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$A\beta_{1-42}$ Oligomers Induced a Short-Term Increase of Glutamate Release Prior to Its Depletion As Measured by Amperometry on Single Varicosities

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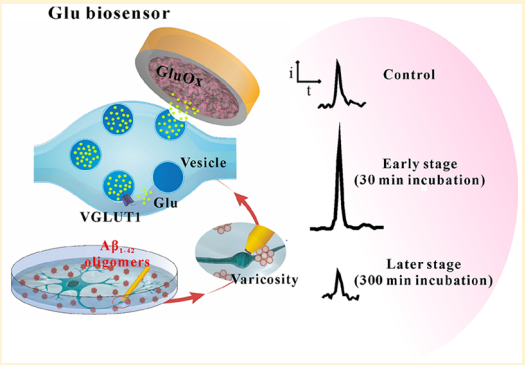
Supporting Information

ABSTRACT: Glutamate (Glu) is a critical neurotransmitter for neuronal communication in the nervous system. In vivo studies have shown that the concentration of Glu is reduced within the brains of those afflicted with Alzheimer's disease (AD), which is also associated with the accumulation of pathogenic amyloid-beta (Aβ). However, the effects of Aβ peptides on the level of Glu release, as well as how Aβ-mediated Glu fluctuation is initiated, remain largely unknown. Here, we fabricated a Glu electrochemical biosensor and in situ quantitatively monitored the release of Glu from a single varicosity of $A\beta_{1-42}$-insulted hippocampal neurons. We found that before the depletion of Glu after 300 min of treatment with $A\beta_{1-42}$, a short-duration (30 min) incubation with $A\beta_{1-42}$ caused a dramatic increase in vesicular Glu release compared to that of a control. Further investigation demonstrated that the density of vesicular glutamate transporter 1 (VGLUT1), which is responsible for transport of Glu into synaptic vesicles, also displayed a significant elevation and then dramatic depletion with the extension of the time of treatment with $A\beta_{1-42}$. These results indicate that at the early stage of AD, $A\beta_{1-42}$ induces excessive Glu release, which may overstimulate the *N*-methyl-D-aspartic acid (NMDA) receptor, resulting in excitotoxicity and damage to neurons. In this work, the amount of Glu released together with its fluctuations under $A\beta_{1-42}$ oligomers toxicity conditions was monitored for the first time, and such monitoring could provide direct and new insights for current research on $A\beta_{1-42}$-induced abnormalities in neurotransmitter release and neuron functions.



INTRODUCTION

Neurotransmitters, the necessary components of neuronal identity released as a result of vesicle exocytosis, are critical for neuronal communication in the nervous system.[1−3] Glutamate (Glu), as the predominant excitatory transmitter in the hippocampus, has a particularly important role in learning and memory. Fluctuations in the amount of Glu released damages synapses by causing receptor-mediated long-term potentiation or long-term depression of the synapses and may play a role in the pathogenesis of chronic neurodegenerative disorders.[4−6] Alzheimer's disease (AD) is one of the most common neurodegenerative disorders and is associated with the accumulation of pathogenic amyloid-β (Aβ). Aβ peptides are cleavage products of amyloid-β precursor protein (APP), with this cleavage catalyzed by β secretase and γ secretase complex and resulting in progressive synapse failure, neuronal damage and memory loss.[7,8] Numerous observations have suggested that Aβ may impact glutamatergic transmission by reducing the concentration of Glu in AD patients.[5,9] However, the effects of Aβ peptides on the fluctuation of the levels of Glu released, and how this Aβ-mediated fluctuation of Glu levels is initiated, remain incompletely understood.

Given its prominent role in AD physiology, considerable efforts have been made to take accurate and precise measurements of the changes of Glu concentration induced by Aβ peptides. Traditionally, the concentration of Glu has been determined primarily using high-performance liquid chromatograph (HPLC) with fluorimetric detection after derivatization, and has involved sampling from the bulk of cells. But such sampling fails to reflect the changes in local concentrations of Glu in real time.[4,10] Furthermore, biosensors composed of Glu-binding proteins coupled to a fluorescence readout have been recently developed, and display spatial and temporal resolutions much greater than before.[11,12] A FRET-based glutamate sensor (consisting of GluSnFR-transfected HEK 293 cells) was recently developed for the detection of Glu, and its use showed $A\beta_{1-42}$ oligomers inducing a local release of Glu from astrocytes.[9,13] These studies offer valuable information that Aβ oligomers may viciously regulate the cycle of Glu in neurontransmission and induce glutamatergic

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dysfunction by affecting Glu release from presynaptic terminals or Glu uptake from extracellular fluids. However, neurotransmitter release via exocytosis is quantized and the process is very fast on time scale.[14] Most of these previous systems, being limited in spatial and temporal resolution, could not directly probe and quantify the release of vesicle content, hindering the determination of the reason for rapid vesicle release fluctuation in neurodegenerative disorders. Accordingly, in order to further understand the underlying molecular mechanisms of Aβ on synaptic dysfunctions, it is very important to be able to in situ monitor synaptic Glu release in real time and quantify the changes in its concentration.

