Fly Marathons

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Ever heard of the quote, "Finishing a marathon is a state of the mind that anything is possible"? Well, for a fruit fly, it's more much more than just that.

Buzzing off incessantly in search of food, mates and sites to lay eggs, the humble fruit fly is indeed capable of 'flying' marathons. Researchers from the lab of Prof. GaitiHasan at the National Centre for Biological Sciences, Bangalore, have identified molecules in the fly brain that help it fly for such long periods of time, thereby giving them an advantage in the wild. This work was recently published in PLOS Genetics, grabbing the attention of readers with a striking image on the journal's cover page.

How long are these fly marathons? On average, tethered fruit flies can fly for as long as 10 minutes, uninterrupted! A minor fraction of these flies can fly for much longer, even upto 30-40 minutes, under lab conditions. But how do these flies do it? One would imagine that flying demands tremendous energy. Well, in this study, the authors have discovered that a protein called FMRFa receptor (FMRFaR) helps keep some neurons in the brain active, so that the fly can continue to fly for long periods of time. So, what happens when a fly does not have this protein? The authors found that a fly lacking the FMRFaR was unable to maintain flight for long. In fact,

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the FMRFaR mutants, as they are called, could only sustain flight for half the time as that of their wild-type siblings.

FMRFaR is a G-protein coupled receptor (GPCR) that sits on the plasma membrane of cells. GPCRs are synonymous to our doorbells, in the sense that they transduce messages from outside to the inside. For example, GPCRs receive signals in the form of hormones, neurotransmitters or other small molecules from the cell's exterior and convert them into appropriate responses within the cell. FMRFaR does exactly this job, but in a very specific class of neurons.

Fruit flies have an elaborate neuronal network in the brain, with different neurons making different signaling molecules to help convey specific types of information to one another. Like humans, they too have a class of neurons that are dopaminergic. These neurons typically synthesize and signal using the molecule called dopamine. In humans, dopaminergic neurons are well known because of their association with Parkinson's disease. Similarly, in fruit flies, behaviors such as locomotion, learning and memory, motivation, have all been linked to proper functioning of the dopaminergic neurons.

Interestingly, the authors found that the FMRFaR was specifically enriched in certain dopaminergic neurons of the fly brain. When FMRFaR levels were reduced in these neurons, the flies were unable to sustain flight for long periods. In fact, genetic experiments allowed the authors to identify that loss of FMRFaR in dopaminergic neurons during the adult stages led to severe loss of flying ability, so much so that the flies could only fly for less than 3 minutes.

The question then is: what signal does FMRFaR generate within these neurons? Like many GPCRs, an inactive FMRFaR can be stimulated by specific peptides from the external environment. This results in the production of a small molecule called Inositol trisphosphate (IP_3) within the cell that diffuses and binds to its receptor partner called Inositol-trisphosphate Receptor (IP_3R). The IP_3R,is on an intracellular compartment called the endoplasmic reticulum, where it functions like an ion channel and releases calcium stored within this compartment. The resulting elevated calcium levels in the cell somehow changes the membrane potential of the neurons, making them active. This process termed 'neuronal excitability' facilitates active neurons to release factors such as neurotransmitters,which are the signaling messengers between neurons.

Loss of FMRFaR hampers this very process of neuronal excitability. To understand this aspect, the authors introduced two different fluorescent proteins, one that reports the levels of cytosolic calcium and another that reports changes in membrane potential.Fluorescent proteins are probes that change fluorescence intensity to reflect changes in levels of molecules of interest. Dopaminergic neurons lacking the FMRFaRshowed reduced ability to respond to a stimulus that would otherwise cause membrane excitability. However, when these neurons were genetically supplemented with a protein that would enhance neuronal excitability, flies lacking the FMRFaR in dopaminergic neurons could fly for moderately longer. These experiments convinced the team that FMRFaR stimulated release of calcium was required in dopaminergic neurons to maintain optimal membrane excitability and thereby flight.

Membrane excitability primarily depends on the function of ion channels that are present on the plasma membrane and that allow influx and efflux of calcium and other ions such as potassium

and sodium. Thus FMRFaR stimulation can directly or indirectly affect membrane excitability by altering the function of these ion channel proteins. To test this idea, the authors introduce us to another molecule called, Calcium-calmodulin dependent Protein Kinase (CaMKII), which is a calcium sensitive proteinthat adds phosphate groups on other proteins to make them either active or inactive. Supplementing FMRFaR deficient dopaminergic neurons with CaMKII ameliorated the flight defect observed in adults. This led the authors to propose that CaMKII is an active participant and functions downstream of the FMRFaR signaling cascade. In fact, genetic and imaging experiments led the authors to believe that CaMKII could be activated upon FMRFaR stimulation in these neurons. Interestingly, inhibition of CaMKII in dopaminergic neurons also led to reduced flight bouts in adult flies.

The authors put forth a model wherein stimulation of FMRFaR in dopaminergic neurons leads to calcium elevation that activates CaMKII. Further down, this could either directly or indirectly influence plasma membrane resident ion channels that are the key regulators of neuronal excitability. Many more questions have sprouted from this new discovery, keeping the authors on the hunt for answers. But one question that is deeply intriguing is: what is the initial trigger that stimulates FMRFaR and where is it coming from? The authors believe that although the peptide, FMRF is known to activate the receptor, the exact neurons that release it or the context in which it is released remains to be identified.

Overall, this study puts FMRFaR at the critical interface of receiving and transmitting information in neurons, thereby enabling the neuron to be in an excited state -a state that enables flies to fly marathons! So then, just like a protein supplement for humans, would providing more FMRFaR genetically help the flies fly even longer? “Well, that is a completely different story for another day!” says Preethi, the lead author of the paper.

This work was conducted by Preethi Ravi, a graduate student, under the guidance of Prof. GaitiHasan at the National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore. This work was also assisted by Dr. DeeptiTrivedi at the Fly Facility, NCBS.
