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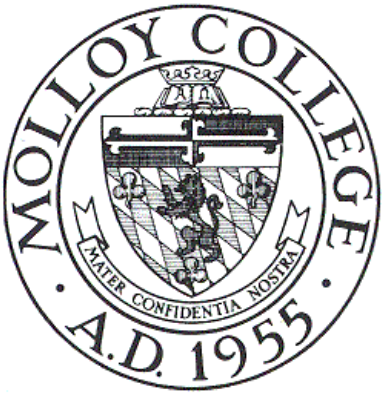

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In This Issue:

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Abnormal GLT-1 Trafficking in Alexander Disease Astrocytes, as shown by Total Internal Reflection Fluorescence Microscopy

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Abstract

Mutations in the gene for glial fibrillary acidic protein GFAP, which encodes the major intermediate filament protein of astrocytes, result in Alexander Disease (AxD), a fatal neurological disorder in which myelin and neurons are lost. The uptake of glutamate (Glu) via the major glutamate transporter (GLT-1) of astrocytes is important for controlling the extracellular concentrations of Glu, thus limiting Glu-mediated toxicity to other cell types. Previous work has defined a loss of GLT-1 in AxD and in mouse models of AxD. Effective. Glu uptake requires a high density of GLT-1 transporters localized to the plasma membrane, but the direct visualization of GLT-1 trafficking in astrocytes has been a major challenge. Here, we describe an optical imaging approach, based on total internal reflection fluorescence, to examine the dynamic remodeling of RFP-GLT-1 fusion protein at the cell surface of live astrocytes on a timescale of minutes. Quantification of the density, size and morphogenesis of RFP-GLT-1 proteins in astrocytes expressing the R239C GFAP mutant revealed a significantly reduced number, size and motile redistribution of GLT-1 proteins compared to the wild type, particularly at transient, spine-like membrane protrusions of the R239C astrocytes. This suggests dysfunctional Glu transport may be due, in part, to a defective membrane organization of GLT-1. Our study provides the first structural insights into the intercellular mechanism underlying an astrocytic encephalopathy caused by a mutation in GFAP that compromises survival of their neighbors.

Introduction

Alexander Disease (AxD), caused by mutations in the *GFAP* gene encoding an astrocyte-specific intermediate filament protein, is a progressive and fatal neurodegenerative disorder. Astrocytes accumulate massive amounts of protein in inclusions (Rosenthal fibers) and survive in a state of chronic oxidative stress[1, 2, 3]. Yet the neuropathology of AxD also shows a loss of oligodendrocytes and myelin and a variable loss of neurons, cells that do not express *GFAP*. Thus, an interesting unsettled question is how dysfunctional astrocytes in AxD cause the death of other cells in the CNS.

Up to 80% of synaptic transmission in brain is mediated by Glu, and low extracellular levels of Glu are essential for appropriate excitatory signaling as well as for limiting an excessive activation of Glu receptors, which can cause excitotoxic neuronal death[4]. Studies of a family of high affinity, Na+-dependent Glu

transporters in the CNS have demonstrated an important role for the astrocyte Glu transporter protein GLT-1 as an endogenous intercellular regulator of glutamatergic synapses[4,5]. The translocation of GLT-1 from subcellular compartments to the cell surface, facilitating active transport of Glu over the cell membrane of astrocytes, is the predominant route for removal of Glu released into the synaptic cleft[6]. Malfunctions of GLT-1 have been implicated in a variety of neurological diseases, including ALS, ischemia, epilepsy, and trauma[7]. Our previous work has defined a loss of GLT-1 in AxD and in a mouse model of AxD, the GFAP-R236H knock-in mice (equivalent to R239C mutation in human AxD). In cultured astrocytes, the overexpression of the R239C GFAP mutation results in a significant decrease in GLT-1 protein and an attenuation of Glu uptake, measured by patch clamping[8].

The relative amount and pattern of GLT-1 organization at the plasma membrane not only reflects a regulated balance between membrane insertion and endocytic removal, but also directly relates to functional perturbations[9,10,11]. To gain insight into the details of this essential process, we applied total internal reflection fluorescence microscopy (TIRFM) to acquire high-resolution images recording the movement of RFP-GLT-1 proteins at or near the plasma membrane of live astrocytes. A detailed quantification of spatial and temporal dynamics of RFP-GLT-1 organization, revealed robust motility and membrane remodeling of RFP-GLT-1 clusters, particularly at sites of transient, retractable spine-like protrusions, in astrocytes expressing WT *GFAP.* In contrast, astrocytes expressing the R239C *GFAP* exhibited less motility of small RFP-GLT-1 particles with little or no transition to large clusters as well as a significantly reduced motion at membrane protrusions. Our data suggests a structural explanation for a dysfunctional GLT-1 protein in astrocytes of AxD and further substantiates the hypothesis that disrupted Glu uptake is one of the causes of neuronal death and demyelination in AxD.

