

Submitter Name: Makoto Taniguchi, Ph.D., PI.

Submitter Email: Taniguch@musc.edu

**A long non-coding eRNA forms R-loops to shape emotional experience-induced behavioral adaptation**

Rose Marie Akiki<sup>1,2</sup>, Rebecca G. Cornbrooks<sup>1</sup>, Kosuke Magami<sup>1</sup>, Alain Greige<sup>1,2</sup>,  
Kirsten K. Snyder<sup>1</sup>, Daniel J. Wood<sup>1,2</sup>, Philip Mace<sup>1</sup>, Kyle Blidy<sup>1</sup>, Nobuya Koike<sup>3,4</sup>,  
Stefano Berto<sup>1</sup>, Christopher W. Cowan<sup>1</sup>, Makoto Taniguchi<sup>1</sup>

<sup>1</sup> Department of Neuroscience, Medical University of South Carolina; Charleston, SC 29425, USA; <sup>2</sup> Medical Scientist Training Program, Medical University of South Carolina; Charleston, SC 29425, USA; <sup>3</sup> Department of Neuroscience, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; <sup>4</sup> Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine; Kyoto 602-8566, Japan.

Emotional experiences often evoke neural plasticity that supports adaptive changes in behavior, including maladaptive plasticity associated with substance use disorders. These adaptations involve the experience-dependent activation of immediate-early response genes, including *Npas4*. The protein-coding genes (mRNAs) are important factors for the functional regulation of brain activity and are targets for many neuropsychiatric conditions; however, they are a minor component of the mammalian genome. Indeed, human and rodent genomes encode a much larger number of long-non-coding RNAs (LncRNAs). Emerging evidence demonstrates significant roles for lncRNAs in multiple processes of gene expression, but the physiological and pathological functions of individual lncRNAs are only beginning to be explored. We discovered that a conserved, lnc-eRNA produced from an activity-sensitive enhancer, produces RNA:DNA hybrid three stranded genomic structures, called R-loops. The R-loops support the formation of 3D chromatin-looping of the enhancer and proximal promoter, and stimulus-induced rapid *Npas4* gene induction in the NAc *in vivo*. We also show that this *Npas4* lnc-eRNA is required for the development of cocaine reward conditioned behavioral adaptations, revealing a critical role for this new genomic regulatory mechanism in the transmission of emotional experiences, such as drug use, to adaptive behavioral responses.