Video Article

Live Images of GLUT4 Protein Trafficking in Mouse Primary Hypothalamic Neurons Using Deconvolution Microscopy

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Abstract

Type 2 diabetes mellitus (T2DM) is a global health crisis which is characterized by insulin signaling impairment and chronic inflammation in peripheral tissues. The hypothalamus in the central nervous system (CNS) is the control center for energy and insulin signal response regulation. Chronic inflammation in peripheral tissues and imbalances of certain chemokines (such as CCL5, TNFα, and IL-6) contribute to diabetes and obesity. However, the functional mechanism(s) connecting chemokines and hypothalamic insulin signal regulation still remain unclear.

In vitro primary neuron culture models are convenient and simple models which can be used to investigate insulin signal regulation in hypothalamic neurons. In this study, we introduced exogeneous GLUT4 protein conjugated with GFP (GFP-GLUT4) into primary hypothalamic neurons to track GLUT4 membrane translocation upon insulin stimulation. Time-lapse images of GFP-GLUT4 protein trafficking were recorded by deconvolution microscopy, which allowed users to generate high-speed, high-resolution images without damaging the neurons significantly while conducting the experiment. The contribution of CCR5 in insulin regulated GLUT4 translocation was observed in CCR5 deficient hypothalamic neurons, which were isolated and cultured from CCR5 knockout mice. Our results demonstrated that the GLUT4 membrane translocation efficiency was reduced in CCR5 deficient hypothalamic neurons after insulin stimulation.

Video Link

The video component of this article can be found at https://www.jove.com/video/56409/

Introduction

Type 2 diabetes mellitus (T2DM) is a global health crisis. T2DM is characterized by insulin signaling impairment and chronic inflammation in peripheral tissues. The hypothalamus is the control center which regulates the body's energy homeostasis, appetite, and circadian rhythms. Most importantly, the hypothalamus also mediates insulin signal responsiveness to regulate systemic metabolism[1,2,3,4,5]. Disrupting the hypothalamic insulin signaling pathway could induce insulin resistance[6,7]. The hypothalamus coordinates cellular energy status and secretion of hormones, such as insulin and adipokines (*e.g.*, leptin) from the peripheral tissues, to regulate systemic glucose metabolism, insulin responsiveness, and food intake. Insulin binding to the insulin receptor activates insulin receptor substrate (IRS) proteins, which then activate insulin downstream signaling molecules, such as PI3K (phosphatidylinositol 3-kinase) and AKT (protein kinase B (PKB/AKT)), to induce GLUT4 membrane translocation. Neurons are not the major target for glucose uptake in response to insulin; however, significant levels of GLUT4 expression have been identified in the hypothalamic arcuate nucleus (ARC) region. Therefore, the regulation of GLUT4 in hypothalamic neurons may play an important role in insulin signaling in the brain-peripheral axis.

Many studies have suggested that chronic inflammation and inflammatory chemokines in hypothalamus also play an important role in the development of diabetes and obesity, and inhibition of hypothalamic inflammation can reverse diet-induced insulin resistance[8,9,10]. Moreover, chemokine-CCL5 (C-C motif ligand 5, also known as RANTES, Regulated-on-Activation-Normal-T-cell-Expressed-and-Secreted) and its receptor CCR5 levels also correlate with the development of T2DM[11,12]. The roles of CCL5 and CCR5 in insulin function and glucose metabolism remain unclear. One study reported that CCR5 deficiency protected mice from obesity-induced inflammation, macrophage recruitment, and insulin resistance[11]; in contrast, another study reported that CCR5 deficiency impairs systemic glucose tolerance, as well as adipocyte and muscle insulin signaling[12]. CCL5 is found to increase glucose uptake in T-cells and to reduce food intake through its action on the hypothalamus[13,14], however, both the mechanism of action and the receptors involved are yet to be identified.

It is difficult to study the cellular mechanisms underlying the effect of peripheral tissue inflammation on insulin functioning in hypothalamic neurons. This is due to cellular heterogeneity and neuron circuit feedback regulations. For this reason, an *in vitro* cell culture model provides a clean model to investigate the effects of the chemokine on hypothalamic insulin signal regulation. Although there are many established immortalized hypothalamic neuronal cell lines for research use, these cell lines expressed different markers, and therefore, represent different types of hypothalamic neurons[15]. Even though primary hypothalamic cultures can be difficult to maintain, they can provide the most realistic response of hypothalamic neurons upon insulin stimulation, and can also avoid the potential unknown effects which come into play when maintaining cells long-term in culture medium with artificial growth factors.