Electrochemical methods with carbon fiber electrodes based on amperometry have, due to their high sensitivity and excellent temporal resolution, been applied for the detection of exocytosis from small vesicles.[15−20] Great advances have been achieved in carbon fiber microelectrode sensors designed to be used for amperometric measurements of exocytosis events near neuron cells.[21−26] However, these studies have mainly focused on the detection of electroactive molecules such as dopamine, epinephrine, and norepinephrine. Glu is a nonelectroactive small molecule, and hence, cannot be directly detected by a bare carbon fiber electrode. Recently, microelectrochemical biosensors of Glu with high sensitivity and specificity have been developed by modification of glutamate oxidase on carbon fiber microelectrode, and such a strategy realized quantitative measurement of synaptic Glu release from cultured hippocampal neurons[27] or brain slices,[28] as well as the number of Glu molecules in single synaptic vesicles.[29] This development has provided a promising tool for the monitoring of the concentration of Glu and the changes in this concentration at the source of its release sites under various physiological and pathological conditions.

In the present study, this microelectrochemical sensor of Glu was applied to investigate the abnormities of Glu release from Aβ damaged glutamatergic neurons. Aβ_{1-42} oligomers (250 nM) were added into the cell culture medium to mimic low Aβ toxicity in AD rat, and vesicular Glu releases from single varicosities with different Aβ-treatment times were then quantitatively monitored and analyzed. Our results showed that short-term incubation (30 min) of Aβ_{1-42} oligomers induced a significant elevation of the release of Glu, before its depletion following a long-term (300 min) treatment. Further investigation demonstrated that the vesicular glutamate transporter 1 (VGLUT1), which facilitates the transport of Glu across vesicles,[30,31] showed a similar trend. This result indicated that VGLUT1 is closely involved in the Aβ_{1-42}-induced abnormalities in Glu release. This work represented the first attempt to electrochemically monitor vesicular Glu release and its fluctuations under Aβ_{1-42} oligomer toxicity conditions in real-time, and could hence provide crucial information and new insights for current research on the etiology of AD.

EXPERIMENTAL SECTION

Materials. β-Amyloid (1−42) was purchased from ChinaPeptides Co., Ltd. (Shanghai, China). DMEM/F-12 medium and B_{27} for cell culture were bought from GIBCO (U.S.). Poly-L-lysine (PLL), trypsin, laminin, L-glutamine, bovine serum albumin (BSA), GluOx, and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) were purchased from Sigma (St. Louis, MO). Antibodies against VGLUT1 and GADPH were purchased from Abcam Co., Ltd. (Shanghai, China). Antirabbit IgG horseradish peroxidase (HRP)-conjugated and nitrocellulose membranes were bought from Beyotime Co., Ltd. (Shanghai, China). All other chemicals of analytical grade, were obtained from Sinopharm Chemical Reagent Co., Ltd. When required, all chemical solutions and buffers were prepared in ultrapure water (18.2 MΩ).

Hippocampal Neurons Culture. A suspension of dissociated primary hippocampal neurons was prepared according to published procedures.[32] Briefly, hippocampus was dissected from the brains of newborn rat pups at postnatal day 1, followed by digestion with 0.125% trypsin for 30 min at 37 °C, and then the serum-free culture medium was added to terminate the action of the trypsinase. Hippocampal neurons were planted on PLL-coated glass coverslips and cultured at 37 °C under 5% CO_2 in DMEM/F-12 supplemented with 2% B_{27}, 100 U/mL penicillin, streptomycin and 50 ng/mL NGF. Experiments were conducted on cells cultured for 3−8 days.

Amperometric Measurements, Date Acquisition, and Analysis. All amperometric monitoring experiments were taken on the stage of an inverted microscope placed in a Faraday cage. First, the sensor that manufactured as described previously[27] was placed onto the varicosity. Second, the high K^+ (62.5 mM) solution was delivered through a glass capillary. Third, the amperometric spikes were recorded via patchclamp amplifier at a constant potential of +700 mV, with an Ag/AgCl wire as reference/counter electrode. Signals were sampled at 20 kHz, and bessel filtered at 2.9 kHz. Last, raw amperometric data were collected using "Pulse" software, and analyzed by a routine in Igor Pro kindly offered by Dr. E. V. Mosharov in Columbia University.

Western Blot Analysis of VGLUT1. Protein concentrations were determined using BCA assays (Thermo Scientific, Waltham, MA). Equal amounts of protein for each sample were bolied for 10 min at 95 °C. Boiled samples were electrophoresed on 10% SDS-poylacrylamide gel electrophoresis, followed by transfer to nitrocellulose membranes. Membranes were blocked for 2 h at 37 °C in 5% BSA containing 0.5% Tween-20 (TBST), followed by incubation overnight at 4 °C with the primary antibodies to VGLUT1 (1:2000) and GADPH (1:10000). Subsequently, membranes were incubated for 1 h with an antirabbit IgG horseradish peroxidase (HRP)-conjugated (1:10000) before detection of immunoreactivity of the bands using enhanced chemiluminescence reagent.

Determination of Cell Viability. Cell viability was assessed by MTT reduction assay as described previously.[33] The assay was used to optimize the Aβ_{1-42} oligomers incubation concentration and time. Briefly, the cells were incubated with a 0.5 mg/mL solution of MTT reagent at 37 °C for 4 h to allow MTT reduction to formazan blue by metabolically active cells. Then formazan crystals were solubilized at room temperature with 200 μL DMSO for 15 min. Optical density at 490 nm was measured in a ThermoMax Multiskan MK3. Six replicate wells were used for each experimental condition in each experiment.

