Behavior of stimulus response signals in a rat cortical neuronal network under Xe pressure

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Supplementary data

A. Change of neuronal-network activity during cell culture period and during control period

For a week, we culture a sample in which about 10^5 of dissociated neurons are seeded in 20 mm^2, then we use the MED system to measure the spontaneous firing rate (SR) of neurons on the electrodes (count per minute, cpm). The resulting SR is shown by the blue line in Fig. S1.

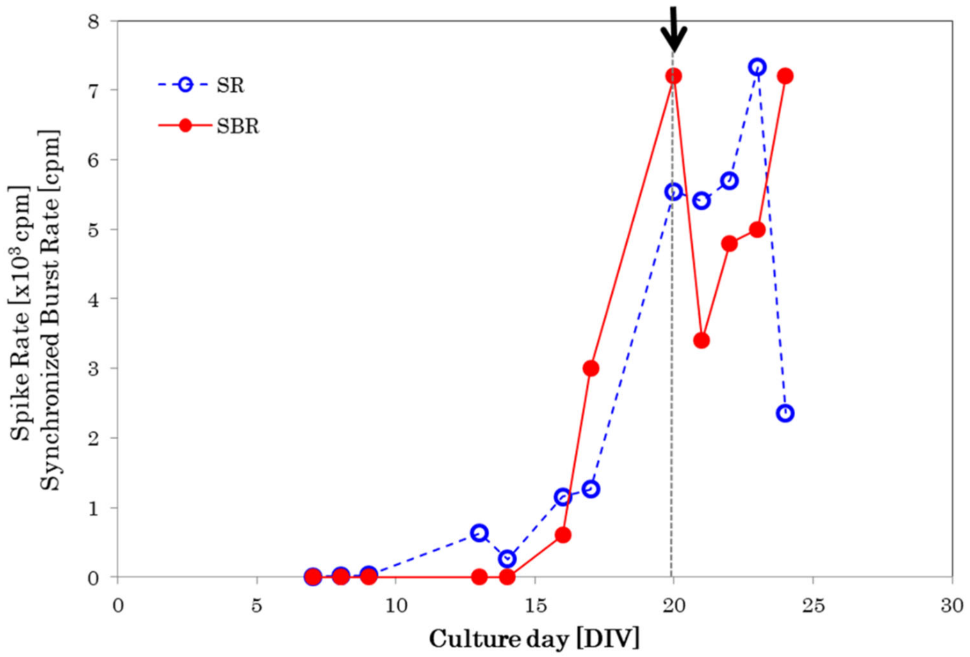

Fig. S1: Changes in spontaneous firing rate (SR: blue open circles and dashed line) and synchronous burst rate (SBR: red solid circles and solid line) for each culture day. The black arrow and dotted line on the 20th day in-vitro (DIV) marks the day at which SBR reached a maximum. On this day, we run a Xe-0.3MPa pressurization experiment.

After about two weeks culture, SR increases and burst firings are observed, indicating a neuronal network is formed. Then, burst signals are observed simultaneously in different electrodes. The synchronous burst rate (SBR), or bursts per minute, is shown by the red line in Fig. S1. The changes in SR and SBR with respect to the culture days are consistent with those in our previous study (Uchida et al., 2012, 2017), as shown in Fig. S1.

Figure S2 shows the waveforms on the 16 electrodes during the period of a synchronous burst. The number ratio of electrodes showing the burst signal at about the same time is 12/15.

Compare the example in Fig. S2 with Fig. 2 and notice that the clear synchronous burst waveforms occur on the electrodes where the neuronal network extends. This example helps to establish that the activity of the network cultured in vitro on the multi-electrode array can be measured by our MED system. Also, we confirmed that the changes of neuronal-network activities during the culture period are in good agreement with our previous studies (Uchida et al., 2012, 2017).

