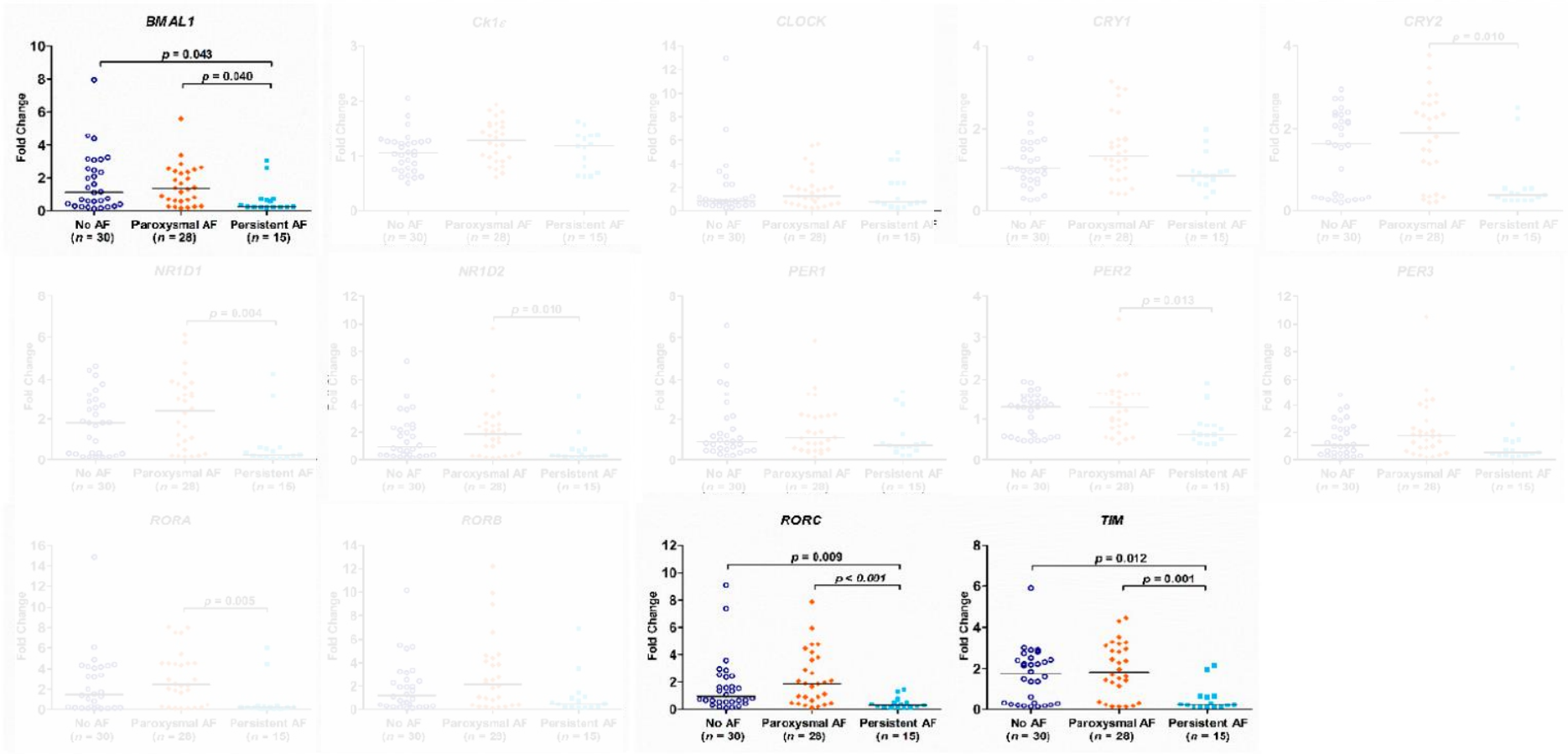
Expression levels of the 14 CCGs



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KHRS 2023

Linear regression model predicting AHREs burden

R2 = 0.633; p < 0.001

Variables	Standardized β Coefficient	*p*-Value
Male sex	0.188	0.066
Age	0.139	0.127
Left atrial volume	0.608	<0.001
BMAL1	0.385	0.050
CRY1	0.386	0.041
CRY2	−0.528	0.382
NR1D1	1.149	0.016
NR1D2	0.248	0.569
PER2	−0.128	0.687
PER3	0.148	0.371
RORA	−1.676	0.025
RORB	0.257	0.112
RORC	−0.470	0.064
TIM	0.265	0.520

AHREs: atrial high-rate episodes

YL Chen, et al. Diagnostics 2021, 11, 90.

