〔博士論文要約〕

# Temperature-dependent modulation of neural activity mediated by change in excitatory/inhibitory contributions
# (興奮性・抑制性入力変化に起因する神経活動の温度依存性)

令和 2 年度

筑波大学大学院人間総合科学研究科

感性認知脳科学専攻

後藤みずほ

筑波大学

Changes in brain temperature have been reported to affect various brain functions. It has long been known that cooling focal regions of the brain inactivates their function. Some pieces of evidence have shown that cooling a cerebral cortex region inactivates specific functions, such as sensory perception, memory, and learning in animals. Furthermore, brain temperature is important in a clinical context. Pathological neural hyperexcitability has be shown to be closely related to temperature.

Previous electrophysiological studies have revealed that temperature changes can also modulate neural activity. However, how change in temperature modulates neural activity remains controversial; some studies have reported that cooling the brain decreases neural activity, while others reported opposite findings. Moreover, little is known about the effects of temperature on neural activity at the network level, where multiple inputs of different neurotransmitters (e.g., glutamate, GABA, dopamine, etc.) are integrated.

In the present study, for the purpose of investigating the mechanism of temperature-dependent change in neural activity in network level, temperature dependency of the cortical evoked potentials and contribution of each neurotransmitter were examined.

To elucidate how changes in brain temperature affect network-level information processing in the brain, cortical evoked potentials were recorded while altering the local brain temperature in anesthetized rats in this study. Electrical stimulations were delivered to the midbrain dopamine area (VTA/SNc) and the evoked potentials in the frontal cortex were measured while the temperatures of which were locally altered using a thermal control device; a coil-shaped stainless steel tube was embedded inside the chamber on the cortex. The chamber was filled with saline or antagonist solution and temperature-controlled water flowed through the stainless-steel pipe to regulate the cortical temperature via thermal conduction. During the experiments, the cortical temperature was monitored using a thermocouple thermometer inserted at a depth of 1 mm. The thermal control system precisely regulated the cortical temperature in all experiments; the relationship between circulating water temperature and brain temperature was in linear function in all experiments.

First, I examined the evoked potentials in the frontal cortex without modifying neurotransmission while body temperature was not controlled (Experiment 1). It was shown that focal cortical cooling increased the amplitude of evoked potentials in the frontal cortex (negative correlation; > 17°C); further cooling decreased their amplitude. This relationship would be graphically represented as an inverted-U-shaped curve. The peak latency was lower (faster peaks) at higher temperatures and higher (slower peaks)

at lower temperatures.

In Experiment 2, a heating pad was introduced to maintain the body temperature at 36°C to eliminate any effect of body temperature decline and fluctuation by anesthesia. When the cortical temperature was modified within the range of 18–36°C, The amplitude increased as the temperature decreased as in Experiment 1. The peak latency was also negatively correlated with temperature.

Next, I investigated the effects of blocking GABAergic inhibitory inputs on the temperature dependency of neural activity. A $GABA_A$ receptor antagonist (gabazine) was applied to the cortices of the same rats that were used in Experiment 1 (Experiment 3; no body temperature regulation). Pharmacological blockade of inhibitory inputs not only increased amplitude, but also eliminated the negative correlation (> 17°C), and even showed a positive correlation. Similar results were obtained when controlling body temperature at 36°C (Experiment 4). Therefore, GABAergic inhibitory inputs are critical for establishing the negative correlation observed in the control condition. In both of Experiments 3 and 4, gabazine administration increased the peak latency, maintaining the negative correlation.

I also demonstrated that the effects of temperature on evoked potentials were dependent on the gabazine concentration (Experiment 5). Amplitude progressively increased as the gabazine concentration increased, finally showing a positive correlation between temperature and amplitude when the concentration of $GABA_A$ receptor antagonist was sufficiently high, whereas they were negatively correlated in the control. Latency also changed in a dose-dependent manner.

Third, I investigated the effects of blocking glutamatergic excitatory inputs (Experiment 6). Blocking the glutamatergic excitatory inputs by glutamate receptor antagonists (NBQX and (R)-CPP) decreased the amplitude but did not cause inversion of the temperature dependency. These results suggest that excitatory and inhibitory inputs play different roles in establishing the temperature dependency of neural activity.

I further examined the effects of dopaminergic inputs (Experiment 7). Modifying dopaminergic inputs using dopamine receptor antagonists (SCH 23390 and raclopride) did not cause a critical change in the temperature dependency of neural activity.