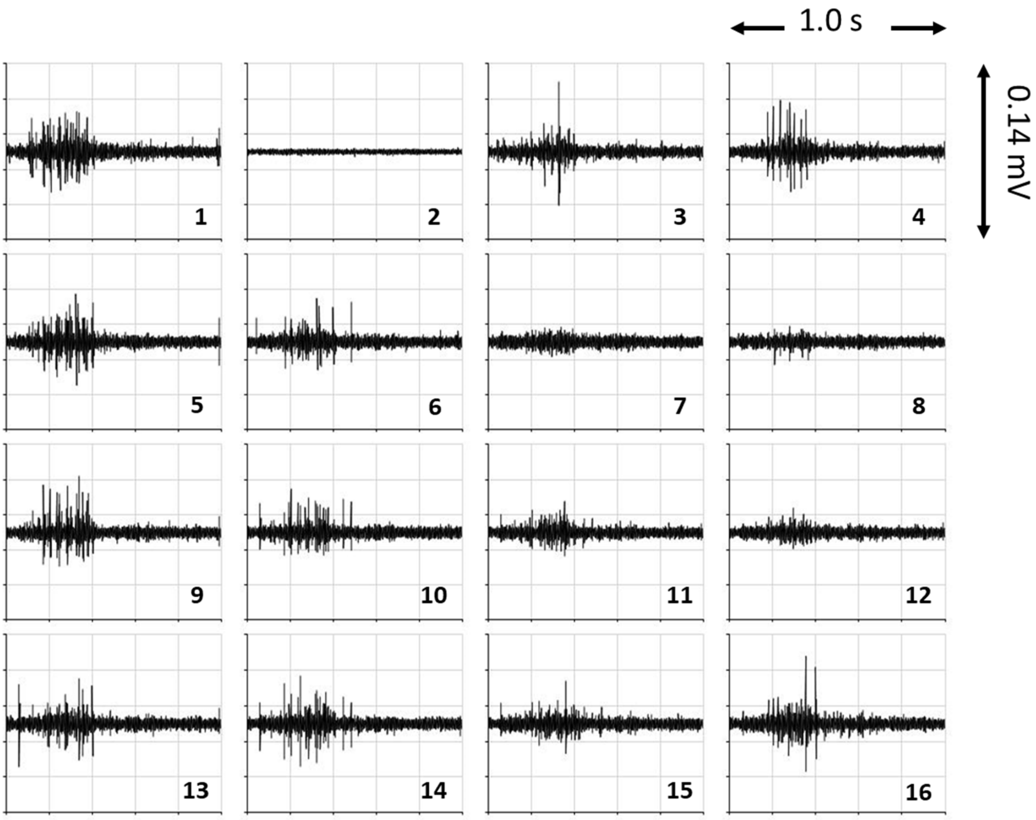

Fig. S2: Typical waveforms of a synchronous burst in the control period. The sample is the same as that in Figs. 2–4 and S1. Clear synchronous burst waveforms occur in channels 1, 3–6, 9–11, and 13–15. (Electrode #2 is used for the stimulus input, so it cannot record the action potential.)

B. Stimulation response behavior with only air pressure by 0.3 MPa

To test whether the stimulus-response signal transduction of the neuronal network is suppressed by a chemical action from Xe or simply by a physical pressure effect, we ran experiments using air pressurized by 0.3 MPa instead of Xe (i.e., having a stage with a total air pressure of 0.4 MPa). Note that these experiments omit the "air replacement stage", and finish 60 minutes after depressurization.

At first, we confirmed that there was essentially no change in the number of synchronous bursts when pressurizing only air (data not shown). This result agrees with our previous study (Uchida et al., 2012).

In the second experiment, we measure ρ and $\Delta\tau$ of the stimulus response signal and construct an integrated distribution of $\Delta\tau$ for each stage. For the integration, we include both signals slower than 2.5 ms (synaptic transmission) and faster than 2.5 ms (axon transmission). The result in Fig. S3 shows almost no change in the distribution diagrams beyond each stage were observed in the measured samples.

Therefore, the experiments confirm that neither the stimulus response nor the signal transmission are affected by the physical condition up to 0.3 MPa pressurization, but instead are both affected by a chemical effect of Xe pressurization.