Hypothesis

The expression of CCGs

- is altered through the change of the light/dark cycle in mice
- influences the gene expression related to energy metabolism、inflammation 、fibrosis and gap junction
- causes the electrical and mechanical remodeling of the mice heart

CCGs: circadian clock genes

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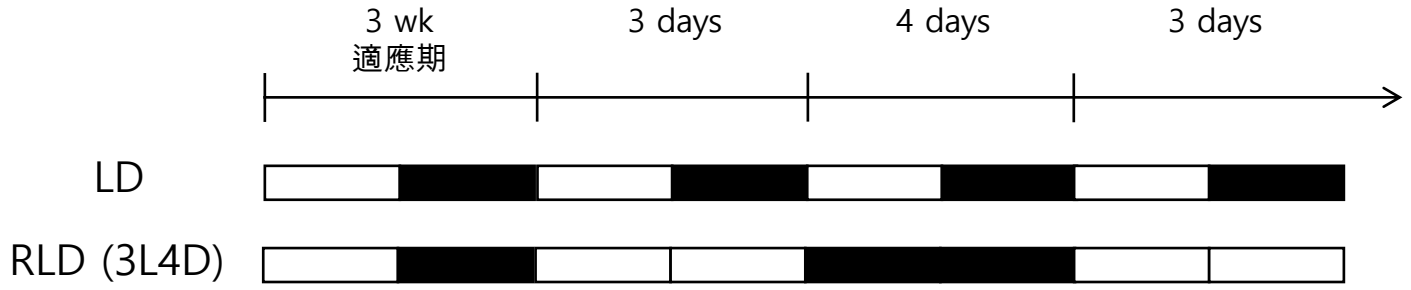
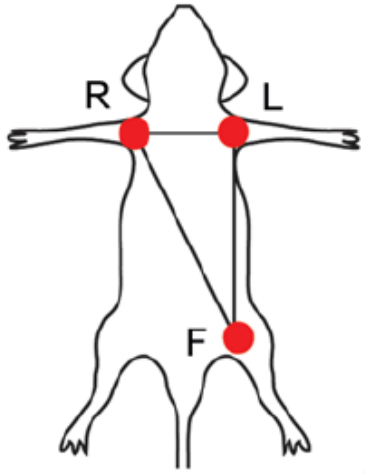
C57BL/6J mice



LD: 12-hr Light/12-hr Dark cycle
RLD (3L4D): 3 days 24hr all-Light, then 4 days 24hr all-Dark

- Sacrificed at 24 weeks: 9 am
- **Gene**:
  a. Metabolism: PPARα. PGC-1
  b. Inflammation: IL-1β. IL-6. IL-10
  c. Fibrosis: Timp1. Smad4. TGF-β1
  d. Gap junction: GJA1

- ➢ Anesthesia： Avertin
- ➢ EKG (iWorx-100B+ LabScribe): 5 mins
- ➢ ECHO (Philips)
- ➢ Transesophageal electrical stimulation



