Circadian Control of Nociception Associated Genes in the Naïve State

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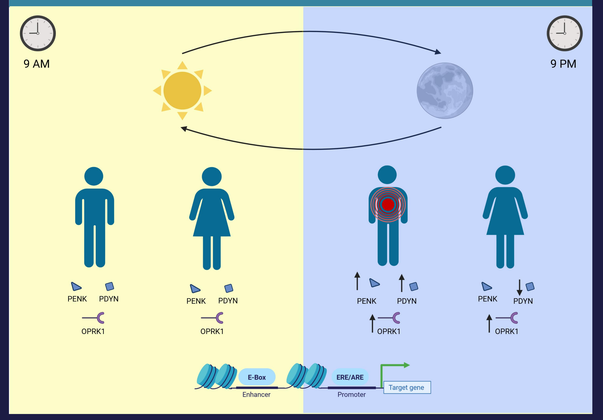
Abstract



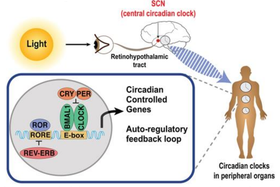
Objectives

- Define enhancer (E-box) sequence location in opioid genes.
- Define estrogen response element (ERE) and androgen response elements (ARE) motif locations in opioid genes.
- Show motif binding by receptors of interest can occur by simulated docking analysis.

Introduction

Circadian rhythms are controlled by a central pacemaker in the brain called the suprachiasmatic nucleus (SCN). This is a small region of the brain that creates and controls diurnal rhythms in mammals. However, peripheral tissues locally control the circadian rhythmicity of their gene expression by their own timed transcription-translation feedback loops (TTFL). (*Segal et al*., 2018)

- Brain and muscle ARNT-like factor 1 (BMAL1) and Circadian Locomotor Output Cycles Kaput (CLOCK) are the main drivers of this cycle.
- BMAL1 and CLOCK form a heterodimer upon expression and bind DNA via the helix-loop-helix domains.
- They bind a specific motif called the enhancer box (E-box) motif that consists of CANNTG where N can be any nucleotide but is often CACGTG (*Muños et al*., 2006).



Specific opioid peptides, opioid receptors, and nociceptive ion-channel expression exhibited circadian rhythmicity in naïve mice.

- Changes in gene expression occurred specifically in male mice, suggesting a sex-linked difference.

This project uses bioinformatic analysis to attempt to uncover underlying mechanisms of circadian control of various nociceptive channels and opioid genes as well as sex-specific differences in the circadian expression of said genes.

Methodology

E-box motif variation possibilities were generated from the canonical sequence with additions from known sequence variants which the BMAL1-CLOCK heterodimer binds. A list of 17 motif variants was produced. Genes of interest were taken from the *Mus musculus* GRCm38/mm10 genome via UCSC. Genes were analyzed for all E-box motifs. Promoter and enhancer DNA sequences had 3D structures generated including:

- Open euchromatin type structure
- Molecular dynamics averaged
- Template-based approach
- Via the SCFBio software

3D DNA structures were individually subjected to simulated docking with varying stages of docking restrictions per run, the top 100 structures were rendered as well as the docking scores and ligand RMSD for each docking orientation. All docking simulations were performed with HDOCK using the BMAL1-CLOCK heterodimer crystal structure (PDB:4F3L). Docking was performed in several rounds to optimize binding

- Blind docking: no indication of sequence recognition site or DNA binding site.
- Multimer docking.
- Masked docking and binding pocket specification docking.

Androgen and estrogen binding sites were uncovered by the following methods:

- 5000 base pair range of the transcription start site using the same method as described above.
- palindromic repeat finder with a tolerance of 1 nucleotide for a 6 base pair inversion.
- JASPAR was used to analyze estrogen response elements (ERE) and androgen. response elements (ARE) to determine key nucleotides and flexible nucleotides from known binding sites.
- This was generated from the sequence logo and frequency matrix produced via JASPAR.

Simulated docking using HDOCK was performed on the few candidates that arose from this analysis.

Results

E-Box Sequence Analysis

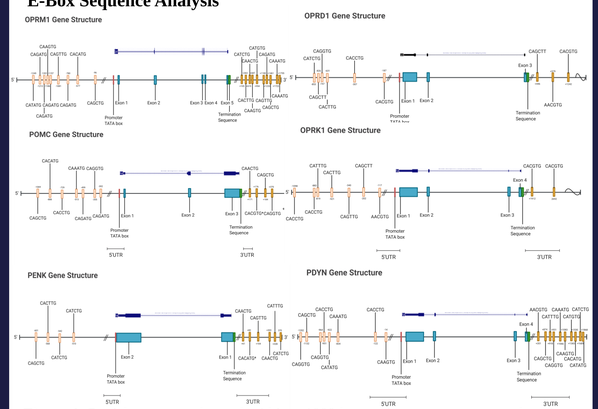


Figure 1. Partial sequences located within a 1000 base pair range were mapped against the gene structure to visualize the array of possible binding locations. The light pink boxes indicate locations of E-box sites upstream of the TSS, orange boxes indicate E-box sites downstream of the termination codon. The blue boxes indicate coding regions within the gene, the red boxes indicate coding region start sites. The wave icon indicates more promotor regions are present in the gene but are not listed.