Material and Methods

Cell Culture and Transfection

The human astrocytoma cell line (U251) stably expressing WT and R239C *GFAP* (tagged with GFP) was maintained in DMEM (Sigma Chemical Co.) with 10% FBS at 37°C in a 5% CO_2 incubator. Cells were plated on acetone-cleaned coverslips, which had a refractive index of 1.78 (Olympus America Inc.), and had been coated with 0.2% gelatin to promote cell adherence. Cells were transiently transfected with the plasmid RFP-GLT-1 with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's protocol. Live images were taken 12h after transfection.

TIR-FM Setup and Image Acquisition

The TIRF capacities at the Nanobiotechnology Center (NBTC) at Cornell University were described previously[12]. In brief, it consists of a Nikon Ti-E/B automated inverted microscope equipped with a high numerical aperture TIRF lens (CFI APO 100X /1.45,) and automated TIRF angle. Live astrocytes (WT and R239C) transiently expressing RFP tagged GLT-1 were incubated in phenol-red free DMEM (21063-029, Invitrogen) supplemented with 10% FBS at 37°C in a homebuilt

thermal insulation box (35 ± 0.5°C, Air-Therm, World Precision Instruments. A 561-nm laser (model 643-AP A01, Omnichrome) and 575-675 nm emission band pass filter were used for RFP-GLT-1 visualization. Time-lapse TIRF Images were captured with an Andor iXon 897 EM -CCD camera (Belfast, UK) and controlled by Nikon Elements software at 1-2min intervals for 15min (http:// www.nbtc.cornell.edu/facilities/tools/ tool_TIRF.htm).

Image Processing and Data Analysis

All images were exported as single TIFF files and further processed using Adobe Photoshop 5.0 or with ImageJ a public domain Java image processing program developed by NIH with an extensible plug-in infrastructure (http:// rsb.info.nih.gov/ij/). All images at a given time series were adjusted identically for brightness and contrast. Images were filtered with Background Subtractor ("Mosaic" plug-in) to reduce noise and were subjected to an automatic subpixel registration algorithm for rigid body motion ("StackReg" plug-in) to correct for any drift in the XY plane. GLT-1 clusters were defined as fluorescent areas of >10 pixels (0.134 μm per pixel) with an intensity at least 3.5 times higher than the background. RFP-GLT-1 proteins that appeared or disappeared from the evanescent field, or moved >1 μm within the evanescent field were classified as motile. Cluster diameter was defined and calculated with image J ("dynamic ROI profiler"plug-in) as the full width at half maximum fluorescent intensity as described[13]. Within a window as specified above, the average fluorescence intensity was determined for each time point captured to determine number, size and perimeter of RFP+ particles/clusters using ImageJ ("threshold segmentation" and "particle analysis"). In regions that automatic thresholding failed to isolate RFP-GLT-1 clusters (a fluorescence detection channel can be saturated by some very large or very bright fluorescent particles) correctly; a double-blind segmentation and counting were performed manually. All data were expressed as the mean ± SEM. Statistical comparisons were performed using Student's t-test (P values < 0.05 were considered to be statistically significant).

Results

Mobile RFP-GLT-1 proteins were detected by TIR-FM with superior resolution in live astrocytes

WT and R239C astrocytes transiently transfected with RFP-GLT-1 were imaged using epifluorescence (EPI) and total internal reflection fluorescence (TIRF) microscopy, respectively. TIRF imaging selectively visualizes the adherent plasma membrane to a depth of about 100 nm into the cell (Fig.1A) and provides high resolution images of the discrete puncta of RFP-GLT-1(Fig.1B, TIR). In contrast, EPI illuminates the entire volume of the cell, partially obscuring the RFP-GLT-1 proteins (Fig.1B, EPI). RFP-GLT-1 proteins are localized in the cell membrane, cytoplasm, and processes of both cell lines. However, copious cytopolasmic puncta or aggregates of RFP-GLT-1 are only observed in the R239C astrocytes (Fig.1B, EPI). Many of these punctate profiles co-localized with lysosomes[14].