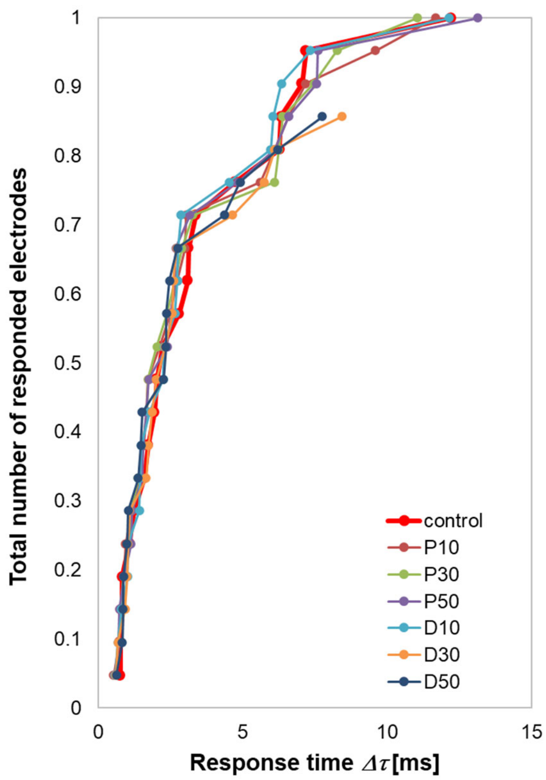

Fig. S3: Same as Fig. 8 except air pressurized to 0.3 MPa (no Xe). The total number of responded electrodes is standardized by that in the control stage.

C. Stimulation response behavior with various Xe pressures

In the main text, the signal analysis and results are described in detail for the 0.3 MPa Xe case as an example. Here we give similar details for the other pressure cases. Results for each pressure condition are averages from measurements on three cultured cell networks, categorized (as with the 0.3 MPa case) with respect to the distance between the observation electrode and the stimulus-input electrode.

(1) Signal transmission suppression when Xe was added at pressures above 0.3 MPa

When Xe of 0.4 or 0.5 MPa was added, we found that the synchronous bursts were suppressed within 5 minutes, yet the spontaneous firing continued. In addition, we confirmed that synchronous bursts did not recover 10 minutes after depressurization. Instead, bursts started to synchronize after at least 30 minutes, but their number density remained low. After replacing the gas with air, the density of synchronous bursts increased, and within 30 minutes after air replacement, the SBR reached the control level. These results are qualitatively consistent with those in our previous studies (Uchida et al., 2017).

Under these same conditions, we measure the spontaneous firing and stimulus response signals. We show here our analyses of the response ratios and the response times from the stimulus signal for all electrodes at each stage of the experiment. For these higher-pressure cases, we plot the average response ratio ρ in Fig. S4 and in the average response time $\Delta\tau$ in Fig. S5, with respect to the distance d between the observation electrode and the stimulus-input electrode.

As in the case of 0.3 MPa, Fig. S4 shows that for these higher pressures, ρ also begins to decrease in the pressure period, becoming roughly constant 30 minutes after pressurization. For the case of 0.4 MPa of Xe (left), ρ decreases to about half that of the control stage for most electrodes. For 0.5 MPa (right), on the other hand, the response signal decreases to zero at most electrodes. Figure S5 shows that $\Delta\tau$ tends to be increased to more than 10 ms soon after the pressurization for most electrodes, which are not plotted. For the 0.4 MPa case (left), a few signals disappear, but many signals that were delayed. For the 0.5 MPa case (right), on the other hand, many signals disappear, and it can be seen that such signals are significantly delayed after pressurization. These results, together with the 0.3 MPa case (Fig. 7), indicate that the change in response increases as the amount of pressurization of Xe gas increases. Here it is noted that there were several signals that changed so that $\Delta\tau$ decreased after pressurization (e.g., green, orange and dark blue signals for 0.4 MPa case, and blue and red signals for 0.5 MPa case). Such signal was almost constant at shorter than 2 ms in the pressurization to depressurized stage. So, there is a possibility that the signal measured in the control stage may differ from the signal observed in the stage after pressurization.

Figure S6 shows the integrated distributions of response time for each stage. At 0.4 MPa of Xe (left), the fast response signals below 2.5 ms, overlapping with the control stage, are neither delayed nor vanished throughout the entire stages. However, the slow response signals shift to longer times with pressurization, with some of them delaying over 10 ms or vanishing. At the end of the pressurization stage, only the fast response signals remain, which make up about half of the original response number. On the other hand, when 0.5 MPa of Xe was pressurized (right), even the fast response signals delay, with almost all signals are vanishing within 30 minutes of pressurization. As the fast response signal is considered to be from axon transmission, the results indicate that sufficiently high Xe pressure can suppress not only the synaptic transmission, but also the membrane transmission. That is, the threshold for inhibition of all signal transmission is about 0.5 MPa of Xe in this system. Even when both synchronous burst and signal transmission between neurons are suppressed, spontaneous firings still occur (with some neurons firing more frequently). Therefore, the activity of neurons themselves is not suppressed by simple Xe pressurization.