Herein, we utilize primary hypothalamic neurons from both C57BL/6 wildtype (WT) mouse and CCR5 knockout ($CCR5^{-/-}$) mouse, and transfect both types of cells with GFP-GLUT4 construct. To investigate the contribution of CCR5 to insulin mediated GLUT4 membrane trafficking, GFP-GLUT4 transfected neurons were treated with insulin or recombinant CCL5. We then characterize the movement of GFP-GLUT4 on the plasma membrane in primary hypothalamic neurons with Deconvolution Microscopy.

Protocol

All the protocols and methods used in animal subjects have been approved by Institutional Animal Care and Use Committees (IACUC) of Taipei Medical University (Protocol numbers: LAC-2013-0278; LAC-2015-0397)

1. Primary Neuron Culture

1. **Preparation before culture**
    1. Coat the culture dishes with poly-D-Lysine (**Table 1**) one day before culture. For a 6-well dish, add 1.5 mL poly-D-Lysine (0.05 mg/mL) into each well. For live-imaging, culture cells on a 12 mm x 12 mm coverslip in a standard coated 6-well plate.
    2. Remove/recycle the poly-D-lysine and wash the dishes twice with 2 mL ddH_2O.
    3. Prepare the surgical tools: a pair of micro-dissecting scissors, curved-tipped forceps, and standard straight-tipped forceps. Sterilize the surgical tools and keep them in 75% ethanol during surgery.
    4. Fill 10 cm petri dishes with 20 mL wash medium (**Table 1**) and keep on ice.
    5. Fill a 15 mL test tube with 15 mL wash medium and keep the tube on ice.

2. **Brain tissue isolation from different brain regions**
    1. Sacrifice 16-19 day old pregnant female mice with standard euthanization protocols by placing mice in a non-ventilated cage and then intoxicating with CO_2. E15.5~E16.5 embryos are recommended for hypothalamic neuronal culture and E16.5~E17.5 are more suitable for hippocampal and cortical neuronal culture.
    2. Sterilize the platform, dissecting microscope, and surgery tools with 75% ethanol to avoid contamination.
    3. Cut around the umbilical cord area (dark red area, **Figure 1A, 1B** dash line) to isolate the pups easily without damaging them (**Figure 1C, 1D**).
    4. Remove the head portion of each pup with a pair of scissors (**Figure 1E, F**), then secure the head portion in place using the fine-tipped straight forceps for the eye region (**Figure 1G**). Use another fine-tipped curved forceps to remove the outer skin and skull from two sides and peel them off from the anterior to the dorsal direction (**Figure 1H**, black arrow points to the direction). The brain should be isolated without any damage (**Figure 1I**).
    NOTE: It is essential to maintain the integrity of the brain to ensure accuracy when isolating different regions of the brain.
    5. Keep isolated brains in a clean petri dish with ice-cold wash medium for the following steps.
    6. Flip the brain and keep the ventral side up. The hypothalamus is a round structure in the middle of the brain (**Figure 1J**, arrow points to the hypothalamic region). Isolate the hypothalamic tissue with fine-tipped curved forceps. Remove the meninges (which have a yellowish-red color) carefully.
    CAUTION: Complete removal of meninges is a critical step to avoid fibroblast contamination.
    7. Hold the olfactory lobe with the sharp forceps and peel off the thin meninges surrounding the whole brain with the curved forceps (**Figure 1K, L**).
    8. The hippocampus is a banana-like shape which is embedded in the lower part of the cortex. Flip to the underside of the cortex and separate the hippocampus from the cortex by pulling it to the side with the curved forceps (**Figure 1M, N,** and **O**). Be sure to remove all the remaining meninges surrounding the brain tissue.
    9. When all regions of the brain have been isolated, chop these tissues in the 15 mL tubes containing ice-cold wash medium with the help of the forceps (step 1.1.5).
    NOTE: Brain tissues can be kept in the ice-cold wash medium for 2-3 h.