A 3D surface plot, another advantage of TIRF for imaging near the cell surface, is illustrated in Fig. 1C. Given that TIRFM optics are significantly more sensitive to the small fluctuations in the contour of the

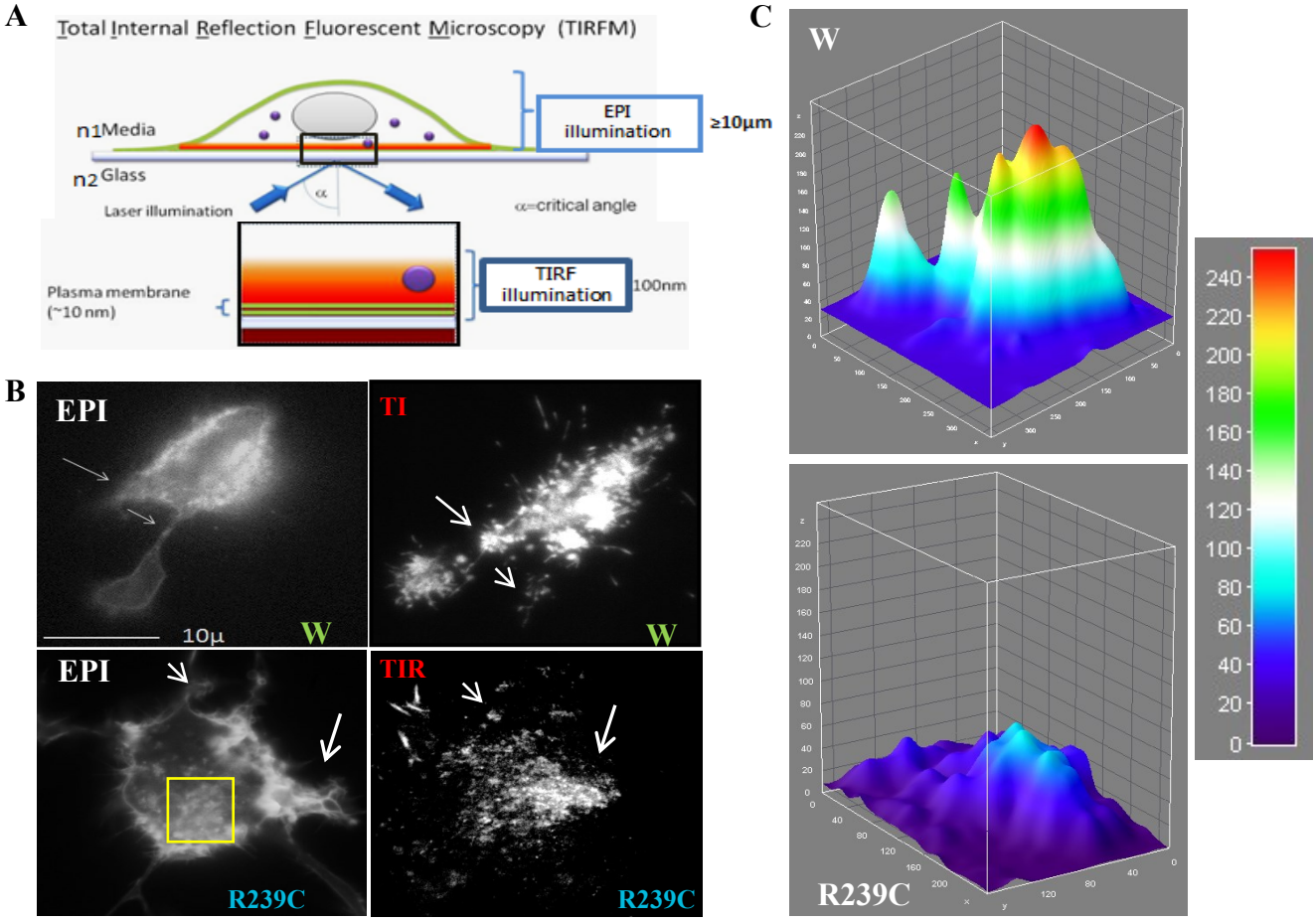

Fig.1. Direct imaging of RFP-GLT1 clusters by TIR-FM in live astrocytes

A. The physical basis of TIRF illumination: In wide-field epifluorescence (EPI) microscopy, laser light excites fluorophores throughout the entire cell volume. In contrast, the evanescent field produced by TIR light excites the fluorescent molecules at the cell substrate interface (~100nm) with minimal exposure of the remaining cell volume. The incidence angle must exceed a critical value and the refractive index of the sample/media (n1) must be less than the index of refraction of the cover slip/glass (n2) to achieve TIR. Images are adopted and modified with permission from Methods Mol Biol. 2011; 756: 325–332.
B. Live cell visualization of the GLT1 clusters by EPI and TIR-FM. GLT-1 fused with red fluorescent protein (RFP-GLT1) was imaged in the same focal plane by EPI (left) and TIR-FM (right) in WT and R239C astrocytes respectively. Arrows point out the same regions with a dramatic increase in visual information achieved by TIR-FM. Note R239C astrocytes displayed copious intracellular puncta or aggregates of RFP-GLT-1. Some of the intracellular puncta were co-localized with lysosomes (Tian et al., 2013).
C. A representative 3D surface plots of RFP-GLT1 proteins (fluorescence intensity of TIRF signal plotted against the surface area of the image in B) are shown in each cell. WT astrocytes appeared elongated with multiple protrusions; whereas R239C mutation results in a more flattened contour with few irregular protrusions. X and Y Axes represent area in pixels with each pixel = 134nm. Z axis represents fluorescence intensity.

membrane/coverslip contact regions, the spatial distribution of image brightness (intensity of fluorescence) can be visualized and measured to provide a 3D conformal mapping of the membrane/coverslip separation distances over the ventral surface of the adherent cell[15]. TIRF images further revealed the preferential membrane localization of RFP-GLT-1 clusters in WT astrocytes (RFP-GLT-1 fluorescence intensity in the membrane are 51% higher than that in the cytoplasm, Fig.1A, TIR). The representative 3D surface map of RFP-GLT-1 in R239C astrocytes also revealed a much more flattened contour lacking abundance and spatial organization of RFP-GLT-1 as

indicated by a dramatic reduction in the size of the cell-coverslip contact area in Fig.1C.

Defective spatio-temporal dynamics of RFP-GLT-1 in the cell membrane of R239C astrocytes

