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Selectively Developmental Synapse Elimination

Key Words:
inappropriate synapse pruning, astrocyte, Ca2+ signaling, Itpr2$^{-/-}$

Unwanted synapse elimination, a pivotal part of the early nervous system development, happening in the early postnatal life, critical for the precise formation and refinement of the neuronal circuits and proper brain function, has been studied for decades that how this process takes place. Unfortunately, the underlying mechanism still remains unclear, therefore, more research in this field is required to uncover this mystery mechanism. The purpose of this short review is to summarize the recent progress in this field research and discuss the future research direction.

Introduction

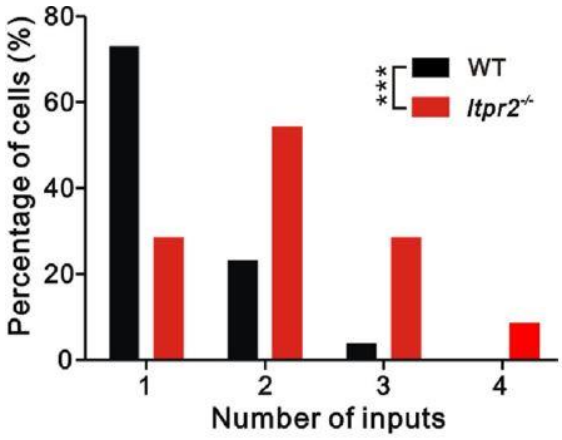
Overproduction of synapses and their subsequent elimination through activity- and experience- dependent processes are critical for refinement of neuronal circuits during development[1]. Disruption of this process is likely to initiate a bunch of neurological diseases, yet, this underlying mechanism of this process is still not clear.

Astrocyte, which has been long viewed to provide structural and nutrient support for the neighboring neurons, however, with more and more evidence suggesting that it plays a significant role in synaptic pruning, communicates with the neighboring neurons via Ca^{2+} signaling to regulate the synapse plasticity and synapse elimination[2]. Gliotransmitters regulated GPCR/IP3R (G-protein-coupled receptor/ inositol 1,4,5-trisphosphate receptor) - Ca^{2+} signalling is generally believed to modulate the neuronal activity. In addition, it has been revealed that IP3R2, exclusively expressed in astrocyte, is of great importance that only by activating of it could initiate the following steps of this pathway and lead to Ca^{2+}concentration increase and further Ca^{2+} signaling[3].

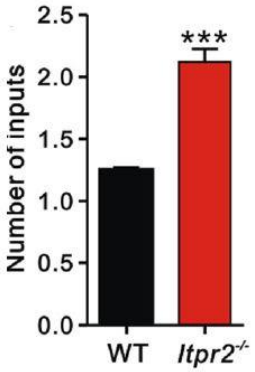
Recent Progress

Yang et al, researchers from Zhejiang University, based on the results of former research, proposed that IP3R2-dependent Ca^{2+} in astrocytes could be involved in the regulating developmental synapse elimination. Via combination of electrophysiological, pharmacological and immunohisochemical methodologies, and using of Itpr2$^{-/-}$ (Itpr2: type 2 inositol 1,4,5-trisphosphate receptor gene) mice model, they found that among these mice, of which the Ca^{2+} signaling was selectively disturbed, their redundant synapse elimination has been heavily impaired. They utilized the whole-cell patch recoding in acute brain slices to examine the developmental synapse elimination. A significant difference in the average number of inputs received by each VPm neuron between WT and Itpr2$^{-/-}$ mice at P16-18 was presented, with the average inputs of Itpr2$^{-/-}$ mice exceeding significantly those of WT and much higher portion of neurons which receive multiple inputs in Itpr$^{2-/-}$ mice (Figure.1), indicated that there were far more synapses in Itpr$^{2-/-}$ mice than WT mice. This result correlates with the immunostaining experiment of VGluT2 (Figure.2).