3. **Tissue digestion, plating and culture**
    1. Rinse the tissues by inverting the tissue-containing 15 mL tube 2-3 times, and then keep the tube straight to allow the tissues to settle down (1-3 min).
    2. Remove the upper medium using the glass Pasteur pipette attached to a vacuum system. To wash the tissue, add 15 mL ice cold wash medium and invert the tube 2-3 times. Repeat wash steps 3 times before the next step.
    CAUTION: Be cautious of the tissues on the bottom when removing the upper medium using a vacuum system.
    3. After the final wash, remove the wash medium with the help of a 1 mL pipette.
    4. Incubate tissues with Papain-Trypsin digestion buffer (**Table 1**) in a 37 °C water bath for 7-14 min. Shake the tubes every two min to ensure all tissues are properly exposed to the digestion buffer.
    NOTE: Incubation time and the volume of digestion buffer can be adjusted based on the tissue size. For hypothalamic tissue collected from 6-8 pups, 300 µL Papain-Trypsin digestion buffer and 7 min incubation time is recommended. More tissue will require more digestion buffer.
    5. Neutralize the enzyme's activity by adding 1 volume of fetal bovine serum. Shake the test tube 3-5 times at room temperature.
    6. Keep the tube on racks and wait for 1-2 min to allow the tissues to settle down; remove the supernatant carefully with a 1 mL pipette.

7. Add 6 mL plating medium (**Table 1**) into the test tube and pipet the tissue up-and-down 50 times with a 5 mL pipette gently (for a 6-well plate, 1.5 mL plating medium per well is recommended). Most cells will dissociate into single cells after repeated pipetting.
   NOTE: Try to avoid the formation of bubbles while conducting this step. Most laboratories use flamed glass pasture pipets to dissociate the tissue. A 5 mL pipette with a narrow tip can be a good alternative for this step.
8. Keep a tube on the rack and wait for 1-2 min to allow the chunky, un-dissociated tissues to settle down. Take the upper phase containing dissociated cells to a new tube and dilute with plating medium (5x volume plating medium per 1x volume of dissociated cells. For example, add 5 mL plating medium for 1 mL dissociated cells).
9. Calculate the cell density using a hemocytometer and seed the required number of cells into the culture plate coated with poly-D-lysine as in step 1.1.1. We recommend 2-4 x 10^5 cells per well for a 6 well dish/35 mm dish were recommended for neuron imaging studies. A higher density would be needed for protein collection and analysis.
   NOTE: Do not add too much plating medium, 1-1.5 mL plating medium in one 6-well dish is sufficient for attachment. An excessive amount of medium will prolong the time required for proper cell attachment.
10. Wait for 2-3 h and check the seeded neurons under the microscope. Neurons will start to grow neuritis when they attach properly.
11. Remove the plating medium and add 2 mL warmed wash medium (37 °C) into the dish to wash off the plating medium. Repeat this step twice.
12. Add 2 mL complete culture medium (**Table 1**) into each well in the 6-well dish.
13. Change half of the medium every 3-4 days. Prepare the complete medium freshly each time. Neurons cultured from different mouse brain regions will have different morphology and characteristics (**Figure 2**).

2. Transfection of Plasmid DNA into Primary Neuron with Liposome System

CAUTION: For neuron transfection, an endotoxin-free plasmid DNA purification kit (**Materials Table**) is recommended for DNA preparation. Additional ethanol precipitation can remove excess solvent and enhances DNA concentration.

1. **Liposome-based DNA transfection in primary neurons.**
   NOTE: The transfection time depends on the maturation status required in experiments. The hypothalamic neurons were transfected at DIV 7-10 (DIV, days *in vitro*).
   1. DNA: Liposome mixture preparation: Dilute GFP-GLUT4 plasmid DNA[16] (2 µg) in a microcentrifuge tube containing 300 µL low serum culture medium. Prepare another microcentrifuge tube with 2 µL liposome in 300 µL low serum culture medium, and incubate for 5 min at room temperature.
   2. Combine the contents of both tubes and incubate for 20-30 min at room temperature.
   3. Remove the neuron culture medium from the culture dish. Add 600 µL DNA-liposome mixture into each well and incubate at 37 °C for 4-6 h.
   4. After 4-6 h, remove DNA-liposome mixture and add 2 mL culture medium.
   5. Fluorescent signals, such as GFP alone and GLUT4 protein conjugated with GFP tag (**Figure 3** and **Figure 4**), can be observed after 18-72 h.