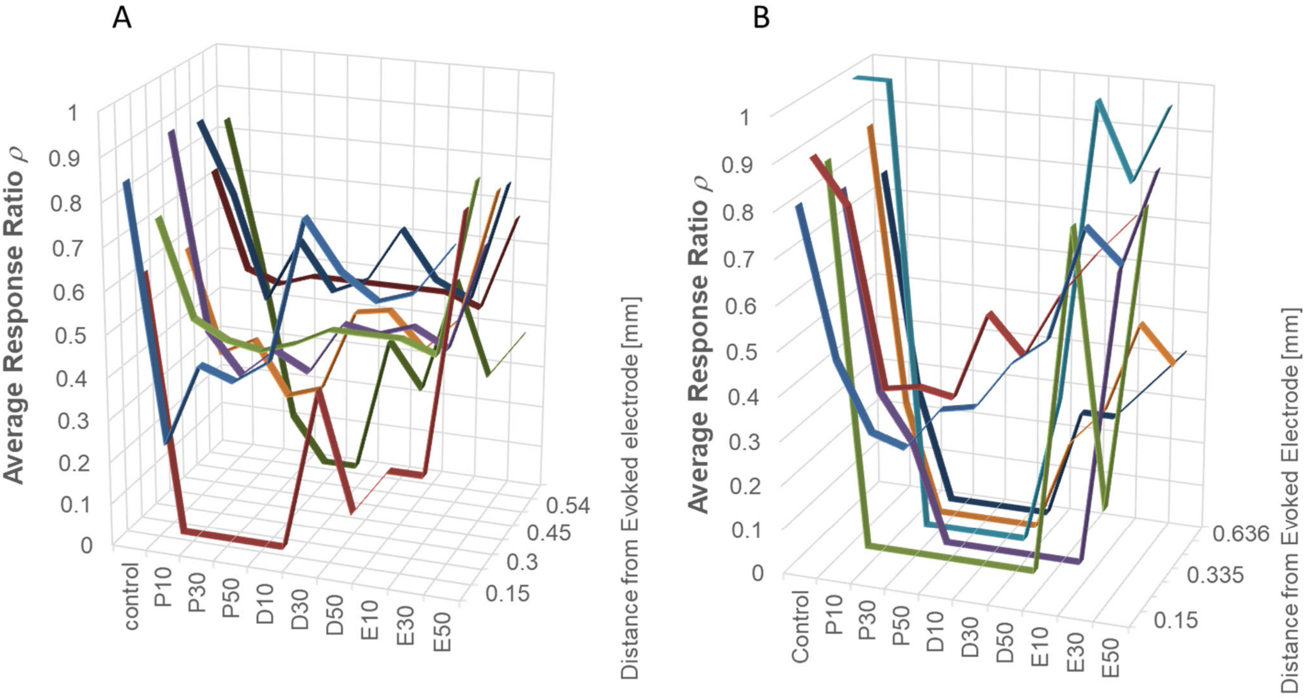

Fig. S4: Same as Fig. 7A except for higher Xe pressure cases. A) Case of 0.4 MPa Xe pressure. B) Case of 0.5 MPa Xe pressure.