Live astrocytes were continuously imaged using TIR-FM for 15min. A frame-by-frame analysis was performed to quantify and characterize the dynamic membrane organization of RFP-GLT-1. By setting the measurement scale and thresholding particles for each image captured in sequential order, we determined the distribution, dimensions and densities of morphologically distinct RFP-GLT-1 protein clusters at the cell surface of WT and R239C astrocytes (see Materials and Methods for details). As illustrated in Fig. 2, WT astrocytes have clear and remarkably robust RFP-GLT-1 remodeling during the recording time: a large increase in fluorescence intensity coupled with a transition from a small punctate appearance to a larger, spreading cloud of clusters with displacements as large as ≥1 μm within the evanescent field on time scales as short as 1 min. Many RFP-GLT-1 clusters move along spine-like membrane protrusions that transiently extend and retract; or oscillate along the z-axis and then rapidly merge, or stay at the same position for up to a minute before merging. Occasionally, it approaches rapidly and becomes visible at fusion (a large, sharp increase in TIR fluorescence). A few clusters emerge and disappear during the recording time.

The dynamic portrait of RFP-GLT-1 proteins at the cell surface of R239C astrocytes, however, is distinctly different with a relatively simple, less mobile fluorescence pattern. The number of transient membrane protrusions are significantly reduced, consistent with earlier findings (Fig.1C) that the membrane of R239C astrocytes has much smaller adhesion (or contact) area. We did not observe the transition between small puncta and larger clouds of clusters seen in the WT cells. The size, amount, density and morphological rearrangements of RFP-GLT-1 particles are also decreased dramatically with only ~50% motility (Fig. 3, quantified in Fig. 3C).

Table 1 summarizes the quantitative measurements of these dynamic parameters in WT and R239C astrocytes. Statistically it is clear that a vast majority of the RFP-GLT-1 proteins (~98%) in WT astrocytes organized into highly motile clusters with an average size of 1.04 μm. About 15% display reversible clustering *(*splits and merges). About 18% of RFP-GLT-1 clusters rapidly move along spine-like membrane protrusions that extend and retract frequently. By comparison, RFP-GLT-1 in the cell membrane of R239C astrocytes formed small protein particles 0.06–0.10 μm in diameter with only about one-third of the density achieved by WT astrocytes. Approximately 50% of these particles are stationary and 6% show directional motion along few membrane protrusions. Quantification of patterns of motility revealed either a simple slow motion of splitting and merging or free lateral movements into the plasma membrane, suggesting impaired structural remodeling of GLT-1 proteins and membrane plasticity in R239C astrocytes.