3. Live Image Recording

1. **Preparing Cells for Live Imaging**
   1. Culture WT and CCR5$^{-/-}$ hypothalamic neuron cells expressing green fluorescence protein labeled glucose transporter 4 (GFP-GLUT4) on the #1.5 (or 0.17mm thickness) glass coverslip which has been pre-coated with poly-D-Lysine in step 1.1.1 in the 6-well plate.
   2. Carefully remove the coverslips with neurons using forceps and place it on the glass slides with caution.
   3. Remove excess medium/liquid with delicate task wipes. Fold the wipes twice, and carefully place them on top of the coverslip. Gently press down the wipes without moving the coverslip.
      CAUTION: Do not press the coverslip against the glass slide forcefully. The purpose of this step is to ensure that the coverslip does not move/drift during image acquisition caused by buoyant force.
   4. Place coverslips and slides on the deconvolution microscope stage, with the coverslip facing down, and secured properly. This step is to ensure that the slide position remains constant so users can track the same set of target cells later.
   5. Observe WT and CCR5$^{-/-}$ hypothalamic neuron cells with a 60x/1.42 NA oil immersion objective lens.
   6. Treat the selected cell samples with CCL5 (10 ng/mL) or insulin (10 U/mL) for one min. Add 1.5 µL of diluted insulin on the edge of coverslip.
   7. Visualize and record the selected cell samples immediately. Program the video recording software to record for 30 min.

2. **Deconvolution microscopy and analysis**
   NOTE: This part of the protocol requires the use of a deconvolution microscope and specialized software for analysis.
   1. Turn on the power of the imaging system, allow microscope stage to initialize properly, and then turn on the LED light source.
   2. Add immersion oil (refractive index 1.520 for live samples at 37 °C) on a 60x 1.42 NA objective lens. Place the sample slide on the microscope with the coverslip facing toward the objective lens, and secure the slide properly.
   3. Use bright-field or fluorescence illumination to identify target cells. Adjust the focus until target cells can be clearly observed. Do not move the objective lens outside of the coverslip area to avoid unnecessary scratch marks.
   4. Identify green fluorescence protein conjugated Glucose Transporter 4 (GFP-GLUT4) by the GFP signal. Identify desired target cells for image acquisition. Selected target cell position can be memorized for future reference (**Figure 4**).
   5. Setup proper experimental parameters (including pixel number, excitation wavelength, transmission percentage, exposure time, stack thickness, time interval, and total imaging time) on each target cell. For this experiment, the image pixel number was set at 512 x 512 (it can be set at 1,024 x 1,024 for higher resolution) for GFP signals. The exposure time was set between 0.025 to 0.05 s for every 5 min. Minor lateral x, y, and z adjustments can be controlled by the recommended software (**Materials Table**).

6. Set the exposure parameter to approximately 2,000 to 3,000 counts to achieve maximum pixel intensity. To minimize fluorescence photobleaching, reduce the percentage of excitation light transmission as much as possible while keeping exposure time less than 1 s. Repeat these steps for each additional fluorescence channel(s) and each individual area of interest.
7. Set the upper and lower limit of the Z-stack on each target cell. This can be achieved by moving the microscope stage until the top and bottom of the target cell are both slightly out of focus. The users can adjust the resolution of the Z-axis by setting the number of images between the upper and lower limit (which can be done by setting the distance between each image).
8. Stacks of images were deconvolved and later analyzed with the help of the respective software – Velocity from PerkinElmer in this case.

Representative Results

The hypothalamic neurons cultured from mice were further identified by immunostaining with hypothalamic specific protein - pro-opiomelanocortin (POMC) antibody and neuronal marker - microtubule-associated protein 2 (MAP2) (**Figure 2A**). We confirmed the primary cultured hypothalamic neurons expressed hypothalamic protein POMC. The expression of the CCR5 receptor and CCL5 in hypothalamic neurons were identified with specific antibodies and co-labeled with POMC antibody (**Figure 2A, 2B**).