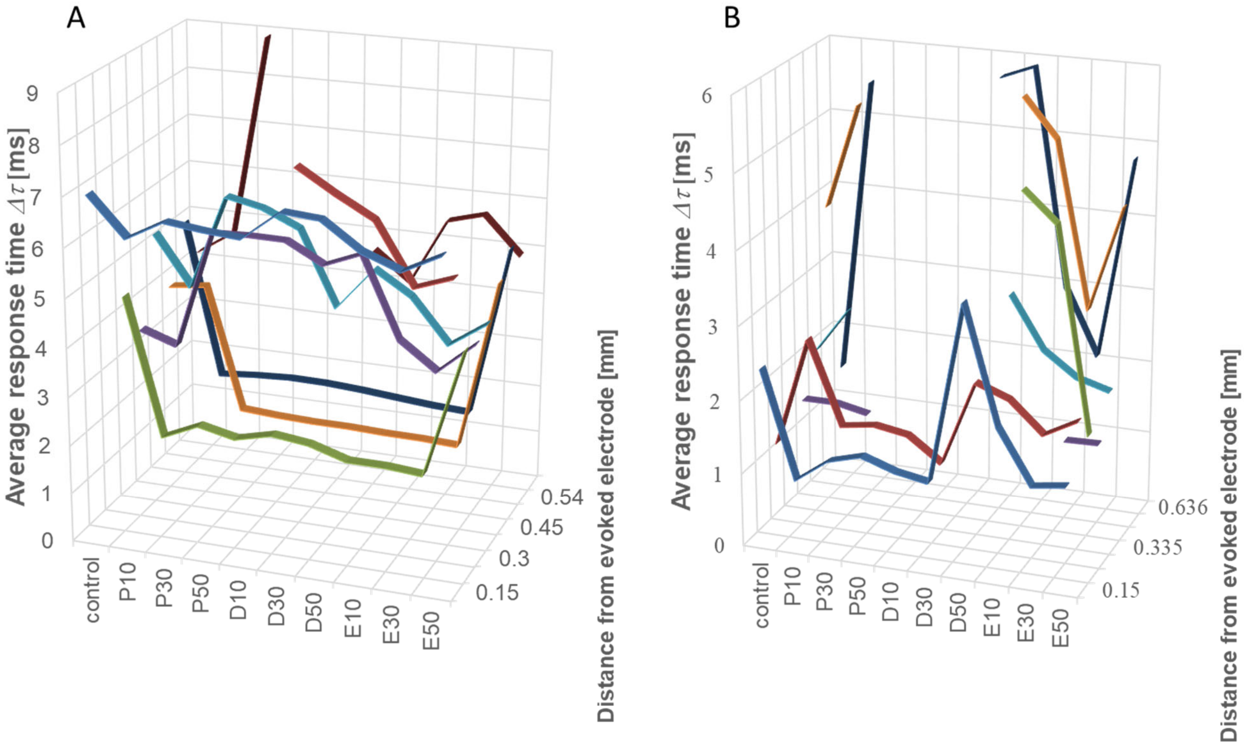

Fig. S5: Same as Fig. 7B except for higher Xe pressure cases. A) Case of 0.4 MPa Xe pressure. B) Case of 0.5 MPa Xe pressure.