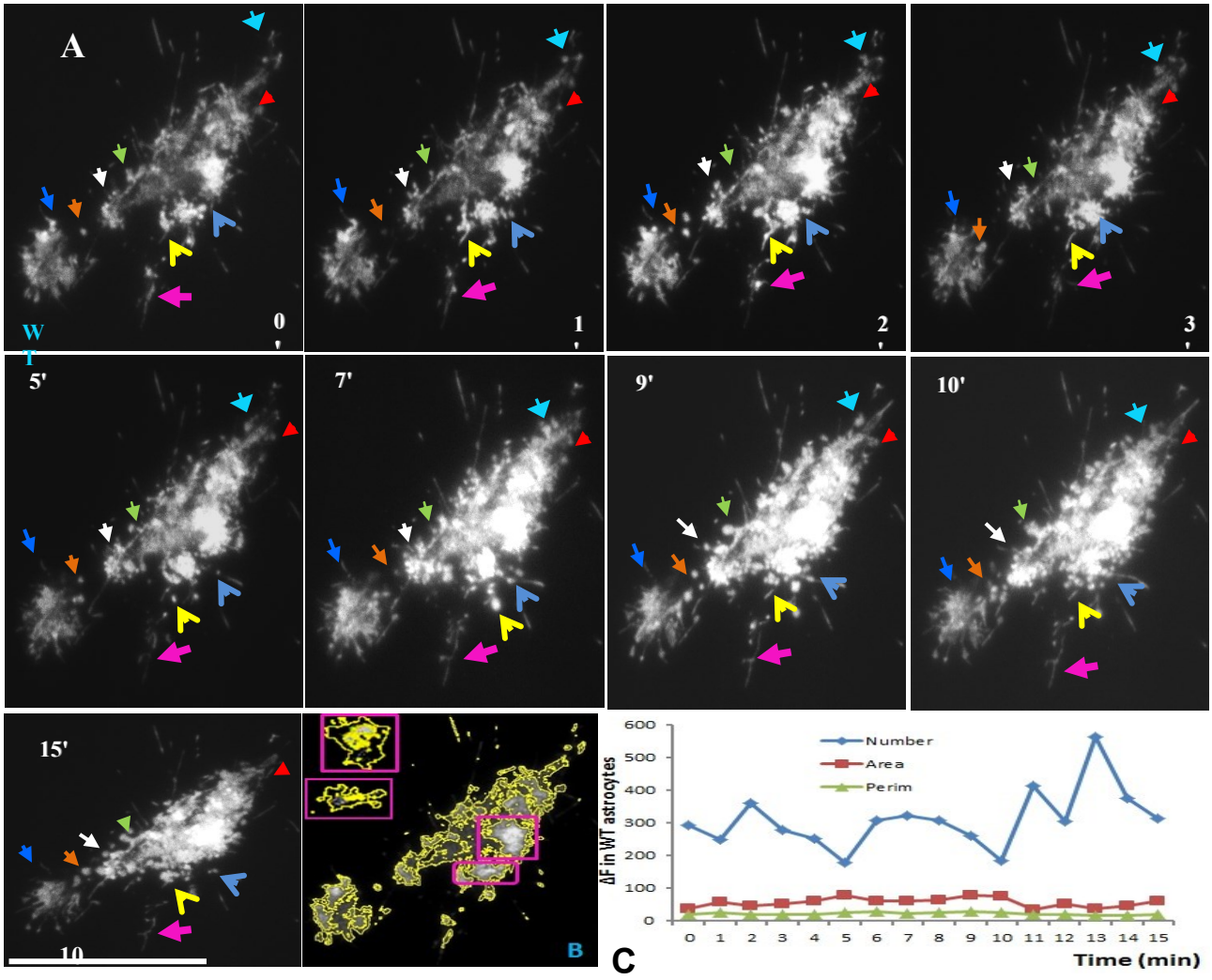

Fig.2. Robust remodeling of RFP-GLT-1 in WT astrocytes by TIRFM
A. Images from a time-lapse sequence (~1min intervals) show strong rapid movements of numerous RFP-GLT-1 clusters over the surface and along short, spine-like structures in WT astrocytes. Motions of representative RFP-GLT-1 clusters are indicated by different color of arrows.
B. An example of particle detection algorithm ("default" plug-in) taken at t = 5min. Insets show suprathreshold regions ("moments" plug -in, purple) that were further segmented for the quantitative analysis of RFP-GLT-1 clusters.
C. The quantification of number, area and perimeter of RFP-GLT-1 clusters over time.
The time is plotted along x axis in minutes, and the relative fluorescence intensity called ΔF is plotted along the Y axis because the value measured in an adjacent (non-clustering) region of the plasma membrane is subtracted. The data were obtained by TIRFM from three WT astrocytes transiently expressing RFP-GLT-1.