Cell Immunostaining. Cells were rinsed twice with phosphate-buffer saline solution (PBS) and fixed for 30 min in 4% paraformaldehyde (in 0.1 M PBS). The cells were then permeabilized (30 min in 0.1 M PBS, 0.1% Triton X-100) and blocked (30 min in 1% Goat Serum) at room temperature. To label VGLUT1, cells were incubated with VGLUT1 antibody labeled with FITC (5 mg/mL) for 1 h at 37 °C.

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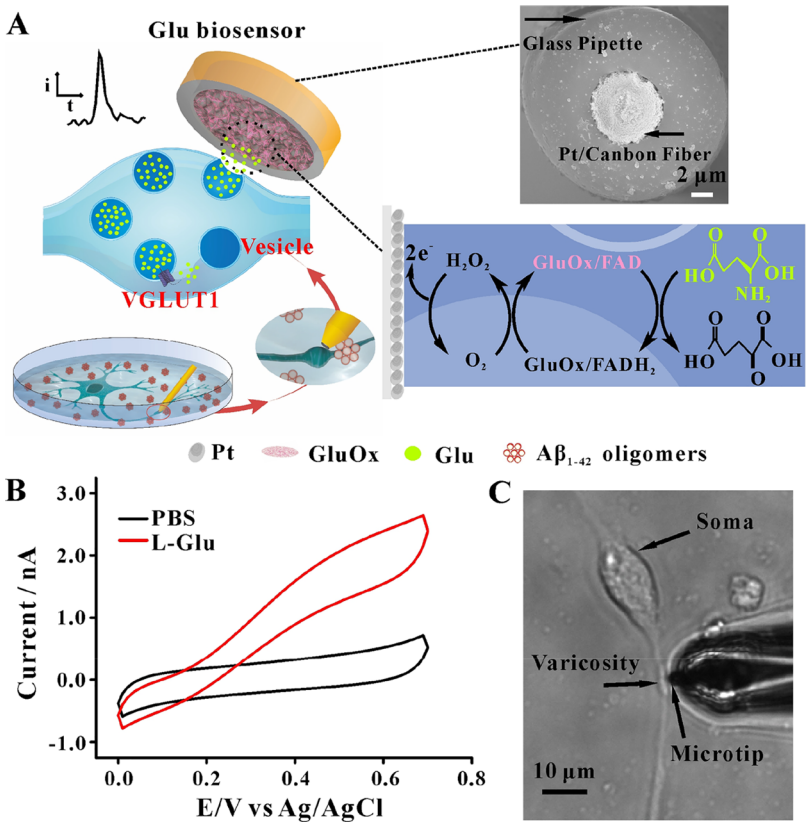

Figure 1. (A) Schematic diagram showing the process of amperometric monitoring of glutamate exocytosis from single hippocampal varicosity, the top-right shows the SEM images of a platinized carbon fiber microelectrode and bottom-right shows the mechanism of Glu detection on the microsensor. (B) Cyclic voltammograms of Glu biosensor responses to 0.1 M PBS (pH 7.0) in the absence (black line) and presence (red line) of 2 mM L-Glu. (C) Bright-field photomicrograph showing the tip of sensor placement on a hippocampal neuron varicosity.

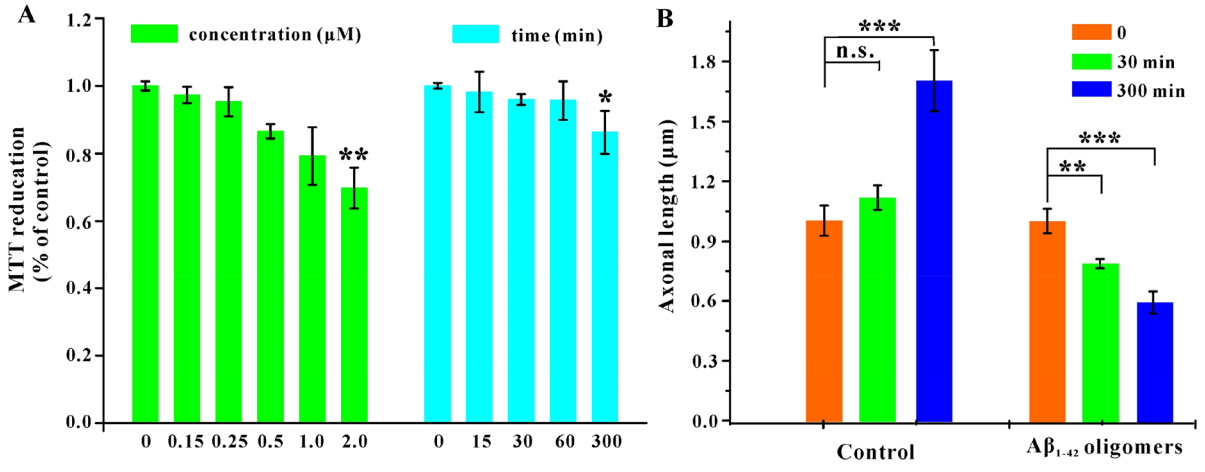

Figure 2. Optimizations of experimental parameters by MTT assay and neuron morphology imaging. (A) Cell viability after exposure to different $A\beta_{1-42}$ oligomers concentrations (0, 0.15, 0.25, 0.5, 1.0, 2.0 μM) for 30 min and the cell viability after incubation for different time (0, 15, 30, 60, 300 min). (B) Statistics of the axonal length in response to $A\beta_{1-42}$ oligomers incubation for different time (30 or 300 min). Data are mean ± standard deviation; *p < 0.05, **p < 0.01, ***p < 0.001, compared with control.