Furthermore, they found that intracerebroventricular injection of ATP could rescue the impaired synapse elimination in Itpr2$^{-/-}$ mice. Among the Itpr2$^{-/-}$ mice which get the intracerebroventricular injection of ATP, The average inputs value and the portion of neurons which receive multiple inputs both decrease significantly. ATP, one of the very various secreted gliotransmitters from astrocytes, function critically during the process of synapse elimination. The concentration of ATP improve a lot at P18 in WT mice, while in the Itpr2$^{-/-}$ mice, it decreases



(a)



(b)

Figure.1 (a) Distribution of number of inputs that each neuron receives. $\chi 2$ test indicates that there is a significant difference in percentages of cell which receive respective inputs in WT mice and Itpr2$^{-/-}$ mice. (b) Histogram of average number of inputs. The result of unpaired t test indicates that neurons in Itpr2$^{-/-}$ mice, on average, receive markedly higher input than those in WT mice.

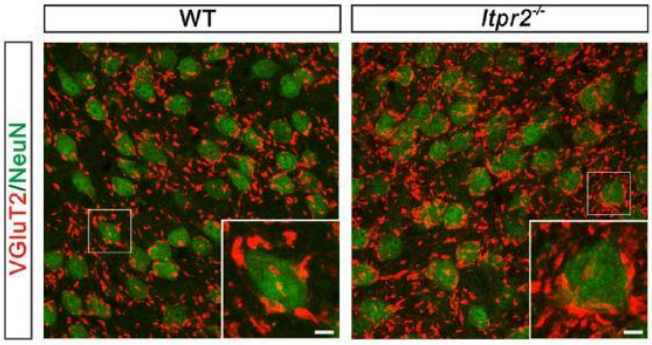


Figure.2 Sample confocal images of immunostained neurons and Pr5 terminals in WT and Itpr2$^{-/-}$ mice. Neurons are stained in green and the terminals are stained in red. Obviously, in Itpr2$^{-/-}$ mice, there are much more terminals.

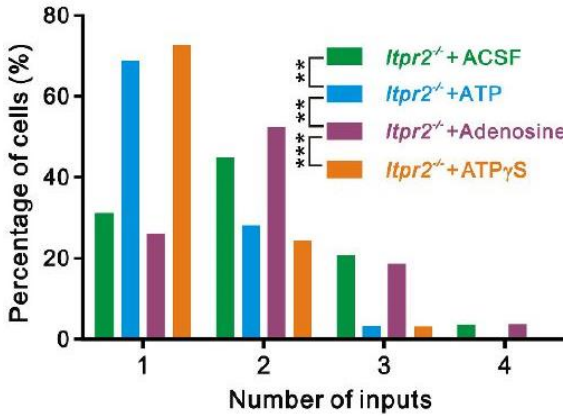


Figure.3 Distribution of the number of input that individual neuron receive. **p<0.01, ***p<0.001, $\chi 2$ test.

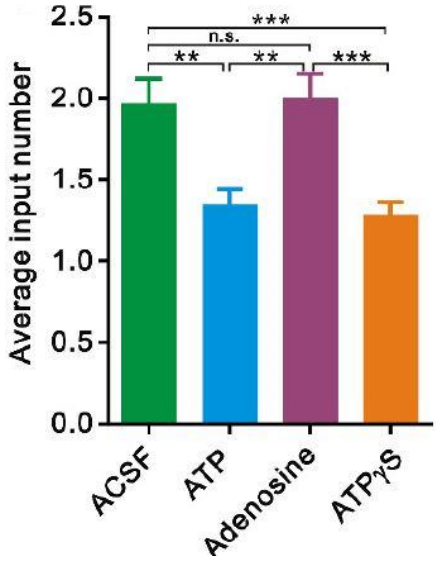


Figure.4 Histogram of average number of inputs received by each VPm neuron in Itpr2$^{-/-}$ mice.***p<0.001 . The one-way ANOVA analysis suggests that in the mice which get injection of ATP and ATPγS, the average of input number decrease significantly..