Phenotypes	Total Number	Density (particles/μm²)	Size (μm)	% Kinetic Styles		
				Stasis	Splits and Merges	Along spine-like protrusions
WT (n=3)	278±36	1.07±0.19	1.04±0.44	1.33±1.20	15.01±2.62	17.96±2.40
R239C (n=5)	82±5	0.31±0.03	0.08±0.02	48.78±1.49	46.13±15.63	5.88±2.83

Table 1. Quantitative comparison of the membrane profiles of RFP-GLT-1 protein in WT and R239C astrocytes.
The two populations (RFP-GLT-1 positive particles in WT and R239C astrocytes, respectively) are significantly different ($p < 0.01$; Student's *t* test). The rest of kinetic styles in WT astrocytes are cloud-like structures, which are not seen in R230C astrocytes. Pixel size, 134nm.

Discussion

This study is aimed at understanding how AxD, an encephalopathy caused by a mutation in *GFAP*, a gene expressed only in astrocytes, compromises the survival of other cell types of CNS. We focused our efforts on the GLT-1 protein because 1) it is expressed primarily in astrocytes; 2) it is the major Glu transporter of the CNS, mediating a sodium-dependent uptake of Glu into astrocytes to control extracellular levels of this excitatory neurotransmitter and prevent excitotoxic cell death; 3) decreased levels of GLT-1 protein and low Glu-induced seizure threshold are found in the hippocampi of infantile AxD patients and a knock-in mouse model of AxD[8,16].

The number of Glu transporters (GLT-1) in the plasma membrane reflects the balance between their membrane insertion and removal by endocytosis[11]. The relative amount of GLT-1 protein translocating to the cell surface is important in maintaining the transporter density necessary for effective function. To determine whether loss of GLT-1 function in AxD results from defective membrane targeting, we were interested in investigating GLT-1 proteins that are at or near the plasma membrane. Therefore, we applied a specialized microscopy technique, TIRF, to detect and monitor the mobility of fluorescently tagged fusion protein RFP-GLT-1 at the cell membrane. The advantage of TIRF for imaging near the cell surface with high resolution lies in the physical basis of this technique. The TIRF excitation field decreases exponentially with distance from the coverslip the cells are attached to. Thus, fluorophores close to the cover slip (e.g. within ~100 nm) are selectively illuminated, highlighting events that occur within this region (Fig 1A). This allowed us to eliminate out-of-focus fluorescence from the bulk of the cell and to reveal details at or near the plasma membrane (Fig 1B).

Our time-lapse TIRFM images were then processed and quantitatively analyzed using Image J. Parameters of spatial organization investigated included the presence of protein clusters, their size, density, abundance as well as morphogenesis of RFP-GLT-1 in the plasma membrane. Our study revealed an unprecedented detailed comparison of the dynamic profiles of GLT-1 protein in WT astrocytes and AxD astrocytes (Table 1). We observed a significant reduction in the size, density, mobility and membrane distribution of RFP-GLT-1 in AxD astrocytes, consistent with our findings of impaired Glu uptake in AxD astrocytes[8].

We do not have stoichiometry data for RFP-GLT-1 clusters observed in WT astrocytes. However, similar clusters were observed when a GFP tagged GLT-1 was transiently expressed in developing hippocampal astrocytes from tissue slices[13]. Given a significantly diminished GLT-1 current recorded by whole cell patch clamp in AxD astrocytes[8], it is possible that clustering (~1.04μm in diameter) may be a requirement for a fully functional GLT-1 protein. Future work with resolved X-ray crystal structure of human GLT-1 protein would clarify whether RFP-GLT-1 particles 0.06–0.10 μm in diameter in AxD astrocytes are dispersed RFP-GLT -1 clusters with relatively low number of RFP-GLT-1 proteins presumably indicative of a decreased number of Glu uptake sites (Table 1).