Immunofluorescence was visualized with an inverted fluorescent microscope (Axiovert 200 M and AxioObserver Z1, Carl Zeiss).

RESULTS AND DISCUSSION

Strategy for Real Time Monitoring of Glutamate from Single Varicosities. The hippocampal neurons varicosities are glutamatergic with diameter of 2−6 μm and occupy a superficial space on the axons. Importantly, most of them are responsible for Glu release and fast synaptic transmission.[21,34,35] Emerging evidence suggests that the injurious effects of soluble oligomers of $A\beta_{1-42}$ in AD may mediate changes in the concentration of Glu in the hippocampus.[36,37] Hence, to explore the effects of $A\beta_{1-42}$ oligomers on the level of synaptic Glu release in the hippocampus, a kind of microelectrochemical sensor of Glu was prepared and used

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to monitor the quantal release of Glu from single varicosities and to specifically monitor the changes in this release caused by Aβ_{1-42} (Figure 1A). Here, for the electrochemical detection of Glu, Pt nanoparticles (NPs) were electrodeposited onto the bare carbon fiber to improve the H_2O_2 oxidation performance (Supporting Information (SI) Figure S-1) followed by immobilizing the GluOx onto the electrode. Cyclic voltammetric characterization of Glu on this sensor indicated that Glu was oxidized by the GluOx/FAD modified on the electrode to produce GluOx/$FADH_2$, which further reacted with molecular oxygen to produce H_2O_2 (Figure 1B, and SI Figure S-2). The oxidation current of H_2O_2 nearly reached its maximum at ca. 0.5 V, which ensured that in the next series of experiments, Glu can be well monitored using amperometry at 0.7 V, and the high sensitivity and fast response of this sensor (SI Figure S-3) suggested its capability in real-time monitoring of Glu released by transient exocytosis. Furthermore, visualization under a microscope of an individual varicosity from axon allowed the precise positioning of the microelectrode to the varicosity for amperometric monitoring (Figure 1C).

Effect of Aβ_{1-42} Oligomers on Neuron Damage and Neurite Atrophy. Recent stuides suggested that Aβ_{1-42} toxic oligomers appear as spherical aggregates at early stage of AD,[7,38,39] which was further evidenced by the AFM imaging in this work (SI Figure S-4). To mimic the low toxicity of Aβ_{1-42} oligomers at the early stage of AD mouse, we pretreated the cultured hippocampal neurons with Aβ_{1-42} oligomers, and their concentration and incubation time were optimized by MTT assay and neuron morphology imaging, respectively.

As shown in Figure 2A, the cell viability was not obviously affected following short-term exposure to Aβ_{1-42} oligomers with low concentrations (less than 1 μM), whereas higher concentration of Aβ_{1-42} caused significant decrease of the cell viability in a short period of time. Considering that at the earliest clinical phase of AD, Aβ_{1-42} triggers progressive loss of dendritic spines, accompanied by a decrease in excitatory synapses, whereas no significant cell damage or death was observed;[40,41] hence, 250 nM Aβ_{1-42} oligomers were selected to treat the hippocampal neurons, which is consistent with the previously applied concentration.[42] The incubation time was then evaluated ranging from 15 to 300 min to preliminarily mimic the different stages of AD disease. The results show that the significant decrease in cell viability occurred only when the incubation time exceeded 300 min (Figure 2A).

Memory dysfunction in the human brain with dementia is linked to neuritic dystrophy and neurons death.[43] We further evaluated the effects of Aβ_{1-42} oligomers on neurites (axons or dendrites) by observing the neuron morphology and measuring average axonal length in cultured hippocampal neurons. The results show that the axonal length of control group increased gradually with the extension of the culture time, while Aβ_{1-42} oligomers incubation caused a continuous decrease of axonal length (SI Figure S-5). It should be noted that obvious neurite atrophy could be found when the hippocampal neurons were treated with 250 nM Aβ_{1-42} oligomers for 30 min (Figure 2B), though the cell viability was not significantly affected at this condition as indicated by MTT assay. Further considering that 300 min incubation caused significant cell damage (Figure 2A) and more serious neurite atrophy (Figure 2B), 30 and 300 min were thereby chosen as the optimal incubation time to represent the early and late stage of AD disease.