J Physiol 592.21 (2014) pp 4613–4626

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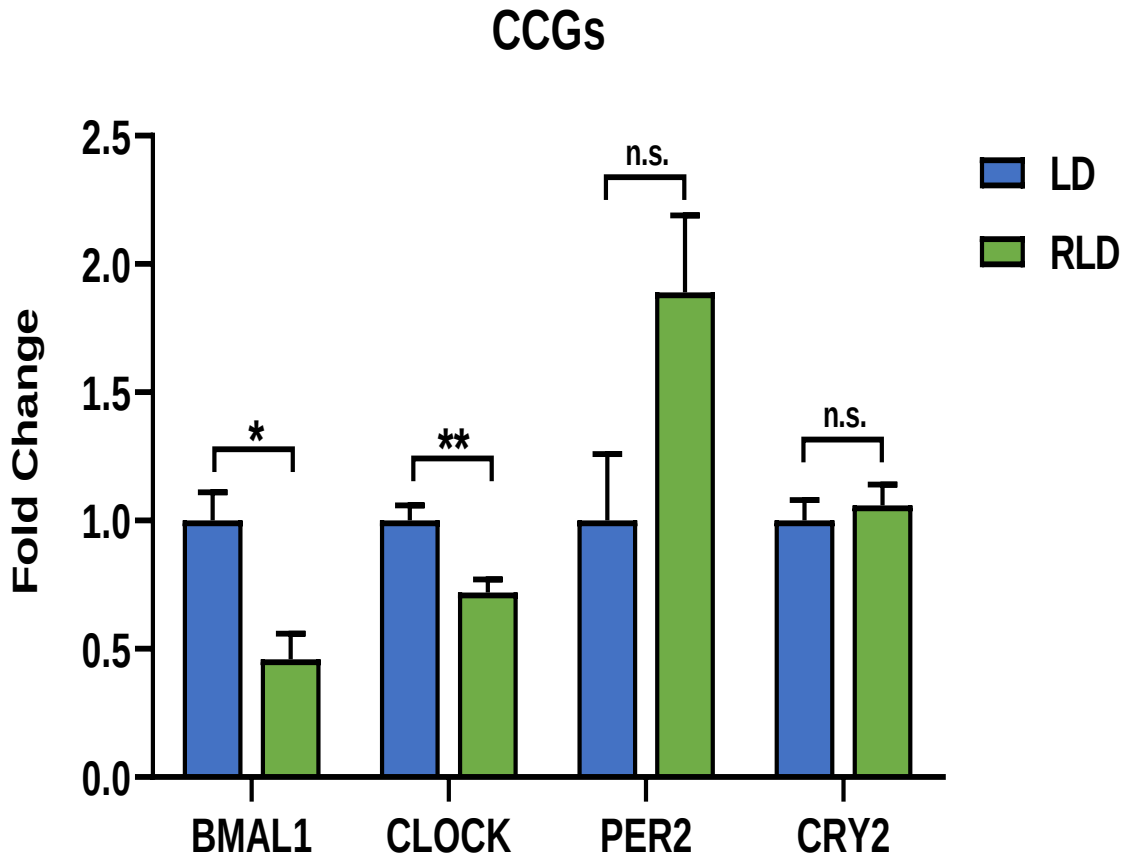