Our study also led to two interesting discoveries. First, a strikingly lower abundance of RFP-GLT-1 protein was observed, particularly at transient, spine-like membrane protrusions of AxD astrocytes (Fig. 5A). While the precise

nature of these protrusions remains elusive, multiple independent studies have shown GLT-1 is enriched in membrane domains that face or surround the areas of Glu release[17,18]. Thus it is tempting to speculate that AxD astrocytes are defective in undergoing rapid membrane remodeling by forming retractable spine-like structures in response to the local changes in Glu concentration. Serial section electron microscopy (EM) combined with a monolayer freeze-fracture technique would provide direct evidence of the number and distribution patterns of GLT-1 protein. Second, we showed that the accumulation of a mutant GFAP caused a change in cell contours, appearing to suppress the multiple protrusions that WT cells were able to generate and resulted in a more flattened cell (Fig. 1C). Biological membrane functions are coupled to membrane curvature, the regulation of which often involves membrane-associated proteins[19]. Further studies with additional astrocyte-specific membrane marker proteins, Aquaporin4, Kir4.1, and Connexin43 are underway to confirm and elucidate the relationship between membrane contour and/or remodeling and membrane protein density in AxD. It is conceivable that the disruption of the cytoskeletal network resulting from *GFAP* mutations in AxD impairs the membrane targeting and subsequent remodeling of multiple membrane proteins, including GLT-1. Alternatively, the GFAP inclusions formed in the cell body of AxD astrocytes may physically disrupt the distribution of membrane organelles and result in abnormal trafficking.

TIRF imaging is a powerful technique that offers the optical clarity needed to understand how membrane proteins physically perform their function over time, and also provides the first structural evidence for the hypothesis that dysfunctional Glu transport in AxD may be due, in part, to a defective membrane organization and trafficking of GLT-1.

Acknowledgments

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Locking Up Your House Helps Keep the Thieves Away - A Student Cheating Primer for Honest Teaching Dummies

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Research estimates that at least 50% of college students admit to cheating and the actual percentage is probably much higher. This is especially true in large classes where a multiple-choice type format is common. As a beginning teacher I assumed that it was mostly the weak and underprepared students that cheated and did not exert much effort to prevent it. Over the years, however, I have observed an increase in student efforts to cheat, which crosses all levels of academic ability.

Cheating is contagious. If you let the cheaters cheat others give in to temptation. Chronic cheaters keep trying even if you've caught and warned them. In addition, despite students recognizing that cheating is ethically wrong, many consider cheating an acceptable way to obtain their academic goals. The need for hard evidence (such as confiscated copies of cheat sheets) in order to formally punish student cheating may inhibit faculty from addressing cheating and even cause them to ignore it[1].

As a community college teacher with 20 years of experience teaching Human Anatomy and Physiology to pre-allied health majors I have seen many varieties of cheating. My novice teaching colleagues often ask me for advice on how to prevent and/or ameliorate cheating in their classes. Many are oblivious to some of the cheating techniques potentially used by students. My 'honest' students thank me for taking precautionary measures and often 'snitch after the fact.' Thus it seems useful to summarize my observations for the use of others.

Common ways that students cheat during tests

For the novice cheating sleuth, below is a list of common cheating strategies that will be used if you do not prevent them.

- Verbally ask the student(s) close to you for the answer.
- Copy the answers of the student next to you, with or without their approval.
- Electronic means such as text messaging (increasingly prevalent if allowed.)
- Swap tests or answer sheets to check answers.
- Crib notes written on: miniaturized flash cards; full-sized pages that fit inside exams; tissues, especially the hard kind from the bathroom; the inside of water bottle labels, caps or other loose headgear, body parts, pocket books and pencil cases. Crib notes may also be swapped between individuals.
- Less common are drum beats, hand-signals, braille codes on pencils etc. Don't ask me how the codes work, I just know they happen.
- Ringers can and do take tests for students, especially in large classes.

Team work examples of cheating during tests

If you let them, teams of students can organize to swap tests, answers and even crib sheets. Stronger students do this to be more competitive with their peers or assist weaker friends.

- One student distracts you by asking a question (with or without bad intentions.) Cheaters immediately consult each other, swap tests and/ or scantrons, text message each other and variously attempt to help each other out.
- Students study different concepts or areas on the test, then swap tests and/or answer sheets between group members.
- Occasionally, one student may completely fill out an exam for another. I've seen identical scantrons and even handwritten exams where the same student completed their own test and the 'friend' they felt sorry for.
- A group of students takes the test and leaves. Following consultation a late arriving student who 'overslept' gets the advantage. Group members switch places for the next test. Alternatively, late arriving student has been text messaging answers to the group.