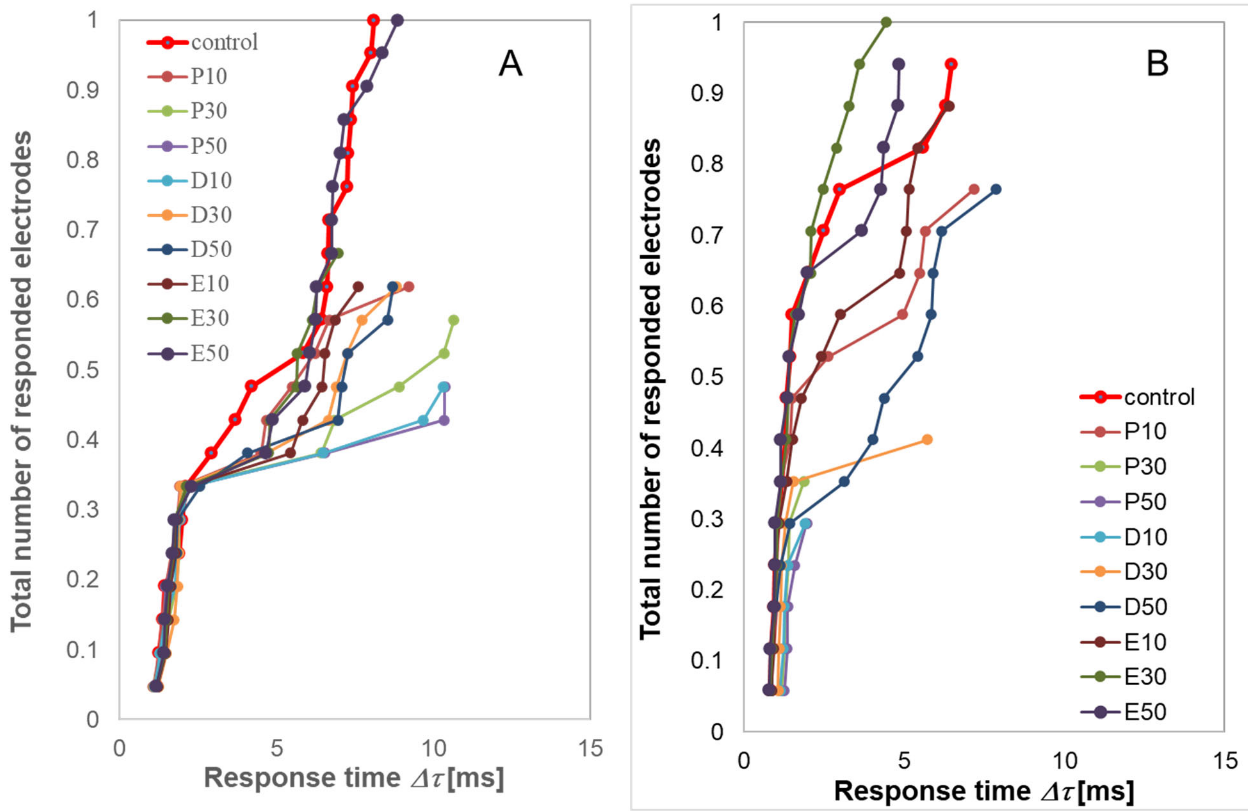

Fig. S6：Same as Fig. S3 except under Xe pressure. A) At 0.4 MPa Xe pressure. B) At 0.5 MPa Xe pressure.

(2) Signal transmission suppression at Xe pressures below 0.3 MPa

For Xe pressurization of 0.1 MPa to 0.2 MPa, the synchronous bursts in the neuronal network remain, but are suppressed by 10–40% of that of the control stage. These bursts would gradually recover about 30 minutes after depressurization, and be completely recovered after the air-exchange stage. These results are qualitatively consistent with the results of our previous studies (Uchida et al., 2017).

For these low-Xe pressure conditions, we also analyzed the response ratio and the response time of the stimulus response signals. Figure S7 shows that the resulting change in the average response ratio ρ is smaller than that at 0.3 MPa. This figure also shows that the pressurization decreases some signals, but others are hardly affected. In addition, the change in the average response time $\Delta\tau$ (Fig. S8) generally increases by pressurization, but there is no significant difference between the stages. Therefore, when Xe gas is applied at less than 0.3 MPa, the signal transmission between neurons is suppressed to a lessor extent than that of the synchronous bursts.

Figure S9 shows the integrated distribution of response time in each stage. When the Xe gas is pressurized by 0.2 MPa (left), the response rate and response time for the fast response signal (less than 2.5 ms) hardly changes, but the long-response times more than 2.5 ms tended to increase, and especially those more than 5 ms exceeded the criteria (more than 10 ms), thus disappeared by 10 to 20% from the late pressurization to the early decompression stages. For the 0.1 MPa case (right), there is almost no change for the fast response signals in the pressurized–depressurized stages. The slow response signals have a delay or vanish due to pressurization as in the 0.2 MPa case, but fewer signals vanish than for the 0.3 MPa case.

