7.5. Experimental Results

7.5.1. Elemental images of neurons

Elemental distribution in untreated neurons

A SEM photograph of a neuron dried on PET film is shown in figure 7.14a. XRF spectra measured at points of the center of the cell body, the root of the axon and dendrite, and the axon are shown in figure 7.15a-d.

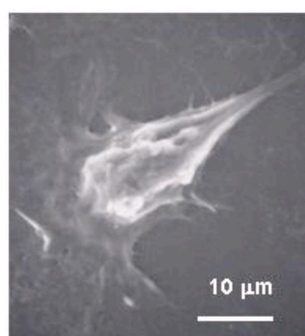

Figure 7.14a

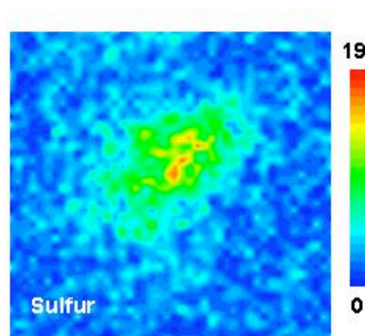

Figure 7.14c

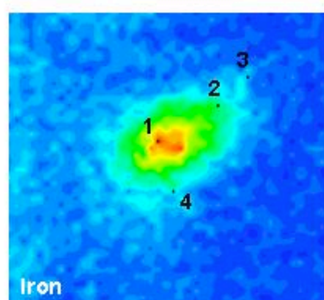

Figure 7.14b

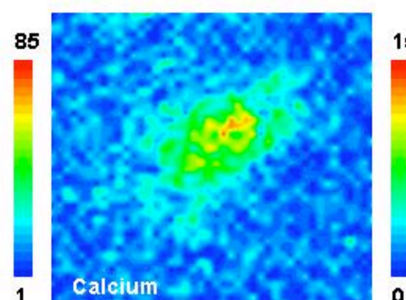

Figure 7.14d

Figure 7.14a-d. A SEM photograph of a neuron dried on a PET film is shown in figure 7.14a. The elemental distribution of Ca, S and Fe within a single untreated neuron (neuron that was cultured in non-metallic environment) are shown in figure 7.14b, 7.14c and 7.14d. In figure 7.14b, the plotted points named No. 1 to 4 are defined as the center of cell body, axon hillock, axon and junction between axon and cell membrane.

The elemental distributions of Ca, S and Fe within a single untreated neuron (the neuron that was cultured in non-metal environment) are shown in figure 7.14b, c and d, respectively. These images are matrices of 45 × 45 pixels of 1 µm resolution. The range of the fluorescent x-ray intensity of Ca is from 0 to 15 counts, and this range is divided into twenty levels. Each level of the measured and interpolated points has been assigned

to a corresponding shade of red, green and blue and plotted as shown in figure 7.14b. Similarly, figure 7.14c shows the distribution of S in the same cell and the range of the intensity of S is from 0 to 19 counts. Figure 7.14d shows a similar plot of the distribution of Fe in the same cell, with the range of the intensity from 1 to 85 counts. While the distribution of Ca, S and Fe in the neuron are almost identical as can be seen in figures 7.14b, c and d, the densities are completely different. These differences is evident in the case Fe, which is the strongest element detected in the x-ray energy range of 1 to 10 keV.