After 3 days of culturing, neurons were transfected with GFP DNA (**Figure 3**) or GFP conjugated GLUT4 (**Figure 4**). GFP expression can usually be found all over the cell without a specific pattern (**Figure 3**) but the GLUT4-GFP will express as a punctate-like structure in the cytosol (**Figure 4**). The transfection kit used in this study is not the most efficient method for neuron transfection; however, it is a less stringent method for better cell survival after transfection, which contributes to better live-cell imaging/recording later. The images of GFP-GLUT4 expressing neurons were taken before time-lapse movies (**Figure 4A-B**, supplementary video 1, 2) upon insulin stimulation or CCL5 stimulation (**Figure 4C**, supplementary video 3). The signals of GFP and GFP-GLUT4 are clear and strong in neurons.

Hypothalamic neurons with GLUT4-GFP transfection were further treated with insulin (40 U) to characterize GFP-GLUT4 trafficking. Reprehensive videos of GLUT4-GFP movement upon insulin stimulation in both WT and $CCR5^{-/-}$ hypothalamic neurons are shown as Video 1 and Video 2, respectively.

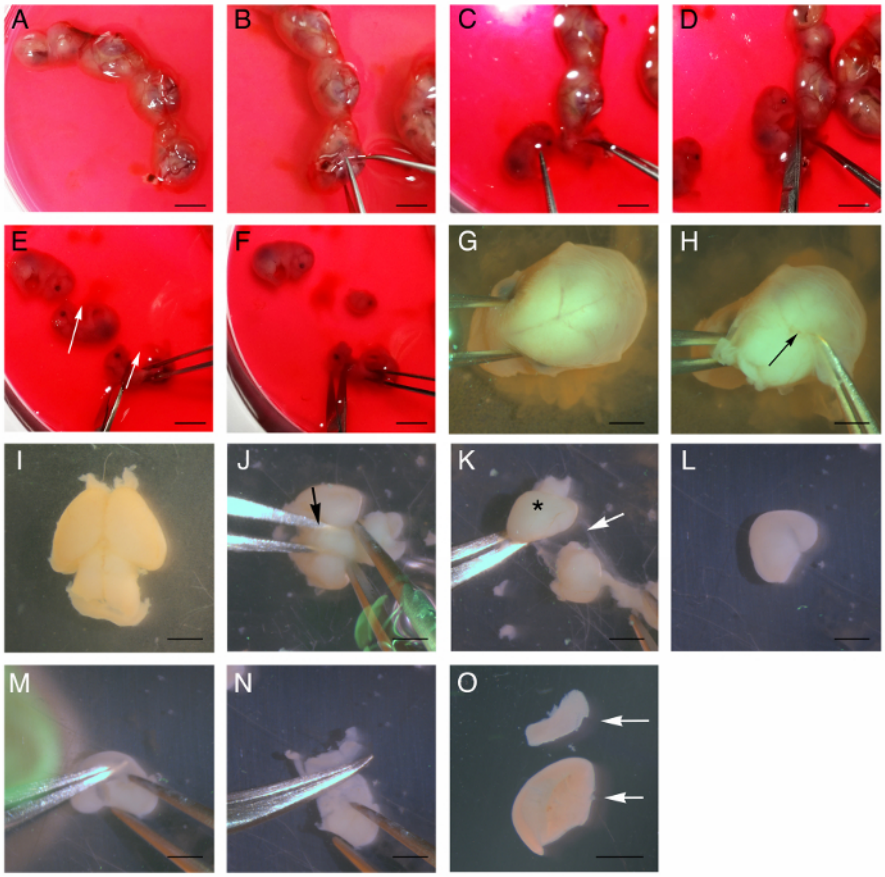

Figure 1: Isolation of tissues from different regions of the mouse brain at the embryonic stage (day 16.5). (**A-D**) The steps involved in the separation of pups from the placenta. (**E, F**) The dissection of a pup head from the body. (**G-I**) The steps involved in the isolation of the whole brain from the skull. The black arrow points in the direction to be pulled while removing the skull using forceps. (**J**) The isolation of the hypothalamus. The black arrow points to the hypothalamic region between forceps. (**K-L**) Isolation of the cortex of the mouse brain. The black asterisk indicates the cortical region of the mouse brain and the white arrow points to the separation of the cortex from the whole brain. (**M-O**) The separation of the hippocampal portion from the cortex. The upper white arrow marks the hippocampal tissue and the lower white arrow marks the cortical tissue. Scale bars = 1 cm (A-F), 200 µm (G-O). Please click here to view a larger version of this figure.