Results

Transcription Changes in Opioid Genes

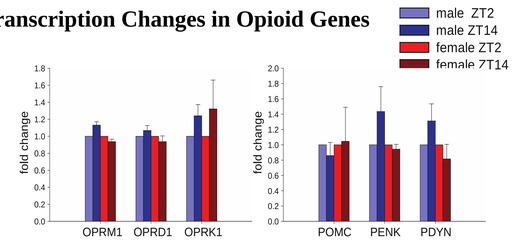


Figure 2. Relative change of opioid receptor and peptide gene expression in naïve male and female mice at 9 AM and 9 PM from previous work in the Ghasemlou lab.

BMAL1-CLOCK E-Box Binding

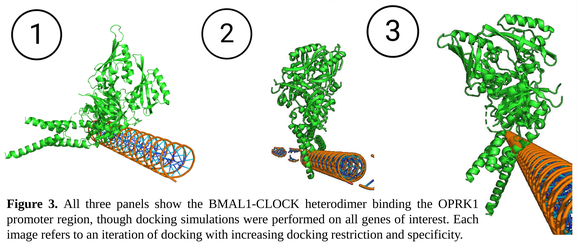


Figure 3. All three panels show the BMAL1-CLOCK heterodimer binding the OPRK1 promoter region, though docking simulations were performed on all genes of interest. Each image refers to an iteration of docking with increasing docking restriction and specificity.

Estrogen Receptor ERE Binding

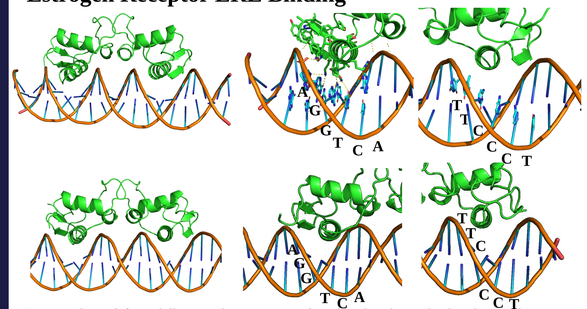


Figure 4. The top left panel illustrates the ERα receptor ligand-binding domain binding the partial ERE site in the PENK promoter region. The top right panels show the sequence being bound, the docking score was -284.06. The bottom panels show the ERE binding motif variant being bound by ERα with a docking score of -298.86.

Androgen Receptor ARE Binding

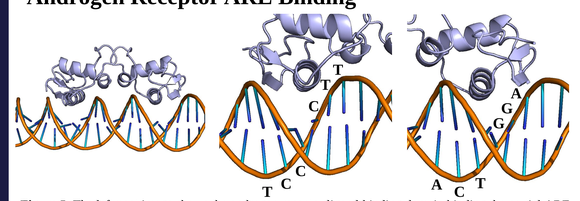


Figure 5. The leftmost image shows the androgen receptor ligand-binding domain binding the partial ARE site on the PDYN promoter region of the gene. The right images show the sequence being bound with a docking score of -315.14.

Conclusion

E-Box Analysis and Binding

- Many potential E-box binding sites were uncovered as far as 5000 base pairs upstream of the transcription start site.
  - Sites within the UTRs, CDs and 1000 base pairs downstream were also identified.
- Global blinded docking was unsuccessful.
  - Binding was off-target on both DNA sequence and protein domain.
- The masked docking approach (2) had better orientation towards the DNA but was unable to bind the correct sequences or utilize the depth of the binding pocket.
  - Docking orientation for many runs was incorrect.
- Binding residue specification led to correct docking orientation and location but produced a very poor docking score and a very high RMSD, suggesting binding was unlikely.

Estrogen and Androgen Motif Recognition and Binding

- Estrogen and androgen recognition sequences were uncovered on various genes.
  - Partial recognition sites were also taken into account.
- Estrogen and androgen DNA binding subunits were used for blind docking analysis on local DNA segments containing binding motifs.
- Sequences with high identity to literature motifs were recognized by receptor dimers and presented a high docking score.
  - Docking scores reached approximately -300 for high sequence identity matches.
- Binding contacts for ERE and ARE sequences on the PENK and PDYN genes mostly matched literature with more distant matches for other genes.
  - Binding orientation was identified to be correct.
- This evidence suggests that transcriptional changes caused by hormone receptor binding is a likely mechanism and reinforces findings.
- Because ERE and ARE sites can be located a great distance away from the TSS, it is difficult to verify which motifs correspond to specific genes.
- These findings provide some evidence for short-range estrogen and androgen activation of various opioid peptides.

Limitations and Future Directions

To continue the research work on this topic, the next steps would likely consist of:

- Chromatin accessibility
- Histone markers
- CHiP Seq
- Behavioral studies

More time analyzing all partial estrogen and androgen binding sites with the simulated docking approach would allow for a thorough understanding of each gene's potential to be under hormonal control. Moreover, most of the potential E-box sites were unable to undergo specific docking analysis due to time constraints. In future work, each site would ideally be analyzed more thoroughly in addition to the global docking analysis.

Acknowledgements

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