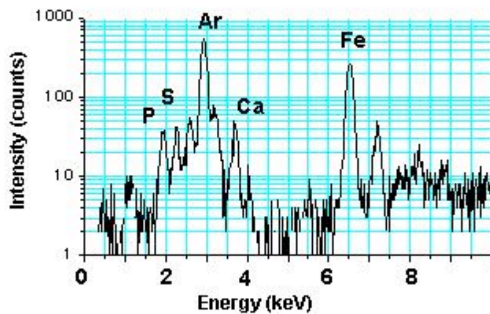

Figure 7.15a

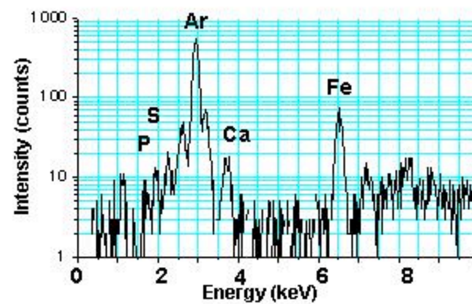

Figure 7.15c

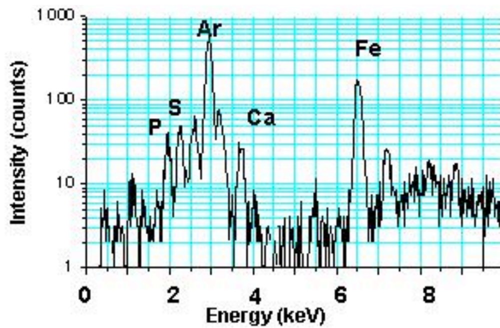

Figure 7.15b

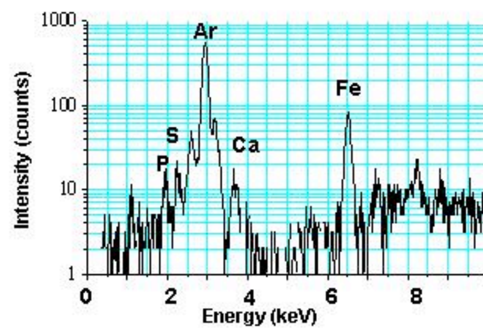

Figure 7.15d

Figure 7.15a-d. XRF spectra measured at the points of the center of the cell body, axon hillock and the junction between dentrites and cell membrane, are shown in 7.15a-d, respectively. The measuremnt time was 200 sec and the excitation energy was 14.2 keV.

Exposure to vanadium chloride solution

<u>Elemental distribution in treated neurons</u>

Treated neurons were cultured in a vanadium chloride solution environment with different exposure times to investigate the difference in uptake of vanadium and also the variations in the distribution of the intracellular elements. The exposure times were 4 and 24 h. As for the cells cultured in the V chloride solution environment, four images for each cell - showing the distributions of P, S, Ca and Fe - were obtained with the

exposure time as a variable. There was no evidence of internalization of V into the neurons.

The images of Fe, S and Ca within the neuron cultured in a vanadium solution (0.04 g/L) for 4 h. are shown in figure 7.16b-d. These images are matrices of 45 × 45 pixels with a resolution of 1 μm. As before, the ranges of density of Fe, S and Ca are each divided into twenty levels, and each level assigned to a shade of green, red and blue. The ranges of the florescent x-ray intensities of Fe, S and Ca are from 0 to 208, 0 to 20 and 0 to 12 counts respectively. The measurement time was 5 sec. The experimental results shown in figures 7.16b-d reveal that these distributions have almost identical patterns as in the untreated cells. However, in figure 7.16c, the density of Ca within the cell decreased and the distribution patterns became obscure.

The images for Fe, S and Ca from neurons cultured in a V solution (0.04 g/L) for 24 h. are shown in figure 7.17b-d. The matrices of these images are 45 × 45 pixels. As before, these images measured in 5 sec, are of 1 μm resolution and the ranges of the intensities of Fe, S and Ca are from 0 to 86, 0 to 12, 0 to 8 counts respectively. These results show that these distributions have almost identical patterns to those in untreated cells. Except for figure 7.17c, where the density of Ca within the cell decreased and the distribution pattern became obscure. In this case, the depletion of Ca from inside the cell is more remarkable than that in the case of the neurons cultured in a V solution for 4 h.

Quantification of density of intracellular elements as a function of time and dose

For the quantification of the density of the intracellular elements, XRF spectra were obtained at the axon hillock (the junction of the dendrite and the cell membrane), the center of the cell body and the axon in each cell. The measurement time was 200 sec. for each point. Each point is shown in figure 7.14, 7.16 and 7.17. In order to explain the increase or decrease in the density of the elements in relation to the effect of vanadium, it is necessary to invoke the parameter: “relative density” again, defined as the ratio of the integrated value of fluorescent intensities of each element at the point in the treated neuron divided by that of the same element at the same point in the untreated one. A relative density of less than 1, implies that the density of that element in the treated cell is lower than that in the untreated one, conversely a value higher than 1 implies higher.

The intensities, normalized values and relative densities of elements at the points in the untreated and treated neurons are calculated and shown in table 7.1 to 7.12. The results from the untreated cell are shown in table 7.1 to 7.4. The results from the treated cell, which was cultured in a vanadium solution environment for 4 h., are shown in table

7.5 to 7.8. The results from the treated cell, which was cultured in a V solution environment for 24 h., are shown in table 7.9 to 7.12.