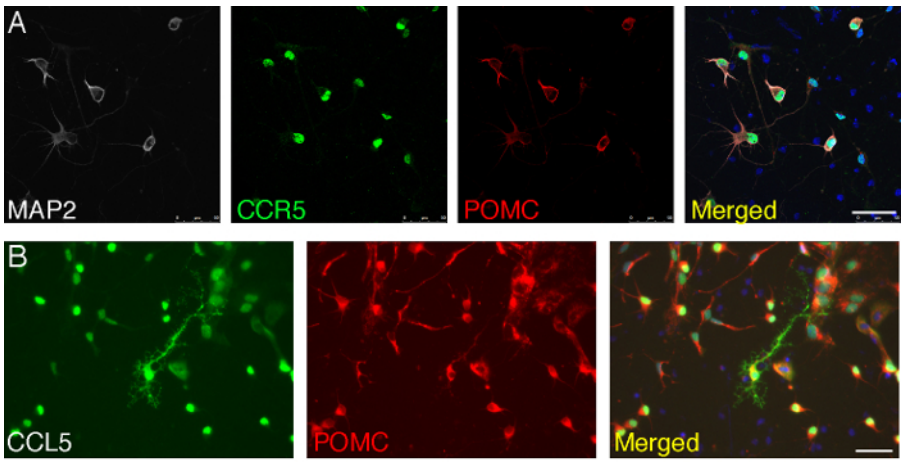

Figure 2: Characterization of hypothalamic neuronal marker - POMC and the co-expression of CCL5 and CCR5. (**A**) Primary cultured hypothalamic neurons were labeled with hypothalamic neuronal marker - POMC (red), the co-expression of CCR5 (green), and neuron marker MAP2 (gray). (**B**) The CCL5 (green) expression in POMC (red) positive hypothalamic neurons (Adapted from the supplementary data of reference[17]). Here, DAPI labeled the nucleus with blue color. Scale bars = 50 µm in (A) and (B). Please click here to view a larger version of this figure.

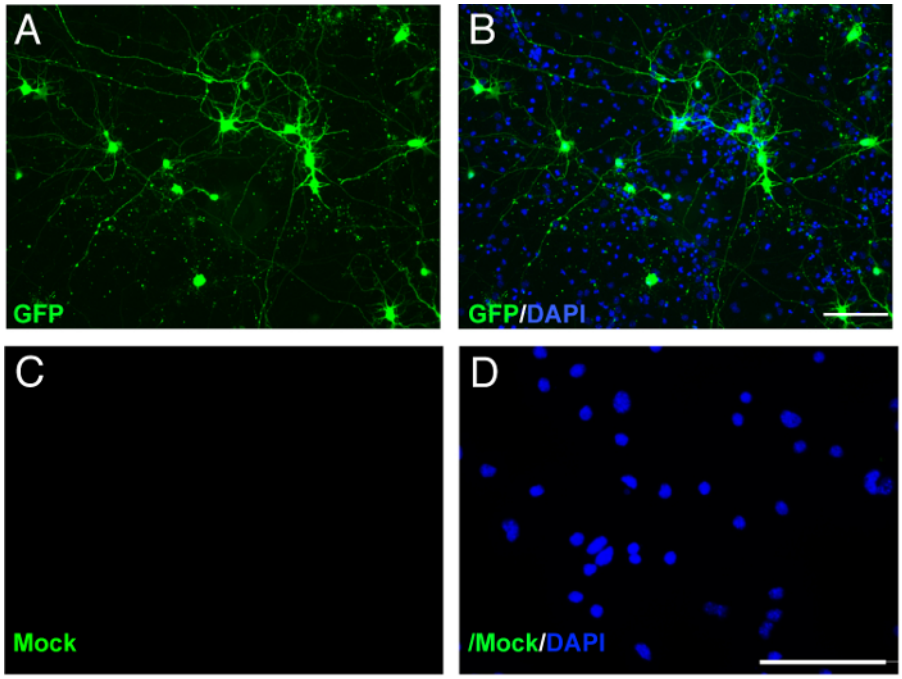

Figure 3: GFP protein expression in mouse primary neurons. GFP plasmid DNA transfected into primary cultured neurons after 4 days culture (DIV4) with liposome and expressed for another 3 days (DIV7). (**A-B**) GFP is expressed in both neuritis and soma. (**C-D**) Neurons with mock transfection; DAPI labeled the nucleus in (**B, D**). Scale bars = 100 μm. Please click here to view a larger version of this figure.

