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GnRH neurons: The return of the rat

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Gonadotropin-releasing hormone (GnRH) neurons are hypothalamic neurons that represent the final output cells of the neural network regulating fertility in all mammalian species (1,2). GnRH neurons migrate from the nose to the brain during early embryogenesis and remain scattered throughout the basal forebrain (3). Most of them send long projections (over 1mm) to the median eminence (ME) at the base of the brain, from where they release GnRH into the pituitary portal system in a pulsatile fashion. One interesting feature of GnRH projections in mice is that they can both receive synaptic inputs and initiate action potentials, which they propagate to the synaptic terminal in the ME. Since they share the properties of both dendrites and axons, mouse GnRH projections have been termed “dendrons” (1).

The pulsatile release of GnRH into the portal circulation is critical for the pulsatile, periodic and cyclic secretion of gonadotropins from the anterior pituitary (i.e. luteinizing and follicle stimulating hormones LH and FSH, respectively), that promote gonadal growth and gametogenesis (1,2,4). The mechanisms behind this exquisite control of the GnRH release are being actively investigated. In mice, a neuronal population located in the arcuate nucleus of the hypothalamus that expresses kisspeptin, neurokinin B and dynorphin (i.e. KNDy neurons) was pinpointed as the initiator of the GnRH pulsatile release (1). Both *in vivo* and *ex vivo* studies in mice confirm that kisspeptin directly stimulates GnRH neuron distal dendrons and terminals, and generates GnRH secretion from the median eminence (1). Until now, GnRH dendrons had only been characterized in mice. In other mammalian species like the Rat, which relies less on olfactory cues to control their reproductive physiology, KNDy neurons are organized differently and send projections to the external zone of the median eminence (5). Whether GnRH dendrons exist in such species remained unknown.

In the current issue of *Endocrinology*, Hoong Yip *et al.* from Alan E. Herbinson laboratory describe for the first time a GnRH-cre transgenic rat (6). The model was generated using a zinc finger nuclease (ZFN) technology, by inserting an Internal ribosomal entry site (IRES) sequence that drives the independent translation of the Cre recombinase, downstream from the translational stop site of the *Gnrh1* open reading frame. Thus, this rat expresses the Cre recombinase under the endogenous transcriptional control of the GnRH1 gene without altering its physiological expression or regulation. In their study, Yip and colleagues present a thorough characterization of the model, showing that GnRH-cre allele is expressed in 94% of the GnRH neuronal population and absent elsewhere. When injecting an adeno-associated virus (AAV) expressing a floxed mCherry sequence into the rostral preoptic area, they further demonstrate that the GnRH-cre allele drives not only specific but efficient recombination of floxed sequences, opening a wide range of possibilities for the study of the GnRH system.

The mCherry membrane labelling of the GnRH neurons also allowed a thorough study of the the GnRH projections in GnRH-cre rats. Their morphology is similar to what was already described for mice and congruent with previous descriptions in rats (5,7). However, the authors demonstrate for the first time the existence of dendron-like structures in the rat GnRH neurons, similar to their mouse counterpart. First, combining mCherry immunofluorescence with immunolabelling, they show that GnRH axon terminals are apposed to synaptophysin-labelled nerve boutons in the external zone of the ME and speculate that the distal projections of GnRH neurons receive synaptic inputs, as suggested previously by electron microscopic studies demonstrating direct contacts between GnRH and KNDy axon terminals in the rat ME (5). Then, using an AAV expressing a floxed calcium reporter (AAV-Flox-GCamp6), they show *ex vivo* that kisspeptin puffs triggers calcium waves in the distal projections of the neurons, recapitulating the findings in mouse GnRH neurons.

There is no doubt that this novel GnRH-cre rat model will be a powerful genetic tool for the study of the molecular pathways involved in the control of GnRH neuronal activity, in a much-awaited non-mouse genetic model. Rat models may finally take their revenge on scientific history: indeed, over the last 20 years there was a switch from rats to mice as the model organism in biomedical science. Although rat models provided invaluable knowledge in the hypothalamic–pituitary–gonadal axis (4),

the possibility of genetic manipulations offered by mice soon outmoded them. Fortunately, recent advances on rat transgenesis will increase the number of rat models available, and prompt discoveries, especially in neuroscience.

Rats are less dependent on pheromones to regulate their fertility and social interactions, and they are incomparably more valuable than mice for the study of cognition and behavior, making them physiologically more similar to humans. Moreover, the bigger size of rat brains will facilitate *in vivo* imaging (e.g. in vivo Calcium imaging through implantation of a miniscope), electrophysiology or microdialysis to study the neural circuits regulated by GnRH. Brain surgery will not only be eased, but also more accurate and less damaging, resulting in smaller regions affected. In addition, stereotactic drug or virus administrations will target more specific anatomical areas. The tighter control of all these techniques is particularly important for preclinical studies in the central nervous system.

Altogether, the GnRH-cre rat model opens up a whole new world of possibilities to improve our knowledge on the hypothalamic physiology. Combining this tool with cre-dependent AAV will not only allow tracking the GnRH neurons, but also silencing or overexpressing genes in these neurons. Crossing the existing GnRH::GFP rat model (7) with the new GnRH-cre model should also facilitate FACS sorting to study gene expression in genetically modified GnRH neurons. Alternatively, GFP can be used as a reporter of the GnRH promoter activity. Using cre-activated Sh-RNA treatments to silence the expression of transcription factors or miRNAs, one may identify the ones able to regulate GnRH reporter activity (8). Eventually this model will reach its maximum potential when new rat inducible genetic models will become available. Until then, let's explore all the possibilities that this new model brings us. New exciting findings on GnRH system are awaiting! Stay tuned!

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