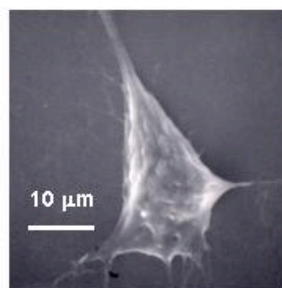

Figure 7.16a

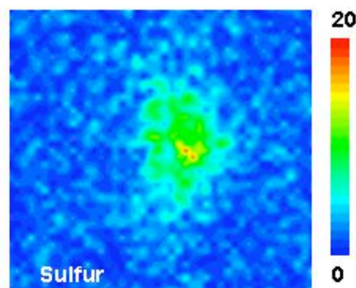

Figure 7.16c

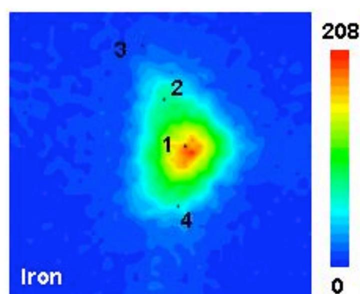

Figure 7.16b

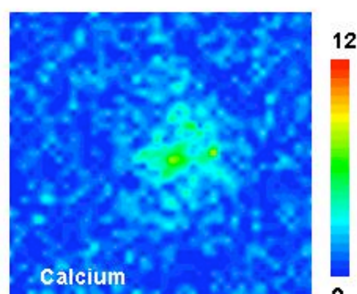

Figure 7.16d

Figure 7.16a-d. A SEM photograph of a neuron dried on a PET film is shown in figure 7.16a. The elemental distribution of Ca, S and Fe within a single cell that was cultured in a 0.04 g/L V solution environment for 4 h. are shown in figure 7.16b, 7.16c and 7.16d, respectively. In figure 7.16b, the plotted points named No. 1 to 4 are defined as the center of cell body, axon hillock, axon and junction between axon and cell membrane.

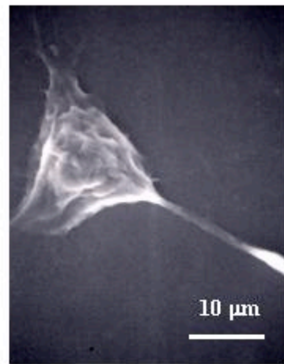

Figure 7.17a

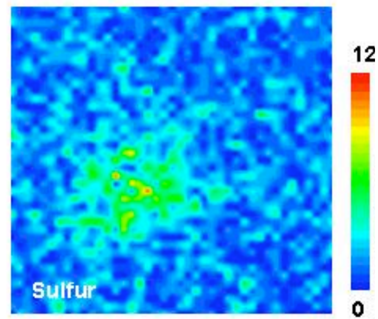

Figure 7.17c

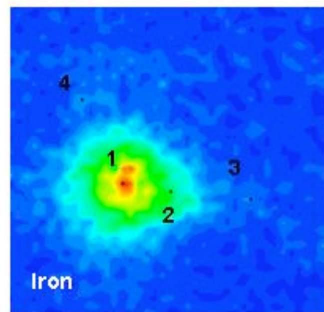

Figure 7.17b

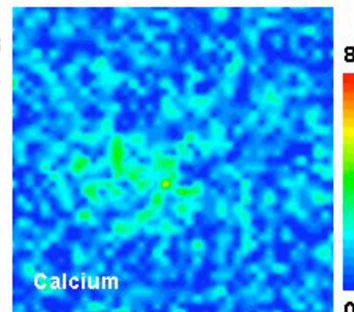

Figure 7.17d

Figure 7.17a-d. A SEM photograph of a neuron dried on a PET film is shown in figure 7.17a. The elemental distribution of Ca, S and Fe within a single cell that was cultured in a 0.04 g/L V solution environment for 24 h. are shown in figure 7.17b, 7.17c and 7.17d, respectively. In figure 7.17b, the plotted points named No. 1 to 4 are defined as the center of cell body, axon hillock, axon and junction between axon and cell membrane.

Table 7.1-7.8. The intensities of the elements within the untreated cells are shown in tables 7.1 to 7.4. The intensities and relative densities of the elements in the treated cells with V solution environment for 4 h. are shown in tables 7.5 to 7.8.

Center of cell body	
Ar	1
P	0.0577874
S	0.065263
Cl	0.0937934
K	0.0495532
Ca	0.0673032
V	0
Cr	0
Fe	0.5865029
Cu	0
Zn	0

Table 7.1

Axon hillock	
Ar	1
P	0.062354314
S	0.089460932
Cl	0.101415143
K	0.078982306
Ca	0.046679999
V	0
Cr	0
Fe	0.309780909
Cu	0
Zn	0

Table 7.2

Axon	
Ar	1
P	0.019229759
S	0.026213471
Cl	0.087331254
K	0.048947706
Ca	0.031951731
V	0
Cr	0
Fe	0.115855142
Cu	0
Zn	0