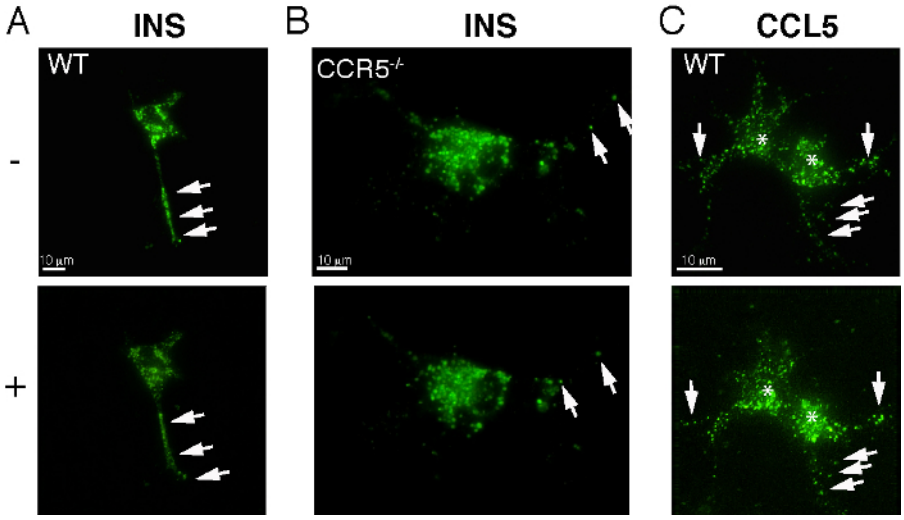

Figure 4: The snapshots of GFP-GLUT4 in hypothalamic neurons. (**A, C**) GFP-GLUT4 protein expressed in Wildtype (WT) hypothalamic neurons and (**B**) CCR5$^{-/-}$ hypothalamic neurons. Neurons were stimulated with insulin (**A, B**) or CCL5 (**C**). The arrows point to the GFP-GLUT4 punctate in the neuritis before (-) and after (+) insulin or CCL5 stimulation and asterisks point to the surface GLUT4-GFP before (-) and after (+) CCL5 stimulation in (**C**)**.** (Figure adapted from reference[17]). Please click here to view a larger version of this figure.

5x Borade Buffer	**Company**	**Catalog Number**	**Volume**
Boric Acid	Sigma-Aldrich	B6768	1.55 g
Borax	Sigma-Aldrich	71997	2.375 g
ddH2O			100 mL
			Filtered, Keep at 4 °C
20x Poly-D-Lysine stock	**Company**	**Catalog Number**	**Volume**
Poly-D-Lysine	Sigma-Aldrich	P6407	100 mg
ddH2O			100 mL
			Filtered, Keep at -20 °C
1x Poly-D-Lysine			**Volume**
20x Poly-D-Lysine			5 mL
5x Borade Buffer			20 mL
ddH_2O			75 mL
Total			100 mL
			Keep at 4 °C
Wash Medium	**Company**	**Catalog Number**	**Volume**
DMEM-High glucose	Gibco	12800-017	495 mL
Antibiotic-Antimyotic	Gibco	15240-062	5 mL
Total			**500 mL**
			Keep at 4° C
Papain-Trypsin digestion buffer:	**Company**	**Catalog Number**	**Volume/Final Concentration**
Papain (10 mg/mL)	Sigma-Aldrich	P4762	200 µL (2 mg/mL)
Trypsin-EDTA (0.25%)	Gibco	25200-072	200 µL (0.05%)
Wash Medium			600 µL
Total			**1,000 µL**
			Keep at -20 °C
Plating medium:	**Company**	**Catalog Number**	**Volume**
Neurobasal medium	Gibco	21103-049	176 mL
Fetal Bovine Serum	Gibco	10437-028	20 mL
L-glutamate (200 mM)	Gibco	25030	2 mL
Antibiotic-Antimyotic	Gibco	15240-062	2 mL
Total			**200 mL**
			Keep at 4 °C
Complete culture medium	**Company**	**Catalog Number**	**Volume**
Neurobasal medium	Gibco	21103-049	95 mL
N2 supplement (100x)	Gibco	17502-048	1 mL
B27 supplement (50x)	Gibco	17504-04	2 mL
L-glutamate (200 mM)	Gibco	25030	1 mL
Antibiotic-Antimyotic	Gibco	15240-062	1 mL
Total			**100 mL**
			Freshly prepared