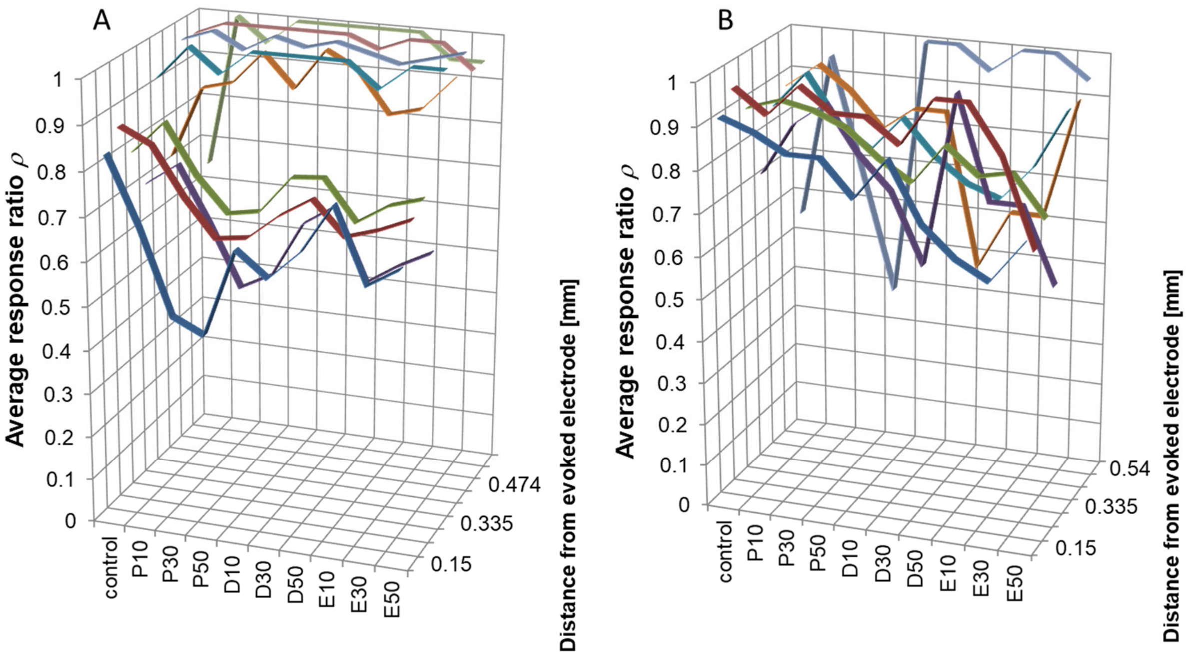

Fig. S7: Same as Fig. S4 except lower Xe pressures. A) At 0.2 MPa Xe pressure. B) At 0.1 MPa Xe pressure.

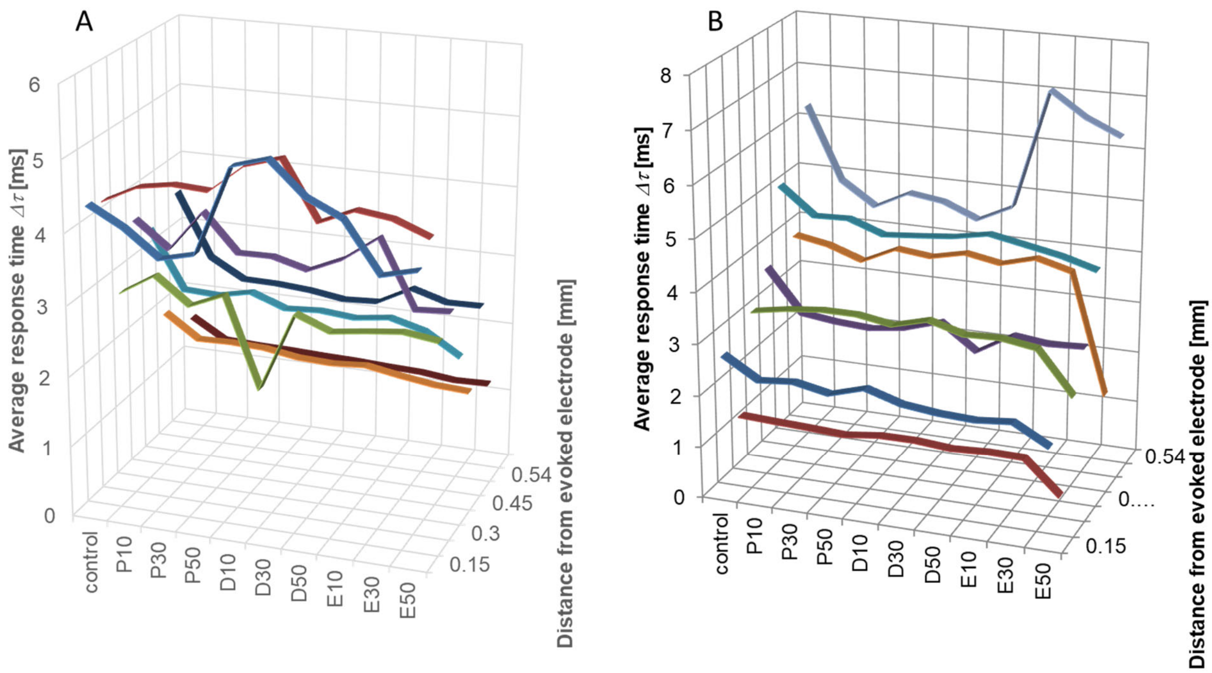

Fig. S8: Same as Fig. S5 except lower Xe pressures. A) At 0.2 MPa Xe pressure. B) At 0.1 MPa Xe pressure.