Table 7.3

Junction	
Ar	1
P	0.014106523
S	0.018333989
Cl	0.075479661
K	0.043152601
Ca	0.015510345
V	0
Cr	0
Fe	0.131122619
Cu	0
Zn	0

Table 7.4

Center of cell body		
Element	Intensity	Relative
Ar	1	
P	0.05367	0.928749
S	0.051489	0.788946
Cl	0.0562	0.599189
K	0.07326	1.478411
Ca	0.020417	0.303359
V	0	
Cr	0	
Fe	1.427985	2.434474
Cu	0.021008	
Zn	0	

Table 7.5

Axon hillock		
Element	Intensity	Relative
Ar	1	
P	0.016004	0.256662
S	0.027547	0.307922
Cl	0.051059	0.503465
K	0.016937	0.21444
Ca	0	0
V	0	
Cr	0	
Fe	0.52575	1.697167
Cu	0	
Zn	0	

Table 7.6

Axon		
Element	Intensity	Relative
Ar	1	
P	0	0
S	0	0
Cl	0.039376	0.450881
K	0	0
Ca	0	0
V	0	
Cr	0	
Fe	0.100283	0.86559
Cu	0	
Zn	0	

Table 7.7

Junction		
Element	Intensity	Relative
Ar	1	
P	0	0
S	0	0
Cl	0.036364	0.481773
K	0	0
Ca	0	0
V	0	
Cr	0	
Fe	0.109181	0.832663
Cu	0	
Zn	0	

Table 7.8

Table 7.9-7.12. Intensities and relative densities of the elements in the treated cells with V solution environment for 24 h.

Center of cell body		
Element	Intensity	Relative
Ar	1	
P	0.051951	0.899002
S	0.050841	0.779017
Cl	0.057341	0.542053
K	0.032988	0.665709
Ca	0	0
V	0	
Cr	0	
Fe	0.96081	1.638202
Cu	0.029053	
Zn	0	

Table 7.9

Axon hillock		
Element	Intensity	Relative
Ar	1	
P	0.039774	0.637873
S	0.036107	0.403606
Cl	0.038667	0.381275
K	0.026088	0.330302
Ca	0	0
V	0	
Cr	0	
Fe	0.617954	1.99481
Cu	0	
Zn	0	

Table 7.10

Axon		
Element	Intensity	Relative
Ar	1	
P	0	0
S	0	0
Cl	0.060561	0.693464
K	0	0
Ca	0	0
V	0	
Cr	0	
Fe	0.102076	0.881066
Cu	0	
Zn	0	

Table 7.11

Junction		
Element	Intensity	Relative
Ar	1	
P	0	0
S	0.008463	0.461604
Cl	0.03808	0.408057
K	0	0
Ca	0	0
V	0	
Cr	0	
Fe	0.098114	0.748262
Cu	0	
Zn	0	

Table 7.12

Center of cell body

The cell body is the metabolic center of the cell. It contains the nucleus, which stores the genes of the cell, and the rough and smooth endoplasmic reticulum, which synthesizes the proteins of the cell.

Fe is the main element of the neuron detected in the energy range from 1 to 10 keV. In the control cell body, a large amount of Fe was observed and its peak intensity was detected at the center. After exposing the neuron to a V solution for 4 h., the density of Fe at the cell body center increased, which is shown in table 4.13. On the other hand, the densities of P, S, Cl and Ca, decreased. The outflow of Ca was especially remarkable. After exposing the neuron to a V solution environment for 24 h., the density of Fe at the center increased even more and so did the depletion of Ca, compared to the case of 4 h. exposure. The densities of P, S and Cl were still at a lower level than those in the control cell.

Axon hillock

Electric disturbances generated in the dendrites or cell body spread to the axon hillock. The various depolarizations and hyperpolarizations move by passive spread along the dendrite plasma membrane from the synapse to the cell body and then to the axon hillock. Whether a neuron generates an action potential in the axon hillock depends on the balance of the timing, amplitudes, and localization of all the various inputs it receives. Action potentials are generated whenever the membrane at the axon hillock is depolarized to a certain voltage called the threshold potential [14].

After exposing the neuron to vanadium solution for 4 h., the density of Fe at the axon hillock in the neuron increased, which is shown in table 4.14. On the other hand, the densities of the other elements at the axon hillock, which are P, S, Cl, K and Ca, decreased after exposing to V. Especially the depletion of Ca from the neuron was the most remarkable. After exposing the neuron to a V solution environment for 24 h., the density of Fe at the axon hillock further increased. The density of P also increased more than that in the neuron cultured for 4 h., but that was still lower level than that in the control neuron. The densities of the other elements were also still at a lower level than those in the control cell.

