

Functional peptidomics: Stimulus- and time-of-day-specific peptide release in the mammalian circadian clock

Norman Atkins, Jr.^{1†#a}, Shifang Ren^{2†#b}, Nathan Hatcher^{2,3#c}, Penny W. Burgoon^{4#d}, Jennifer W. Mitchell^{1,3,4}, Jonathan V. Sweedler^{1,2,3}, and Martha U. Gillette^{1,3,4*}

¹Neuroscience Program, ²Department of Chemistry, ³Beckman Institute for Advanced Science and Technology, ⁴Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

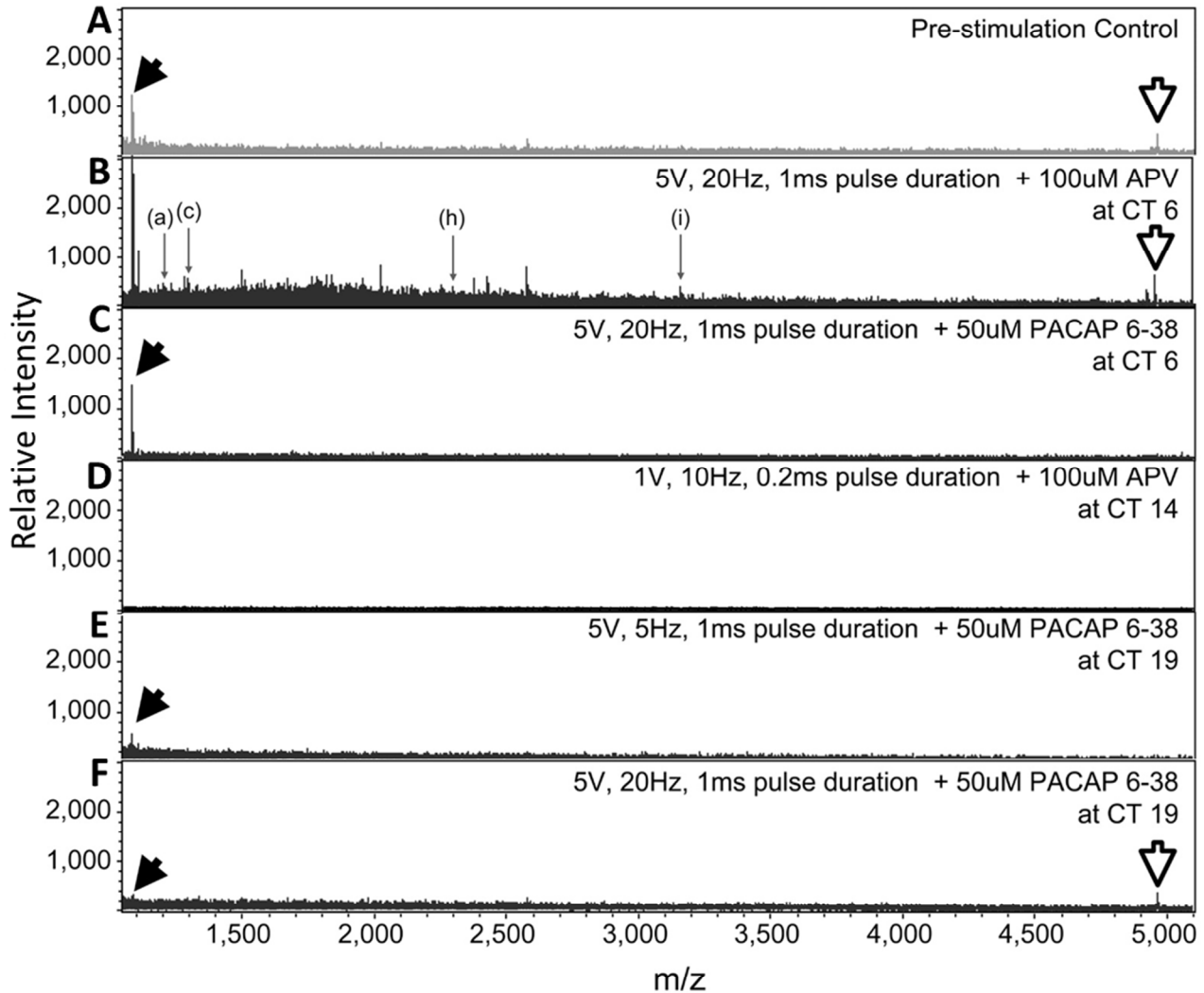
† Authors contributed equally to this work

* Corresponding author. Email: mgillett@illinois.edu. Tel: 217-244-1355

SUPPORTING INFORMATION

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Supplementary Figure 1. Stimulus-evoked peptide release from rat SCN downstream of NMDA receptor (NMDAR) or PACAP receptor (PAC1R) activation is distinct. Releasate was collected during electrical stimulation of the ON following a 10-min incubation of the horizontal SCN slice with either the competitive NMDAR antagonist (2*R*)-amino-5-phosphonovaleric acid (APV; 100 μ M) or the competitive PAC1R antagonist PACAP 6-38 (10 μ M) at respective circadian time-points. **(A)** The pre-stimulation control shows little peptide release, as anticipated. **(B)** At CT 6, the NMDAR antagonist APV was ineffective in blocking stimulus-evoked SCN peptide release. **(C)** Peptide release was inhibited, however, with administration of PAC1R antagonist PACAP 6-38. **(D)** Early subjective nighttime (CT 14) stimulus-dependent peptide release was blocked by APV. **(E, F)** During late nighttime (CT 19), PACAP 6-38 prevented SCN peptide release following both 5 Hz (E) and 20 Hz (F) ON stimulation. In the presence of the glutamate antagonist APV, electrical stimulation at CT 6 triggered peptide release comparable to release observed under ON stimulation only conditions (B). Peptides observed in releasate profiles following pre-incubation in APV, include arginine vasopressin, neurokinin B **(a)**, angiotensin **(c)**, PEN **(h)**, and galanin **(i)**. The peptides arginine vasopressin (filled arrowhead) and β -4 thymosin (hollow arrowhead) were observed in both pre-stimulation control (A) and various stimulation conditions.

Table S1. Peaks detected via MALDI TOF MS from stimulated releasate of the suprachiasmatic nucleus that have not been assigned

| Observed m/z (MH ⁺) | RHT Stimulus-Triggered Releasate Conditions | | | | |
|------------------------------------|---|-----|------|---------------|----------------|
| | Pre-Stim | CT6 | CT14 | CT19 (5Hz) | CT19 (20Hz) |
| 1122.4 | | | | | ● |
| 1179.6 | | | ● | | |
| 1199.7 | | ● | | | |
| 1295.6 | | | | ● | |
| 1329.6 | | | ● | | |
| 1333.6 | | | ● | | |
| 1356.6 | | | ● | | |
| 1383.7 | | | ● | | |
| 1482.0 | | ● | | | |
| 1509.7 | | | | ● | |
| 1572.9 | | ● | | | |
| 1590.7 | | | ● | | |
| 1738.8 | | | ● | | |
| 1856.6 | | ● | | ● | |
| 1900.8 | | ● | | | |
| 1956.8 | | | | ● | |
| 2336.8 | | | ● | | |
| 2380.1 | | ● | ● | ● | |
| 2433.1 | | ● | ● | ● | |
| 2481.2 | | ● | ● | ● | ● |
| 2580.0 | | ● | ● | ● | ● |
| 3065.9 | | ● | | | |