The results in the present study suggest that the negative correlation between the amplitude of evoked potentials and the near-physiological local temperature is caused by the alteration of the balance of contribution between excitatory and inhibitory inputs to the evoked potentials. My data indicate that the temperature dependency of evoked potentials is mainly determined by the temperature-dependent change in excitatory (i.e., glutamatergic) and inhibitory (i.e., GABAergic) inputs. When GABAergic inhibitory

inputs are fully inactivated (gabazine condition), the net amplitude should be derived from the excitatory inputs. My data demonstrated that gabazine administration eliminated the negative correlation between amplitude and intermediate-to-high temperature, and the regression coefficients were significantly positive. In contrast, when no antagonists are administered (control condition), the net amplitude should be determined mainly by both excitatory and inhibitory inputs. Since my data show that the net amplitudes were lower at higher temperatures, the effect of inhibitory inputs on the evoked potential amplitude may increase monotonically and be sufficiently large to override that of the excitatory inputs. Taken together, the balance of the effects of excitatory inputs and inhibitory inputs on evoked potentials would be altered in a temperature-dependent manner, such that the smaller contribution of inhibitory inputs compared to excitatory inputs cumulatively generates an increased amplitude at lower temperature.

Previous behavioral studies have shown that focal brain cooling induces a reversible inactivation of various brain functions. Those reports are consistent with my results of < 17°C (left side of the inverted-U curve). From this perspective, the increase of the evoked potential amplitudes due to cooling from the physiological temperature would be unexpected. However, some previous electrophysiological studies measuring local field potentials were consistent with my data. Previous studies have shown in anesthetized rats that the amplitudes of cortical somatosensory evoked potentials increase when the cortical temperature decreases approximately 16°C from body temperature. In addition, the amplitudes decreased when the cortical temperature increased 10°C from body temperature. The increase of evoked potential amplitudes by decreasing the temperature has also been observed in the hippocampus. The peak amplitudes of field potentials in the dentate gyrus of rats when they were swimming at low temperature (resulting in low hippocampal temperature) were larger than those when they were swimming at high temperatures. A study which recorded evoked potentials in the CA3 by stimulating the mossy fiber layer of the dentate gyrus in hippocampal slices in guinea pigs reported that the amplitudes of evoked potentials showed an inverted-U relationship with the temperature; the amplitude was the largest at approximately 31°C and was smaller at higher (31 to 37°C) and at lower (15 to 31°C) ranges. Although the peak temperatures are quantitatively different among studies (possibly because different brain areas in different animals have their temperature characteristics), those data are qualitatively consistent in that the amplitudes increased when the cortical temperature decreased around the physiological temperature.

Temperature change affected not only the amplitudes of the evoked potentials, but also their latencies. In contrast to amplitude, latency and temperature showed a

negative, monotonic relationship, which was observed in all the Experiments. Since evoked potentials are the result of a combination of chemical reactions, decreases in their rates can result in larger latencies of synaptic transmission and resultant delayed local field potentials at lower temperatures. When GABAergic inhibitory inputs were blocked, the peak latency increased. Previous studies using intracellular recording and optical imaging with a voltage sensitive dye have showed that midbrain stimulation first induces excitatory post-synaptic potentials (EPSPs), followed by inhibitory post-synaptic potentials (IPSPs) in frontal neurons. The chain of the cortical post-synaptic potential is characterized as an EPSP-IPSP sequence. In the present study, the later inactivation of IPSPs by a $GABA_A$ receptor antagonist may have extended EPSPs and increased the peak latency.

In this study, cortical evoked potentials were recorded while controlling the local temperature. In the frontal cortex, the amplitudes were negatively correlated with local cortical temperatures > 17°C, but this negative correlation was eliminated by the administration of a $GABA_A$ receptor antagonist. These results suggest that the negative correlation between the amplitudes of evoked potentials and the local temperature is caused by an alteration of the balance of contribution between excitatory and inhibitory inputs to the evoked potentials, possibly due to higher temperature sensitivity of inhibitory inputs. Although further investigation is necessary to elucidate how the temperature dependency of excitatory and inhibitory inputs influences brain functions, including cognitive and behavioral aspects, the present study provides a network-level explanation for mechanism of temperature dependency of neural activity that GABAergic inhibitory inputs play a critical role in establishing the temperature dependency.