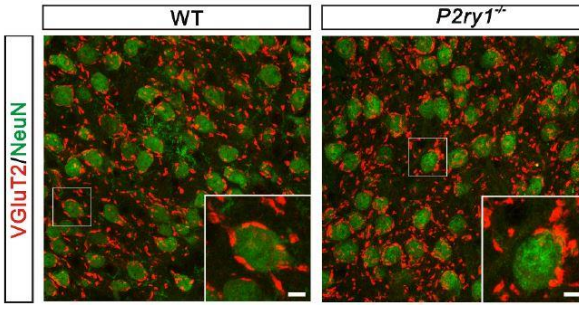


Figure.5 Sample confocal images of immunostained neurons and Pr5 terminals in WT and P2ry1$^{-/-}$ mice. Neurons are stained in green and the terminals are stained in red. Obviously, in P2ry1$^{-/-}$ mice, there are much more terminals, indicating that more synapses are existing.

dramatically due to the impaired Ca^{2+} -dependent manner regulated ATP release. Granted that ATP is easily hydrolyzed, by virtue of utilizing non-hydrolysable ATPγS and the hydrolyzed product adenosine, they find that

ATPγS could repair the impaired synapse elimination, however, adenosine could not, furthering proving that ATP is the substance that could help Itpr2$^{-/-}$ mice to fix the deficiency in the synapse elimination (Figure.3 and Figure.4).

Since researchers detected that ATP concentration was fundamental to the synapse elimination, in the following steps, they did quite a lot experiments to find out how ATP improve the synapse elimination in Itpr2$^{-/-}$ mice. There already is a great volume of literature indicating ATP, thorough activating P2Y or P2X, to modulate the synapse function. Particularly, via activation of P2Y1, which locates in the outer face of the neuron to interact with ATP, could ATP induce a synapse elimination correlated long-term depression (LTD)[1,4]. Most importantly, in P2ry1$^{-/-}$ mice, researchers found that the ATP-induced synapse elimination was impaired as well, with much higher percentage of neurons receiving multiple inputs in P2ry1$^{-/-}$ mice compared to WT mice. In line with the electrophysiological result, experiment of immunostaining for VGluT2 also suggests the deficit synapse elimination in P2ry1$^{-/-}$ mice (Figure.5).

Discussion

With all results above, Yang et al concluded that IP3R could regulate the astrocytes Ca^{2+} signaling, which was responsible for astrocytes ATP release. I feel like that I need to clearly distinguish these two distinct event. Itpr2$^{-/-}$ gene knock out leads to low level of ATP release from astrocytes, resulting in insufficient interaction between ATP and P2Y1, and finally do detriment to synapse elimination mechanism and leave quite a lot redundant synapse in early nervous system development. They proposed that activity-dependent competition for synapse elimination involves both postsynaptic neuronal activation and astrocytic Ca^{2+} elevation[5]. As stated before, GPCR-IP3R-dependent Ca^{2+} signaling is critical pivotal for astrocytes to release gliotransmitters and modulate synaptic transmission, Ca^{2+} is indisposable for selectively synapse elimination.

In the very beginning, ATP concentrations in 2 WT and Itpr2$^{-/-}$ mice are pretty similar, after having developed for several days, ATP concentrations in the 2 models start to differentiate and initiate disparate downstream effects. Lack of IP3R would not influence the very early development, however, could impair the later synapse elimination.

Though the outcomes of Itpr2$^{-/-}$ and P2ry1$^{-/-}$ are extremely similar, that does not make sense that these two events have to take place simultaneously. Itpr2$^{-/-}$ could lead to low level of ATP release, nonetheless, P2ry1$^{-/-}$ would make astrocytes fail to respond to the ATP concentration change.

Though Yang et al have directly proved that astrocytes contributed to synapse elimination in an IP3R-dependent manner through activation of ATP-activation, there exist some other Ca2+ signaling pathways in astrocytes which do not rely on IP3R, still work in the Itpr2$^{-/-}$ mice[6]. Thereby, we could exclude the possibility that other Ca^{2+} signaling pathways could attribute to the proposed synapse elimination mechanism. All the more so, P2Y1 receptors have also been reported to be expressed in the microglia and the ATP activates microglia, so the deficit synapse elimination could be explained by microglia-mediated synapse elimination. Luckily, in their Itpr2$^{-/-}$ models, Yang et al found the P2Y1 receptor protein level in microglia was approximately zero, but they still could not utterly exclude the chance that microglia might contribute synapse elimination through P2Y1 receptors. A more precise model with cell-type specific knock out is demanded to solve this problem.

References

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