Table 1: Digestion buffer and media composition used in this study.

Supplementary Video 1: Insulin stimulated GFP-GLUT4 movement in WT hypothalamic neurons. Please click here to download this file.

Supplementary Video 2: Insulin stimulated GFP-GLUT4 movement in CCR5$^{-/-}$ hypothalamic neurons. Please click here to download this file.

Supplementary Video 3: CCL5 stimulated GFP-GLUT4 movement in WT hypothalamic neurons (Video adapted from reference[17]). Please click here to download this file.

Discussion

The ability to monitor live cells, upon CCL5 or insulin stimulation, is critically important for studying the rapid effect of CCL5 or insulin on GLUT4 movement. In fact, it allows us to visualize the significant difference between WT and CCR5$^{-/-}$ hypothalamic neurons upon insulin stimulation. We have performed the surface labeling of endogenous GLUT4 protein in WT and CCR5$^{-/-}$ hypothalamic neurons at different time points after insulin stimulation[17]. The labeling of cell surface proteins requires high-specificity antibody with low background. In addition, surface fluorescence quantification can also be challenging and time consuming. Thus, time-lapse recording allows us to be certain that the effect of CCL5 or insulin is a true physiological change based on experimental conditions, rather than a statistical variation. Together with surface labeling of endogenous GLUT4, we provide strong evidence and experiments to demonstrate how CCL5 and CCR5 participate in GLUT4 translocation and insulin signaling.

In modern cell biology and molecular biology studies, many experiments require the utilization of fluorescence microscopy. This technology allows scientists to visualize the spatial relationship between proteins and/or cellular organelles, in addition to movement direction and speed, stimulatory effects, morphological changes, and protein trafficking. However, this technology still has its limitation: when fluorophores are excited, signals emitted from the target protein (or area) can be overwhelmed by background fluorescence. As a result, fluorescence images can appear blurry with expected signals buried deep into background signals. This phenomenon is especially apparent for the observation of membrane-bound proteins.

Total Internal Reflection Fluorescence Microscopy (TIRFM) was developed to overcome this difficulty. It allows scientists to visualize the excitation of selected surface-bound fluorophores without affecting the background fluorophores. It allows scientists to selectively characterize features and events on a very thin surface region such as a plasma membrane. Deconvolution microscopy is a computationally intensive image processing technique that is made possible with the help of technological advancements in recent years. It has been frequently utilized to improve digital fluorescence image resolution. As mentioned previously, when fluorophores are being excited by any type of illumination (such as laser or LED), all fluorophores will emit light signals regardless if they are in focus or not, so the image will always appear blurry. This blurring is caused by a phenomenon called "Point Spread Function" (PSF), as light coming from a small fluorescent source (bright spot) will spread out further and become out of focus (blur). In principle, this event will produce an hourglass-like shaped fluorescent signal, and a fluorescence image can be made up of numerous such light signals. The deconvolution process can reassign all the fluorescence signals to its original bright spot form, and eliminate most of the out-of-focus light to improve image contrast.

In recent years, deconvolution algorithms have generated images with comparable resolution to that of a confocal microscope. Moreover, in comparison with TIRFM, which prevents out-of-focus blur from being detected by a limited excitation region, wide-field microscopy allows all light signals to be detected and reassigns them back to their source through the deconvolution process. Therefore, in practice, deconvolution microscopy has become not only a more efficient image acquisition method, but also a more cost-effective method when compared to TIRF microscopy.

Disclosures

The authors have nothing to disclose.

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