Amperometric Detection of Aβ_{1-42}-Regulated Glutamate Release from Individual Hippocampal Neurons Varicosities. Vesicular Glu release was elicited by high-K^+ solution (62.5 mM) puffs, and monitored through oxidation of Glu on the microelectrochemical sensor placed on single varicosities of hippocampal neurons. Well-defined quantal amperometric spikes were recorded from the normally cultured neurons (Figure 3A), and whose amperometric responses were

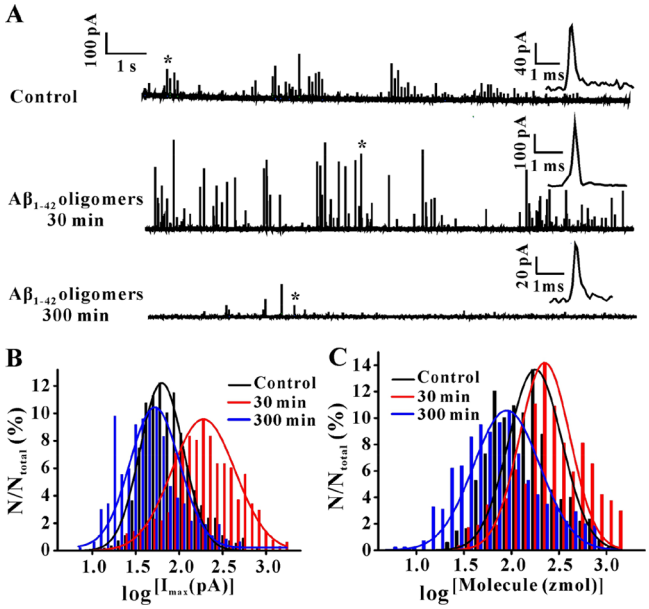

Figure 3. (A) Amperometric traces from Glu sensor placed on a varicosity. A potential of 700 mV (vs Ag/AgCl reference electrode) was applied. (B) Histograms of the distribution of I_{max} of the spikes from control (black), Aβ_{1-42} oligomers-treated for 30 min (red) and 300 min (blue) hippocampal neuron varicosities. (C) Representative histograms of log of the vesicular glutamate amounts quantified for the single vesicle from control (black), Aβ_{1-42} oligomers-treated for 30 min (red) and 300 min (blue) hippocampal neuron varicosities. Line: Gaussina curve fit of the data (black, n = 548 events from 15 hippocampal neurons; red, n = 647 events from 12 hippocampal neurons; blue, n = 470 events from 20 hippocampal neurons).

used as the control in this study. In view of the good selectivity of this Glu sensor (SI Figure S-6) and the undetected amperometric spikes from neurons in a Ca^{2+}-free extracellular medium (SI Figure S-7), we can conclude that the amperometric spikes were corresponded to sequential vesicular Glu release from single vesicles. The individual peak currents (I_{max}) and number of released molecules (N) from single vesicles can be obtained by statistical analyses of the recorded individual current spikes, since N is proportional to the peak area (Q) according to Faraday's law, $Q = 2FN$ where F = 96 500 C is the Faraday constant. The results showed that peak currents and peak area obeyed log-normal distributions (Figure 3B, C) with I_{max} = 100.7 ± 8.0 pA, and N = 109 000 ± 12 000 molecules (mean ± SEM).

When the cultured hippocampal neurons were incubated with 250 nM Aβ_{1-42} oligomers for 30 min, an unexpected but very interesting result was obtained that both the frequency and amplitude of the amperometric spikes were significantly increased (SI Table S-1). Statistical analyses showed that in a given time (60 s), 55 ± 9 (mean ± SEM) spikes were recorded from control groups, which was increased by ca. 112% (117 ±

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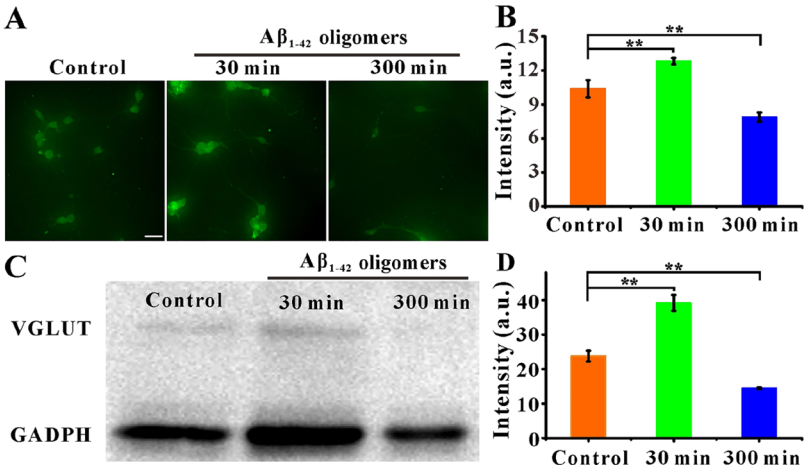

Figure 4. VGLUT1 levels in mature hippocampal neuronal cultures exposed to Aβ_{1-42} oligomers. (A) Representative immunofluorescence images of VGLUT1 levels in hippocampal neurons exposed to vehicle or 250 nM oligomers for 30 or 300 min. Scale bars: 60 μm. (B) Integrated VGLUT1 immunofluorescence levels. (C) Western blots of hippocampal cultures exposed to control or 250 nM oligomers for 30 or 300 min. (D) Graph shows densitometric analysis for VGLUT1 level. GADPH was used as an additional loading control. Error is standard error of the mean (SEM); $^{**}p < 0.01$, compared with control.