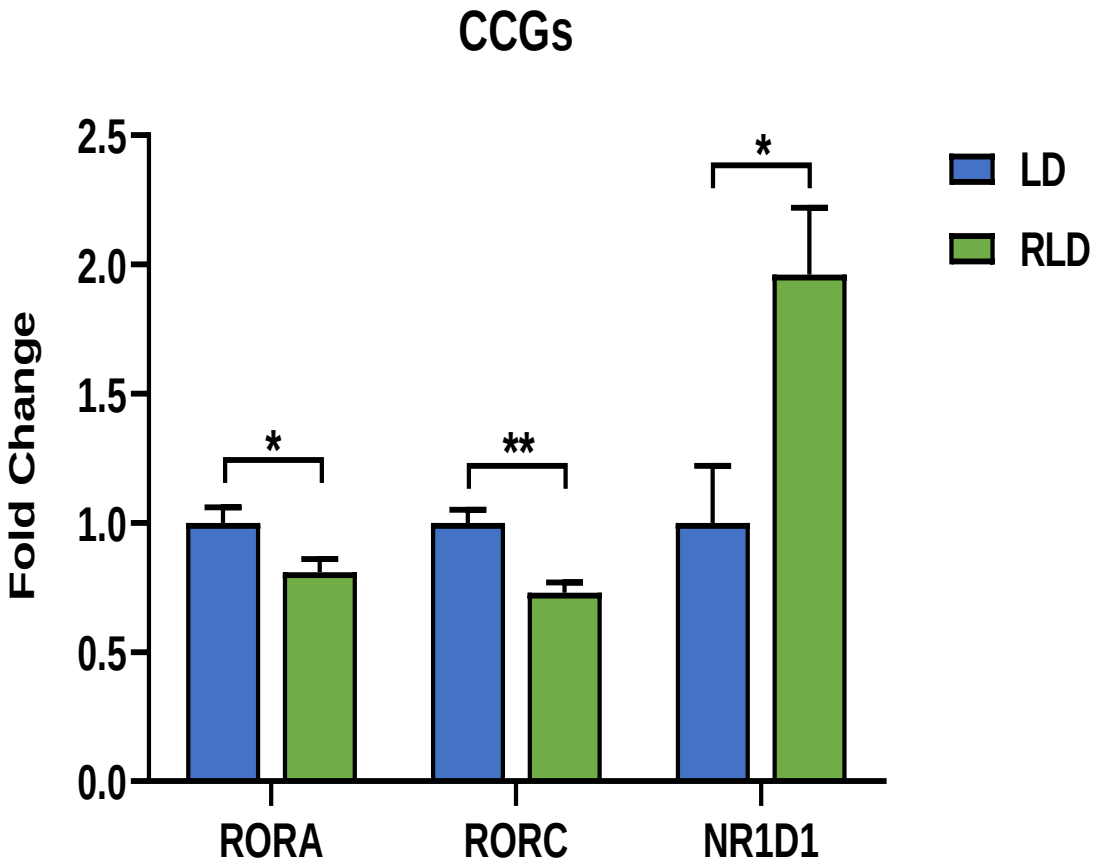
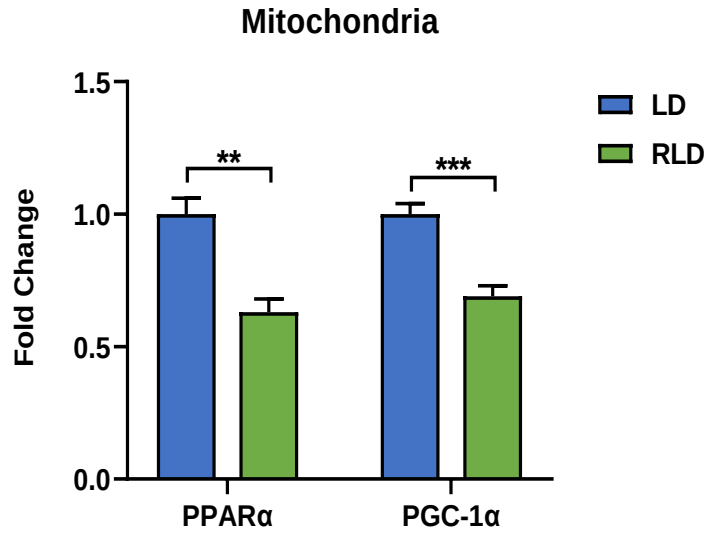
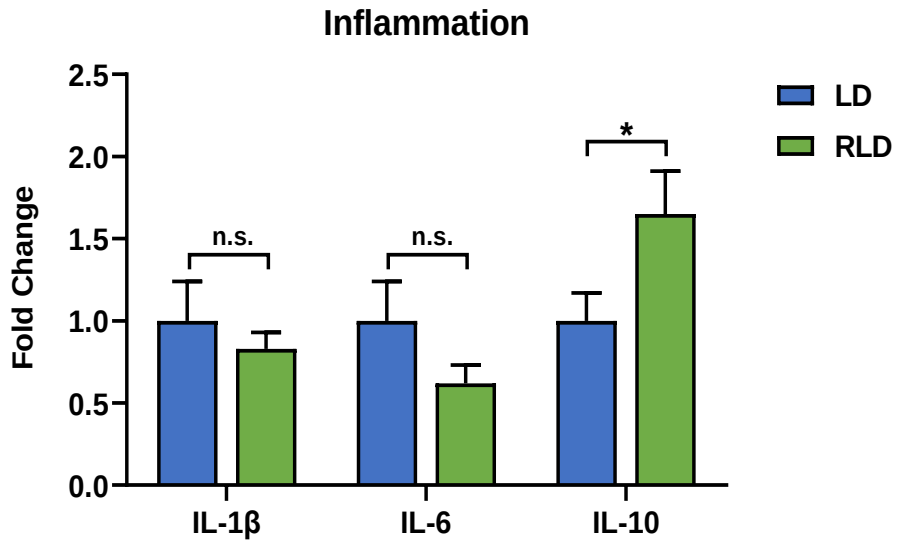
mRNAs expression of CCGs