Compared with the relative density at the center of the cell body, it can be observed that the tendencies of the change of the elemental densities are different. After 4 h., the density of Fe at the axon hillock increased, but the further increase was observed at the center of the cell body. On the other hand, the densities of P, S and Ca decreased at the center of the cell body, but the further decrease was observed at the axon hillock.

Especially, the depletion of Ca from the axon hillock was remarkable. After 24 h., the density of Fe at the axon hillock further increased, but that at the center of the cell body decreased compared with that in the case of the cell cultured in a V solution for 4 h.

Junction of dendrites and cell membrane

Most neurons have several dendrites. These branch out in tree-like fashion and serve as the main apparatus for receiving signals from other neurons. Dendrites are specialized to receive chemical signals form the axon termini of other neurons. Dendrites convert these signals into small electric impulses and transmit them to the cell body.

After exposing the neuron to a V solution for 4 h., the density of Fe at the junction of dendrites and cell membrane in the neuron decreased a little but this value was almost identical to that in the control cell, which is shown in table 4.12 and 4.16. On the other hand, the densities of P, S and Ca decreased. After 24 h., the intracellular state at the junction of dendrites and cell membrane was almost identical to that after 4 h.

Axon

Most neurons have a single axon, whose diameter varies from a micrometer in certain nerves of the human brain to a millimeter in the giant fiber of the squid. Axons are specialized for the conduction of a particular type of electric impulse, called an action potential, away from the cell body.

After exposing the neuron to a V solution for 4 h., the density of Fe at the axon in the neuron was almost identical to that in the control cell, which is shown in table 7.11 and 7.15. After 24 h., the density of Fe at this point had almost the same value. However, the densities of P, S and Ca decreased remarkably.

Summary

As a result of exposing the cell to a vanadium solution, the density of iron increased remarkably. Especially, the conspicuous increase of iron was observed at the axon hillock and center of the cell body. On the other hand, the depletion of calcium was observed in the cell. Internalization of vanadium into the cell was not observed. However, the SEM photographs revealed that many of the dendrites were lost after exposing the neurons to a vanadium solution environment. It is unclear whether vanadium solution directly injured the dendrites or whether the injurious effect to the cell body induced the subsequent degeneration of the dendrites. In either case, it is probable that iron is closely related to the defensive mechanism against foreign metal

elements and the process of cell death.

Exposure to chromium oxide solution

Elemental distribution in treated neurons

Treated neurons were cultured in a chromium oxide (CrO_3) solution environment with different exposure times to investigate the differences in uptake of chromium and also the variations in the distribution of the intracellular elements. The exposure times were 0.5 and 4 h. As for the cells cultured in a Cr oxide solution environment, four images for each cell - showing the distributions of phosphorus, sulfur, chromium and iron - were obtained with the exposure time as a variable.

The images (elemental distributions) of P, S, Cr and Fe within the neuron cultured in chromium solution (0.04 g/L) for 0.5 h. are shown in figure 7.18b, c, d and e. As previously, all these images are measured in 5 sec., of 1 μm resolution and the ranges of density of P, S, Cr and Fe are each divided into twenty levels, each assigned to a corresponding shade (green, red and blue). The ranges of the fluorescent x-ray intensities of P, S, Cr and Fe are from 0 to 21, 0 to 17, 0 to 11 and 0 to 61 counts respectively. The results show almost identical patterns to those from untreated cells.

The images for neurons cultured for 4 h. are shown in figure 7.19b, c, d and e. The ranges of the florescent x-ray intensities of P, S, Cr and Fe are from 0 to 11, 0 to 10, 0 to 12 and 0 to 121 counts respectively. These results also reveal almost identical patterns to those from the untreated cells.

Relative density of intracellular elements as a function of time and dose

For the quantification of the density of the intracellular elements, the notion of relative density as defined previously will be used again. XRF spectra were obtained at the junction of dendrite and cell membrane, and the center of the cell body. In the case of V solution, XRF spectra were obtained at the axon hillock and axon. However, in this case, it was impossible to distinguish the axon from many of the dendrites. The measurement time was 200 sec. for each point. Each point is shown in figure 7.18 and 7.19. The relative densities at the axon hillock and axon were left out of consideration.

The intensities, normalized values and relative densities of elements at the points in the untreated and treated neurons are shown in table 7.13 to 7.16. The results from the treated cell, which was cultured in a Cr oxide solution environment for 0.5 h., are shown in table 7.13 and 7.14, and for the 4 h. in table 7.15 and 7.16.