9/min) after treating the neurons for 30 min. Moreover, the average I_{max} and N are calculated to be I_{max} = 278.8 ± 11.1 pA and 231 000 ± 2000 molecules, which were increased by ca. 177% and 112%, respectively, compared to those from controlled neurons. Since Aβ_{1-42} oligomers are not electroactive molecules (SI Figure S-8), such a significant increase indicates that they must play biological roles that strikingly promote vesicular Glu release from hippocampal neurons.

Long-term exposure to Aβ_{1-42} oligomers causes serious cell damage and neurite atrophy. As expected, only a few amperometric spikes with tremendously decreased amplitude could be detected from hippocampal neurons incubated by Aβ_{1-42} oligomers for 300 min (Figure 3A). Comparing with that of controls, the frequency of amperometric spikes (14 ± 2/min) was significantly decreased by 75%, and I_{max} (63.9 ± 5.1 pA) and N (73 000 ± 400 molecules) were decreased by ca. 36% and 33%, respectively. This indicate that long-term exposure to toxic Aβ_{1-42} oligomers cause neurotransmitter depletion in hippocampal neurons.

These results from amperomtric detection thus directly demonstrated that vesicular Glu release is strongly affected by Aβ_{1-42} oligomers. Especially, short-term (30 min) incubation of neurons with Aβ_{1-42} oligomers induced a striking increase of Glu release before its depletion caused by long-term Aβ_{1-42} oligomers toxicity. Previous studies have shown that excessive Glu leaded to NMDA receptors overactivation, which was interpreted as a reason for glutamatergic synapses damage.[44−46] Our results indicate that initial synaptic dysfunction may have already initiated at the early stage of AD by releasing excessive amounts of neurotransmitters, thus overactivating NMDA receptors, and eventually resulting in excitotoxicity, neuron damage, neurotransmitter depletion, memony impairment, and learning ability decrease at the late stage of AD.

Aβ_{1-42} Oligomers Engages in VGLUT1 Expression Level on Vesicles. At glutamatergic synapses, transport of Glu into synaptic vesicles is achieved by vesicular glutamate transporters (VGLUTs), and the quantal size of Glu release depends on the VGLUT expression level.[47] Of the three known transporters, VGLUT1, VGLUT2, or VGLUT3, VGLUT1 predominates in the hippocampus, and previous histopathological results showed reduced VGLUT1 density and decreased concentrations of Glu in the later stages of AD.[48,49] To investigate the relationship between the expression of VGLUT1 and Glu release from Aβ_{1-42}-insulted hippocampal neuron, the level of VGLUT1 was quantified by carrying out immunofluorescence imaging and Western blot assay. As shown in Figure 4, neurons after being treated with Aβ_{1-42} oligomers for 30 min indeed exhibited an obvious increase in VGLUT1 levels, while neurons incubated with Aβ_{1-42} oligomers for 300 min showed significantly lower VGLUT1 levels than did the control. This rationalized our amperometric results, suggesting that Aβ_{1-42} oligomers may be a potential intracellular player engaged in VGLUT1 expression to influence neurotransmitter release in age-related progressive degeneration of neurons.

CONCLUSION

In summary, we have successfully monitored vesicular Glu release at single varicosities of Aβ_{1-42}-insulted hippocampal neurons by using a microelectrochemical biosensor of Glu for the first time. Our results revealed that a short-duration exposure to Aβ_{1-42} oligomers promotes a marked increase in the release of Glu before the eventual depletion of the neurotransmitter resulting from long-term exposure to the Aβ_{1-42} oligomers. Such a significant increase in Glu may cause neurotoxicity by overstimulating NMDA receptors as observed in early stage AD patients. Further studies demonstrated that changes in the release of Glu are closely related to the expression level of the VGLUT1 protein at the vesicle, indicating that Aβ_{1-42} oligomers may affect the VGLUT1 levels and hence influence the release of Glu. Though our results have provided valuable information for investigations of the etiology of AD at its different stages, the underlying mechanisms of how Aβ_{1-42} oligomers alter Glu release and VGLUT1 expression in a time-dependent manner remain to be further studied. Furthermore, a great deal of research should be performed to better correlate in vivo results with in vivo conditions of AD. First, the occurrence and development of AD is a long-term process, in vitro studies should simulate this process more precisely, and the results from more time points should be obtained; Second, only Aβ_{1-42} oligomers was used in this work, but Aβ can exist in diverse assembly states, such as

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monomers, dimers, trimers, higher-order oligomers as well as protofibrils, so the effects of Aβ with different forms on neurons should be comprehensively studied. Last but not least, in vitro results should be in vivo validated, in this aspect, amperometric detection on brain slices of rats afflicted with AD would be considered in our future work.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b03826.