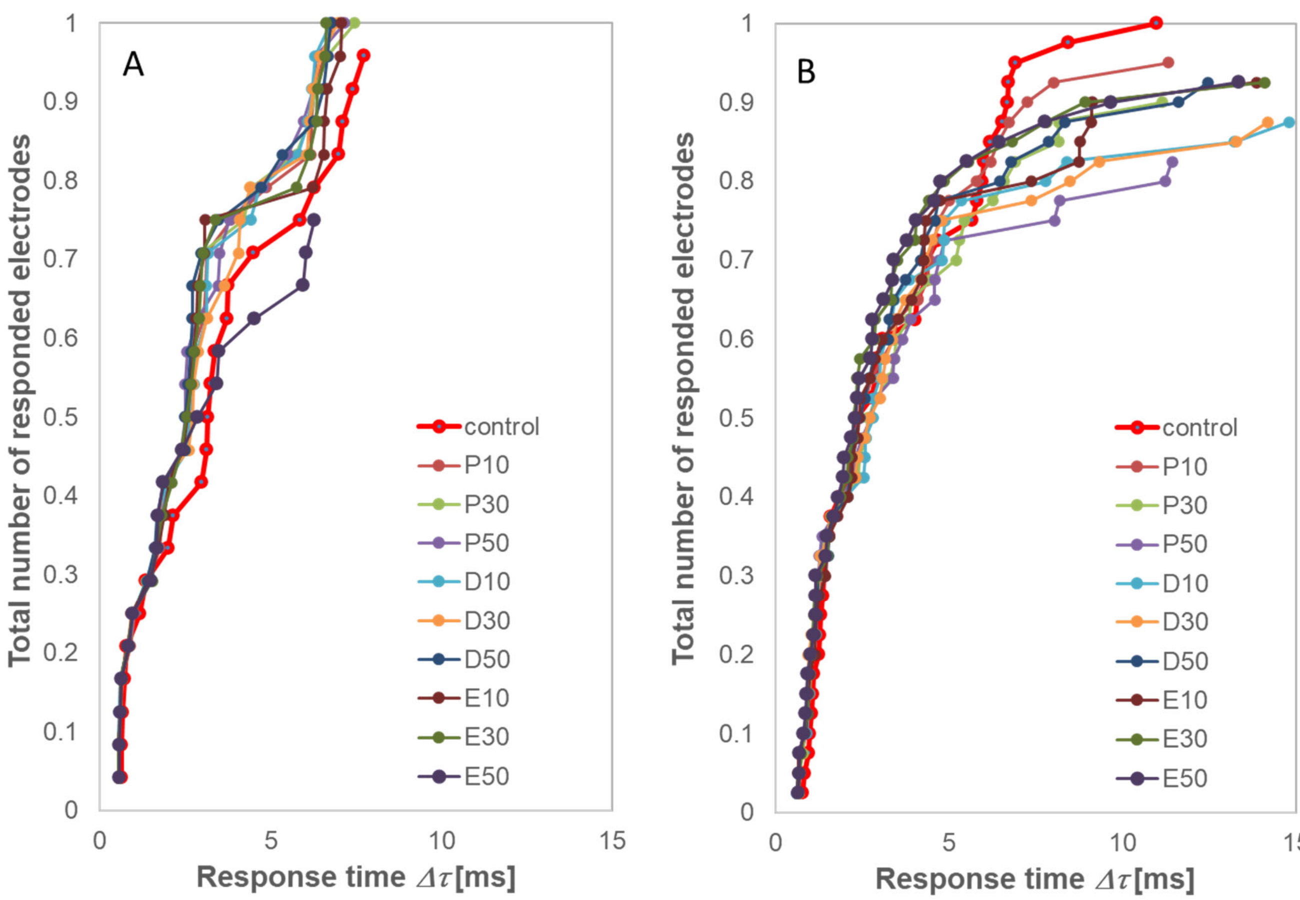

Fig. S9：Same as Fig. S6 except lower Xe pressures. A) At 0.2 MPa Xe pressure. B) At 0.1 MPa Xe pressure.