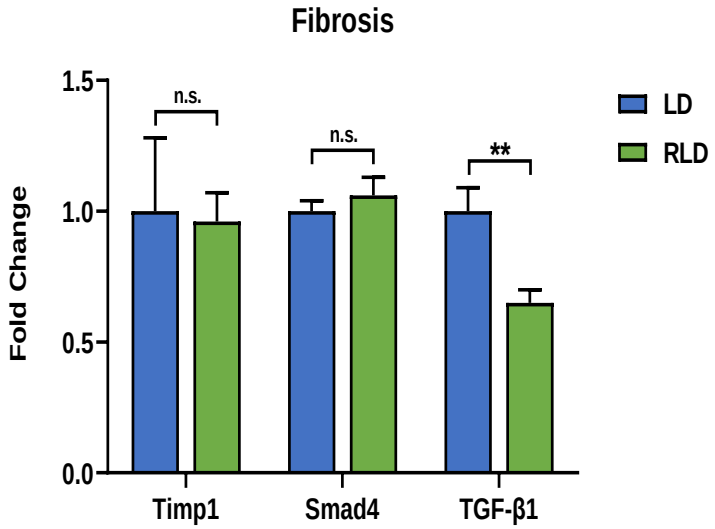


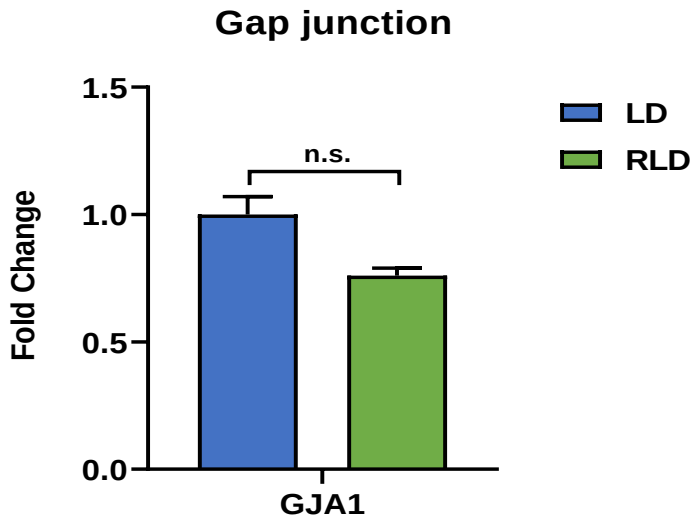


mRNA expression of clock-controlled genes

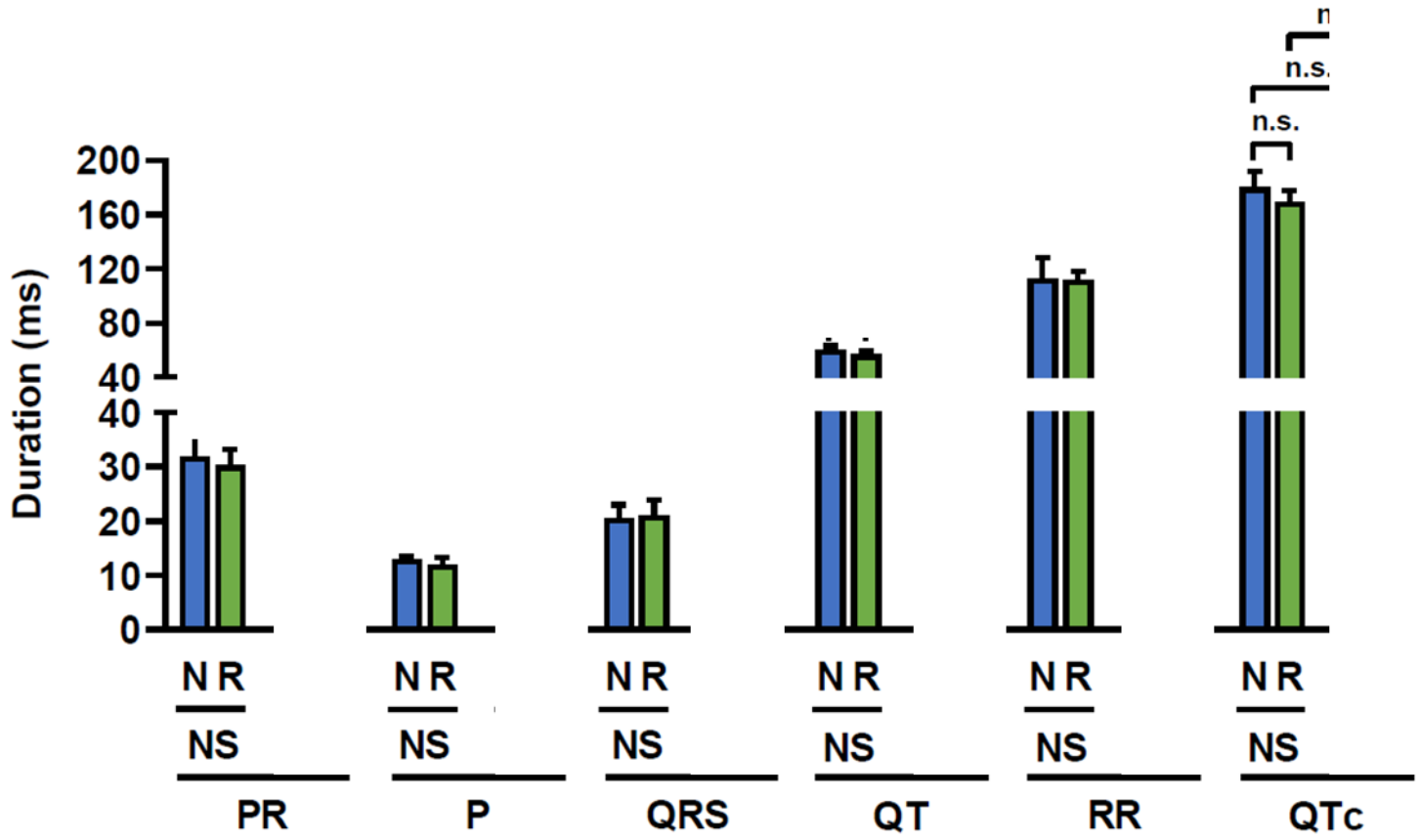


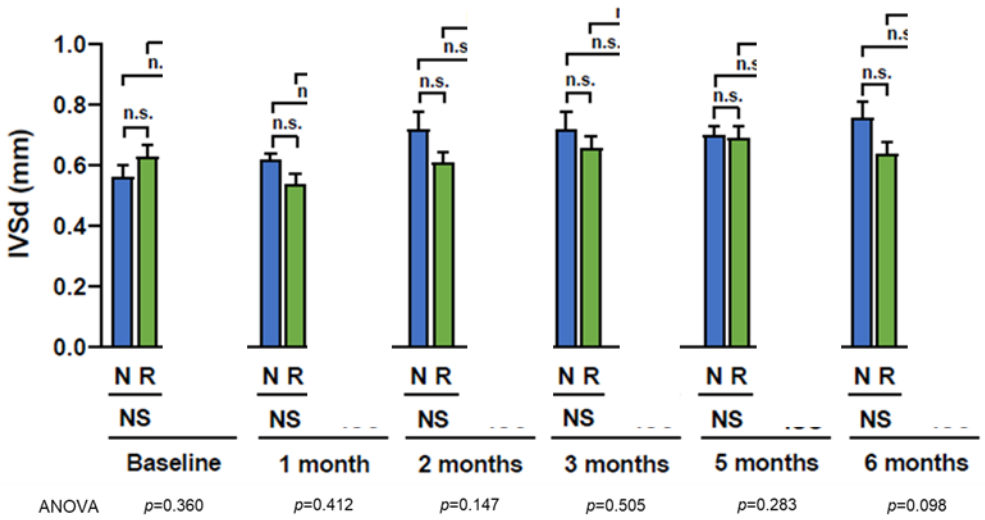


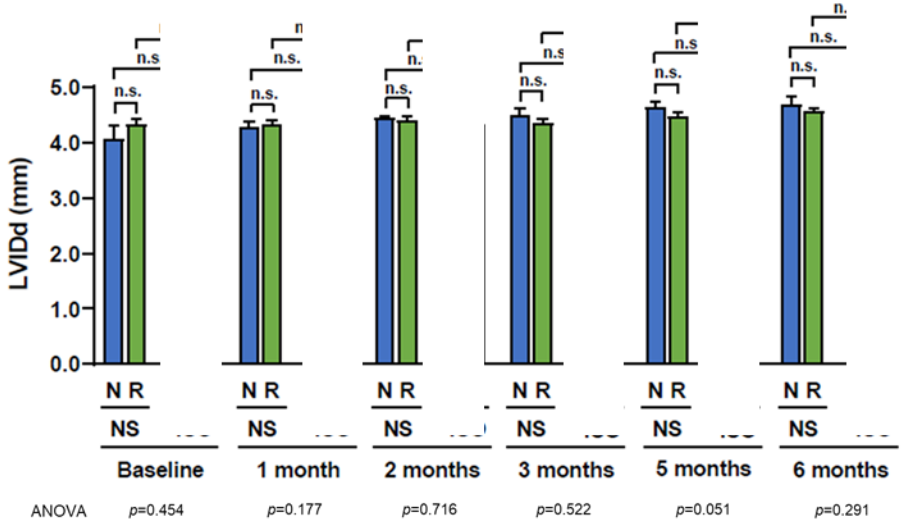