SEM images of Pt/carbon fiber microelectrode (Figure S-1), cyclic voltammograms of Glu biosensor toward Glu (Figure S-2), amperometric response time of the Glu sensor (Figure S-3), atomic force microscope (AFM) images of Aβ_{1-42} oligomers (Figure S-4), the morphological observation of hippocampal neurons after Aβ_{1-42} oligomers manipulation for different time (Figure S-5), selectivity of the Glu sensor (Figure S-6), Aβ_{1-42} oligomers induced glutamate release with or without extracellular Ca^{2+} (Figure S-7), electrochemical activity of Aβ_{1-42}oligomers (Figure S-8), and different parameters calculated for single exocytosis events (Table S-1) (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Spitzer, N. C. *Annu. Rev. Neurosci.* **2017**, *40*, 1−19.
(2) Zádori, D.; Veres, G.; Szalárdy, L.; Klivényi, P.; Toldi, J.; Vécsei, L. *J. Alzheimer's Dis.* **2014**, *42*, S177−S187.
(3) Rodriguez-Perdigon, M.; Tordera, R. M.; Gil-Bea, F. J.; Gerenu, G.; Ramirez, M. J.; Solas, M. *Hippocampus* **2016**, *26*, 1303−1312.
(4) Brito-Moreira, J.; Paula-Lima, A. C.; Bomfim, T. R.; Oliveira, F. B.; Sepulveda, F. J.; De Mello, F. G.; Aguayo, L. G.; Panizzutti, R.; Ferreira, S. T. *Curr. Alzheimer. Res.* **2011**, *8*, 552−562.
(5) Abramov, E.; Dolev, I.; Fogel, H.; Ciccotosto, G. D.; Ruff, E.; Slutsky, I. *Nat. Neurosci.* **2009**, *12*, 1567−1576.
(6) Francis, P. T. *J. Geriatr. Psychiatry* **2003**, *18*, S15−S21.
(7) Chen, G. F.; Xu, T. H.; Yan, Y.; Zhou, Y. R.; Jiang, Y.; Melcher, K.; Xu, H. E. *Acta Pharmacol. Sin.* **2017**, *38*, 1205−1235.
(8) Zott, B.; Simon, M. M.; Hong, W.; Unger, F.; Chen-Engerer, H.; Frosch, M. P.; Sakmann, B.; Walsh, D. M.; Konnerth, A. *Science* **2019**, *365*, 559−565.
(9) Talantova, M.; Sanz-Blasco, S.; Zhang, X.; Xia, P.; Akhtar, M. W.; Okamoto, S.; Dziewczapolski, G.; Nakamura, T.; Cao, G.; Pratt, A. E.; Kang, Y.; Tu, S.; Molokanova, E.; McKercher, S. R.; Hires, S. A.; Sason, H.; Stouffer, D. G.; Buczynski, M. W.; Solomon, J. P.; Michael, S.; Powers, E. T.; Kelly, J. W.; Roberts, A.; Tong, G.; Fang-Newmeyer, T.; Parker, J.; Holland, E. A.; Zhang, D.; Nakanishi, N.; Chen, H. S. V.; Wolosker, H.; Wang, Y.; Parsons, L. H.; Ambasudhan, R.; Masliah, E.; Heinemann, S. F.; Piña-Crespo, J. C.; Lipton, S. A. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, E2518−E2527.
(10) Hashimoto, A.; Nishikawa, T.; Oka, T.; Takahashi, K.; Hayashi, T. *J. Chromatogr., Biomed. Appl.* **1992**, *582*, 41−48.
(11) Marvin, J. S.; Borghuis, B. G.; Tian, L.; Cichon, J.; Harnett, M. T.; Akerboom, J.; Gordus, A.; Renninger, S. L.; Chen, T.; Bargmann, C. I.; Orger, M. B.; Schreiter, E. R.; Demb, J. B.; Gan, W.; Hires, S. A.; Looger, L. L. *Nat. Methods* **2013**, *10*, 162−170.
(12) Okumoto, S.; Looger, L. L.; Micheva, K. D.; Reimer, R. J.; Smith, S. J.; Frommer, W. B. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 8740−8745.
(13) Hires, S. A.; Zhu, Y.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 4411−4416.
(14) Ren, L.; Mellander, L. J.; Keighron, J.; Cans, A. S.; Kurczy, M. E.; Svir, I.; Oleinick, A.; Amatore, C.; Ewing, A. G. *Q. Rev. Biophys.* **2016**, *49*, No. e12.
(15) Wightman, R. M.; Jankowski, J. A.; Kennedy, R. T.; Kawagoe, K. T.; Schroeder, T. J.; Leszczyszyn, D. J.; Near, J. A.; Diliberto, E. J.; Viveros, O. H. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 10754−10758.
(16) Mosharov, E. V.; Sulzer, D. *Nat. Methods* **2005**, *2*, 651−658.
(17) Wightman, R. M. *Science* **2006**, *311*, 1570−1574.
(18) Amatore, C.; Arbault, S.; Guille, M.; Lemaître, F. *Chem. Rev.* **2008**, *108*, 2585−2621.
(19) Ganesana, M.; Lee, S. T.; Wang, Y.; Venton, B. J. *Anal. Chem.* **2017**, *89*, 314−341.
(20) Phan, N. T.; Li, X.; Ewing, A. G. *Nat. Rev. Chem.* **2017**, *1*, 48.
(21) Majdi, S.; Berglund, E. C.; Dunevall, J.; Oleinick, A. I.; Amatore, C.; Krantz, D. E.; Ewing, A. G. *Angew. Chem., Int. Ed.* **2015**, *54*, 13609−13612.
(22) Barlow, S. T.; Louie, M.; Hao, R.; Defnet, P. A.; Zhang, B. *Anal. Chem.* **2018**, *90*, 10049−10055.
(23) Li, X.; Dunevall, J.; Ren, L.; Ewing, A. G. *Anal. Chem.* **2017**, *89*, 9416−9423.
(24) Li, X.; Majdi, S.; Dunevall, J.; Fathali, H.; Ewing, A. G. *Angew. Chem., Int. Ed.* **2015**, *54*, 11978−11982.
(25) Li, Y.; Zhang, S.; Wang, L.; Xiao, R.; Liu, W.; Zhang, X.; Zhou, Z.; Amatore, C.; Huang, W. *Angew. Chem., Int. Ed.* **2014**, 12456−12460.
(26) Li, Y.; Zhang, S.; Wang, X.; Zhang, X.; Oleinick, A. I.; Svir, I.; Amatore, C.; Huang, W. *Angew. Chem., Int. Ed.* **2015**, *54*, 9313−9318.
(27) Qiu, Q.; Zhang, F.; Tang, Y.; Zhang, X.; Jiang, H.; Liu, Y.; Huang, W. *Electroanalysis* **2018**, *30*, 1054−1059.
(28) Wang, Y.; Mishra, D.; Bergman, J.; Keighron, J. D.; Skibicka, K. P.; Cans, A. *ACS Chem. Neurosci.* **2019**, *10*, 1744−1752.
(29) Wang, Y.; Fathali, H.; Mishra, D.; Olsson, T.; Keighron, J. D.; Skibicka, K. P.; Cans, A. *J. Am. Chem. Soc.* **2019**, *14*117507.
(30) Wojcik, S. M.; Rhee, J. S.; Herzog, E.; Sigler, A.; Jahn, R.; Takamori, S.; Brose, N.; Rosenmund, C.; Südhof, T. C. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 7158−7163.
(31) Martineau, M.; Guzman, R. E.; Fahlke, C.; Klingauf, J. *Nat. Commun.* **2017**, *8*, 2279.
(32) Kaech, S.; Banker, G. *Nat. Protoc.* **2006**, *1*, 2406−2415.
(33) Ciapetti, G.; Cenni, E.; Pratelli, L.; Pizzoferrato, A. *Biomaterials* **1993**, *14*, 359−364.
(34) Bennett, M. R. *J. Auton. Nerv. Syst.* **2000**, *81*, 25−30.
(35) Umeda, T.; Ebihara, T.; Okabe, S. *Mol. Cell. Neurosci.* **2005**, *28*, 264−274.
(36) Moreno, H.; Yu, E.; Pigino, G.; Hernandez, A. I.; Kim, N.; Moreira, J. E.; Sugimori, M.; Llinas, R. R. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5901−5906.
(37) Selkoe, D. J. *Science* **2019**, *365*, 540−541.
(38) Liu, H.; Zhou, X.; Shen, Q.; Xing, D. *Theranostics* **2018**, *8*, 2289−2299.
(39) Feng, L.; Watanabe, H.; Molino, P.; Wallace, G. G.; Phung, S. L.; Uchihashi, T.; Higgins, M. J. *J. Mol. Biol.* **2019**.*4312687*