STOP IT BEFORE IT STARTS

- Monitor and Proctor
  - Make it obvious you mean business
  - Don't sit up the front of a test doing other work
  - Space seats as far apart as possible
  - Don't give up and ignore cheating
  - Furtive eye glances mean watch out something's up
- Randomly seat students for all major tests
  - Consider using a pre-prepared student roster with assigned seats and change it regularly.
  - Preferably, also use similarly pre-numbered tests and answer sheets.
  - Don't assume students sit where you assign them; groups can and do switch places.
- No electronic devices, pocket books, pencil cases etc. on desk or close to body.
  - Ideally, only visible writing supplies should be allowed within arm's reach.
  - Phones, if permitted at all, should be on vibrate only.
- Absolutely no 'late' arrivals or bathroom breaks.
- For scantron grading, have students circle or strike through final answer in pen.
- Absolutely no make-up tests using the same test ever.
- Use two versions of the test (more if you can track them).
  - Don't make it obvious which version is which. Some students will try to switch them.
  - Using the same question order, with a reordered answer selection is surprisingly effective.
  - On tests with diagrams consider asking the same questions but use different picture labels. This also works surprisingly well.
  - Do not confuse yourself by using too many versions.

- Make the same test look different, even if it is not.
  While different versions are preferable, as long as they don't catch on most students will assume that their version is different if it looks different.
  Try:
  Using a different first page only
  Printing test out with different fonts
  Putting version A, version B etc. on front page.
  Using different colored paper
- Number the tests and answer sheets
  This curtails swapping and may also help prevent the occasional student from taking the test out of the room without turning it in.

Cheating and old tests

Some students go to considerable efforts to gain an unfair advantage on their peers by obtaining (and not universally sharing) old copies of your tests.

- Do you give back test questions?
- Do you give back answer sheets, but not the test questions?
- Do you recycle your old tests?
- Do you use the same test for different sections of the same course?

We all do some, or all of the above, at least some of the time. Old tests are out there, regardless of whether you give them back or not. I've seen collated copies of my tests dating back a decade. Unless you have accounted for all pages and copies and can guarantee illicit pictures were not taken, someone will eventually obtain a copy. Even if you collect tests there can be team efforts organized to get a copy. For example, each member of a group removes one page from their test, one student removes second page, another the third etc. Then they compile the test later (and maybe supply a copy to the 'makeup' buddy who had 'flu.')

If you want to curtail this type of 'cheating' and don't want to rewrite all of your tests all of the time reorder the questions and the answers for different sections of a course. Even using identical questions, this works surprisingly well.

Catching the cheaters: (or you just want to know who the cheaters are likely to be)

- Generally the cheaters will most likely be sitting at the back or sides of the class.
- You can usually spot likely cheaters by their furtive glances in your direction.
- Ask the laboratory instructor, or prior instructors, who they suspect.
- Using alternative test versions, without obvious visual differences, will also catch a few
- Photocopy answer sheet(s) before returning them if you suspect post-test modifications.

Other Considerations

- Few faculty will have the time, energy, or motivation to keep ahead of all the cheating strategies all the time. If nothing else, 'random' seating, with the proctor(s) sitting out of the line of sight and going on periodic patrols goes a long way.
- Make sure your policy on cheating, is clear, consistent and impartially enforced. You do not want to deal with claims of discrimination.

- Student(s) who complain about others cheating are not always innocent.
- Don't think that if you catch someone and let them off with a warning, or a lousy grade, they won't do it again. The chronic cheaters will keep trying, but hopefully not well enough to get an undeserved grade. Those that think cheating is their only solution will keep trying to the end.
- Don't think that just because you've prevented cheating in one setting e.g. the lecture test, you've prevented it in another e.g. the laboratory practical exam.
- Take home exams, quizzes and homework should be used for what they're worth. Similarly, online tests are likely compromised if the testing environment is not secure. In my experience, what happens is the academically weak but honest ones lose out.

Remember, cheating is contagious. The mostly honest but weak-willed will most likely not cheat too much if you take precautionary measures. If you don't, they will soon cave into temptation if others are cheating without penalty, and who can blame them.

The best policy is still an inflexible attitude. You cannot cheat.

References

[1]Smyth, M.L. and J.R. Davis, 2003. An examination of student cheating in the two-year college. Community College Review **31:** 17-32.

2014 Benjamin Cummings/MACUB Student Research Award Winners

Heather Renna of Molloy College
The Role of the Tissue Resident Mesenchymal Stem Cell in the Inflammation Driven Process of Atherosclerous

Tisha Paul of Molloy College
Linking Inflammation of Neuronal Cell Lines with Betacasmorphin 7 (BCM-7) and Betacasmorphin 9 (BCM-9)

Monique Willoughby of Queensborough Community College
Analysis of Micro RNA's in APL Autoantibodies Induced Tumor Malignancy

Krima Patel of Monmouth University
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