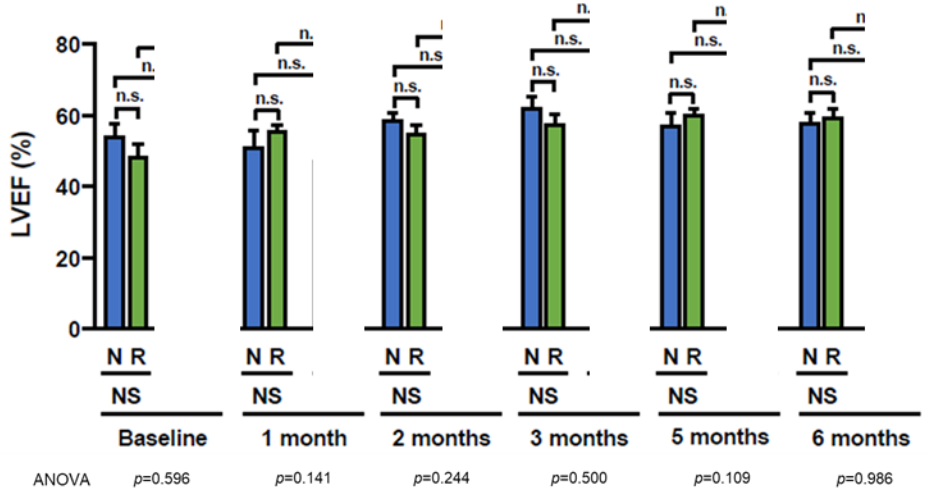


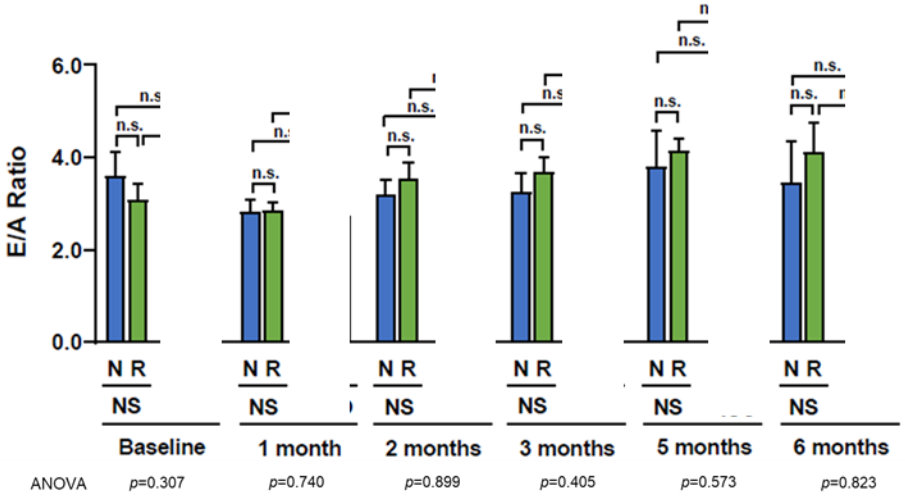
ECG parameters





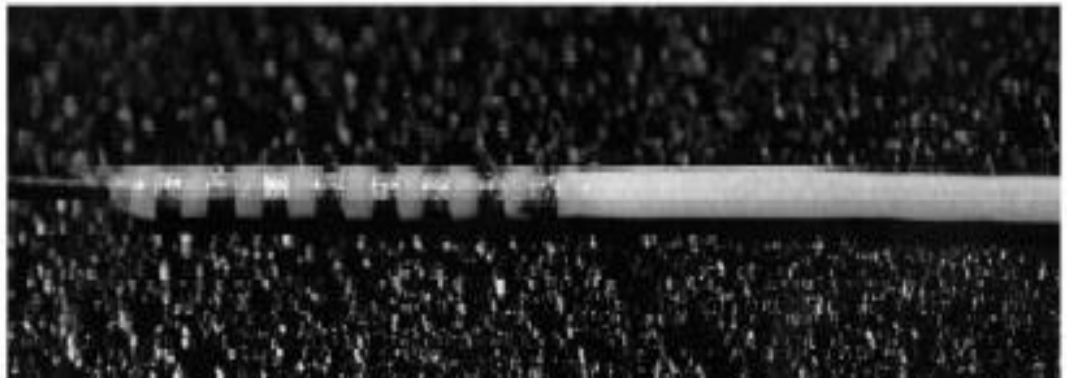


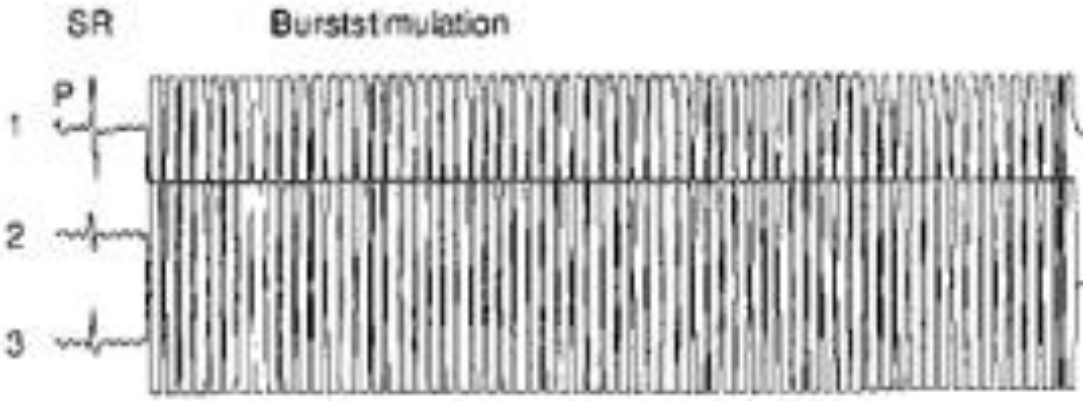




N: Normal LD
R: Reverse LD
mean±SEM

Transesophageal Electrical Stimulation Study





Burst pacing 30 secs with atrial 1:1 capture till 25 ms

No AF lasting 1 sec was noted

Conclusion

- In our altering light-dark cycle mice model, the expression of CCGs was influenced. (*BMAL1, Clock, ROR-A, ROR-C, NR1D1*)

- The expression of clock-controlled genes, including *PPAR*α, *PGC-1*α, *IL-10* and *TGF-*β*1* were also influenced.

- However, the electrical and structural remodeling was not found in our altered light-dark cycle model.

THANKS FOR YOUR ATTENTION

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KHRS 2023