DOI: 10.1021/acs.analchem.9b03826
Anal. Chem. 2019, 91, 15123−15129

(40) Shankar, G. M.; Bloodgood, B. L.; Townsend, M.; Walsh, D. M.; Selkoe, D. J.; Sabatini, B. L. *J. Neurosci.* **2007**, *27*, 2866−2875.
(41) Selkoe, D. J. *Science* **2002**, *298*, 789−791.
(42) Chang, J.; Liu, F.; Lee, M.; Wu, B.; Ting, K.; Zara, J. N.; Soo, C.; Al Hezaimi, K.; Zou, W.; Chen, X.; Mooney, D. J.; Wang, C. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9469−9474.
(43) Jović, M.; Lončarević-Vasiljković, N.; Ivković, S.; Dinić, J.; Milanović, D.; Zlokovic, B.; Kanazir, S. *PLoS One* **2019**, *14*, No. e216726.
(44) Chang, J.; Liu, F.; Lee, M.; Wu, B.; Ting, K.; Zara, J. N.; Soo, C.; Al Hezaimi, K.; Zou, W.; Chen, X.; Mooney, D. J.; Wang, C. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9469−9474.
(45) Hardingham, G. E.; Bading, H. *Nat. Rev. Neurosci.* **2010**, *11*, 682−696.
(46) Canas, P. M.; Simões, A. P.; Rodrigues, R. J.; Cunha, R. A. *Neuropharmacology* **2014**, *76*, 51−56.
(47) Daniels, R. W.; Collins, C. A.; Chen, K.; Gelfand, M. V.; Featherstone, D. E.; DiAntonio, A. *Neuron* **2006**, *49*, 11−16.
(48) Kashani, A.; Lepicard, È.; Poirel, O.; Videau, C.; David, J. P.; Fallet-Bianco, C.; Simon, A.; Delacourte, A.; Giros, B.; Epelbaum, J.; Betancur, C.; El Mestikawy, S. *Neurobiol. Aging* **2008**, *29*, 1619−1630.
(49) Fremeau, R. T.; Kam, K.; Qureshi, T.; Johnson, J.; Copenhagen, D. R.; Storm-Mathisen, J.; Chaudhry, F. A.; Nicoll, R. A.; Edwards, R. H. *Science* **2004**, *304*, 1815−1819.

DOI: 10.1021/acs.analchem.9b03826
Anal. Chem. 2019, 91, 15123−15129