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RESEARCH HIGHLIGHT

Getting a Sense of ATP in Real Time

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Adenosine 5′-triphosphate (ATP) is a purinergic signal that can be sensed by all major cell classes in the brain. ATP is a key signal released during periods of physical damage/injury, inflammation, and neurological events, such as seizures. Understanding ATP signaling in the living brain is complicated by its fast enzymatic degradation, its action at multiple receptors, and its somewhat elusive mechanism of release across cell classes. Luckily, the Li lab has recently developed a highly optimized, genetically encoded ATP sensor with the potential to greatly improve our understanding of ATP signaling mechanisms and dynamics [1] (Fig. 1).

Termed $GRAB_{ATP1.0}$, the sensor developed by Wu et al. is based upon the circularly-permutated GFP concept underlying GCaMP sensors. However, $GRAB_{ATP1.0}$ is fused to the ATP- and ADP-sensing G-protein coupled receptor, P2Y1 (Fig. 1). This ~1.9 kb construct has been successfully packaged into a number of plasmids and can be selectively expressed in neurons and astrocytes through AAV transfection. What makes $GRAB_{ATP1.0}$ a particularly excellent resource is its selectivity for ATP and ADP over all other purines and neurotransmitters. In addition, $GRAB_{ATP1.0}$ senses ATP in a dynamic range spanning 1 nmol/L to 1 mmol/L, matching the extracellular concentrations of ATP expected during physiology and pathophysiology [2]. Finally, upon ATP stimulation, $GRAB_{ATP1.0}$ demonstrates a 5- to 10-fold fluorescent response in culture or zebrafish, with responses reaching 3-fold in the mouse cortex. Based upon its dynamic range, fluorescent signal, and reliable expression, $GRAB_{ATP1.0}$ exhibits ideal qualities for a robust *in vivo* sensor for ATP and ADP (Fig. 1).

One of the major questions surrounding ATP signaling is how it is released by different cell classes. In the spinal cord, ATP can be released through a vesicular mechanism to influence chronic pain conditions [3]. However, in the brain, the ATP release mechanism is largely obscure. In zebrafish, ATP can be released through the hemichannel Pannexin-1 [4]. In mouse, neuronal hyperactivity can induce ATP release through a hemichannel of as yet unknown identity [5]. Using the newly developed $GRAB_{ATP1.0}$ sensor, Wu *et al*. demonstrate that extracellular ATP responses induced by swelling in neuronal culture are not impacted by tetanus toxin light chain, a synaptic release inhibitor. Thus, swelling-induced ATP release is likely non-vesicular. Moving forward, $GRAB_{ATP1.0}$ should allow for robust, high-throughput pharmacological screening of hemichannels and volume-sensitive channels to identify the major mediator(s) of neuronal or glial ATP release.

The second major question surrounding ATP signaling is the timing of its extracellular actions. As a small molecule with endonucleases for its extracellular degradation [2], ATP is expected to be a relatively short-lived signal. Previously, the timing of ATP signaling has been studied using microdialysis, which does not allow for real-time measurements or high sensitivity. In characterizing their new ATP sensor, Wu *et al*. provide some intriguing insights into the signaling time of ATP [1]. In culture, a transient ATP application evokes relatively rapid

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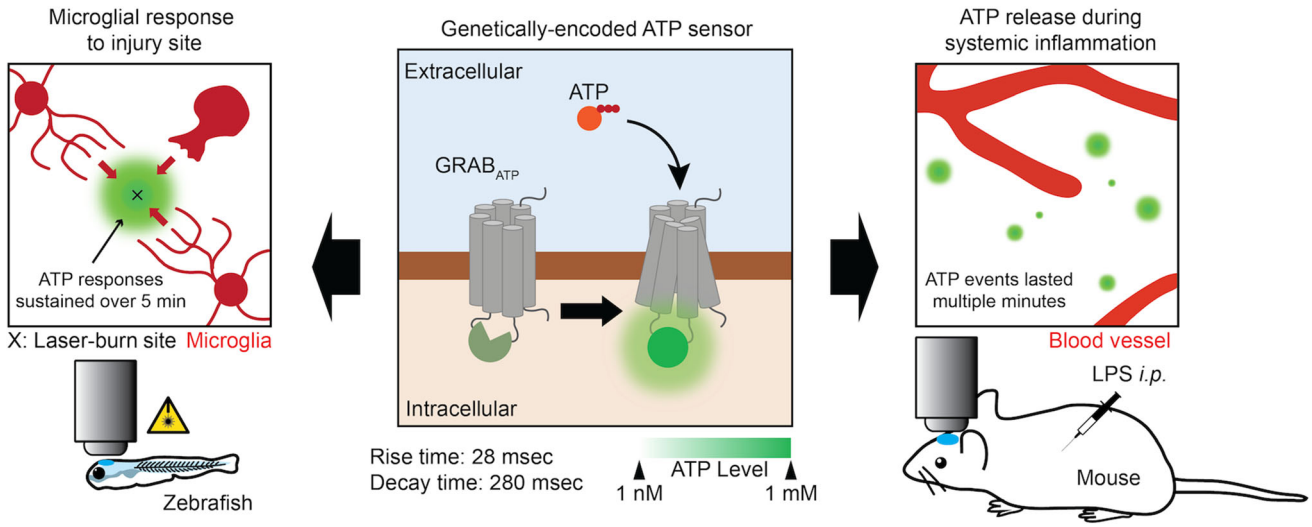

Fig. 1 Development of the genetically-encoded ATP sensor ($GRAB_{ATP1.0}$) and its responses to ATP release through *in vivo* imaging. $GRAB_{ATP1.0}$ is an ATP (and ADP)-specific sensor that increases green fluorescence upon extracellular ATP binding. $GRAB_{ATP1.0}$ is validated *in vivo* in zebrafish and mouse brain. In zebrafish, microglia respond to radially spreading ATP release from the site of injury. In mouse cortex, ATP release events occur during LPS-induced systemic inflammation.

fluorescent sensor dynamics, including an activation time in the tens of milliseconds, with a deactivation time in the hundreds of milliseconds. The inactivation likely reflects a dispersion or degradation of ATP, because the fused P2Y1 construct does not appear to desensitize, even with 2 hours of continued ATP exposure. Thus, $GRAB_{ATP1.0}$ can rapidly sense ATP and inactivate upon its loss/dispersion. However, ATP events reported in zebrafish and mouse can be exceptionally long-lasting. Local, transient application of ATP to the zebrafish optic tectum can produce a signal lasting 30–40 s, while laser-burn injury can induce a sustained and spreading ATP response for >5 min (Fig. 1). In mouse cortex, systemic inflammation by LPS injection can evoke ATP events lasting several minutes (Fig. 1). The impetus for such long-lived events likely reflects sustained ATP release, but the cell classes involved and the potential for feed-forward signaling are all worthy of study. These key aspects of sustained ATP signaling could be dissected thanks to the robust nature of the Li lab's new sensor. Sustained purine signals are known triggers of pro-inflammatory cytokines and many neurological disorders [6], positioning this construct as an informative tool for future *in vivo* longitudinal studies of brain and spinal cord diseases.

Signals like ATP are still incompletely understood when it comes to their downstream signaling impact on complex biology. ATP can evoke neuronal and glial Ca^{2+} activity, microglial process motility, and ionic flux at the receptor level [6]. Thereafter, there is a somewhat incomplete picture of how these receptor-mediated responses impact biology at the circuit or systems level. Recent studies have demonstrated that local degradation of ATP into adenosine during seizure activity provides an important negative feedback loop that reduces pre-synaptic release and seizure severity [7]. This is a positive first step in understanding how purines regulate the local environment, beyond their known roles in cytokine production. The fact that purines are such a ubiquitous damage and danger signal, potentially sensed across all brain cell classes, suggests that they play a key role in coordinating complex brain responses to disease, injury, and infection. Wu *et al.* provide an early example of how to harness an ATP sensor in this domain, by showing the coordinated motility of dsRed-labeled microglia towards an ATP-rich injury site. Thus, the $GRAB_{ATP1.0}$ sensor would be extremely useful in studying microglial process convergence towards neurons during periods of heightened or lowered brain activity [5, 8, 9]. In addition, understanding how ATP and other signals, such as glutamate, interact and potentially reinforce each other could have great benefits in understanding hypersynchronous disorders such as epilepsy, stroke, migraine, and chronic pain. The unique pattern of ATP fluorescence, including its radial spread over many microns has interesting parallels to the glutamate plumes recently described during migraine [10]. At the same time, it will be interesting to know which aspects of basal physiology involve elevated ATP signaling. In particular, it will be intriguing to study if certain aspects of cognition involve ATP signaling in the learning, consolidation, or retrieval phases of task performance.

At this stage, $GRAB_{ATP1.0}$ has a few minor limitations. First, the high end of the dynamic range (1 mmol/L extracellular ATP) could represent a saturated signal during pathological events such as stroke or seizure.

Towards this end, the authors have also developed a lower affinity variant with faster off-time kinetics, called ATP1.0-L, which should help in detecting faster ATP events during pathology. The second limitation of any extracellular ATP sensor is its inability to pinpoint the cellular source(s) of ATP release without additional pharmacological or genetic approaches. This would be particularly important to determine in their studies of ATP release in LPS-induced systemic inflammation. The third limitation of $GRAB_{ATP1.0,}$ as noted by the authors, is its inability to distinguish between ATP and ADP in real time. The final limitation of $GRAB_{ATP1.0}$ is its current AAV packaging under hSyn and GFAP promoters. In future, a Cre-dependent viral construct (*i.e.*, "FLEX") would allow for a greater range of expression, while a knock-in mouse is currently needed to study microglia. Nevertheless, having a robust ATP sensor opens up countless possibilities to better understand an elusive signaling molecule noted for its roles in disease and injury. The $GRAB_{ATP1.0}$ sensor and its potential combination with red-shifted sensors and pharmacology should allow researchers to uncover new mechanisms governing ATP release and regulation, as well as its impact on acute or chronic disease progression.

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