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研究課題名（和文）Identification of brain regions involved in the arousal from coma and sleep.

研究課題名（英文）Identification of brain regions involved in the arousal from coma and sleep.

研究代表者

マリシェフサカヤ　オリガ（MALYSHEVSKAYA, Olga）

筑波大学・国際統合睡眠医科学研究機構・研究員

研究者番号：２０７３９４２９

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研究成果の概要（和文）：昏睡状態では、意識の消失、不動化、覚醒レベルの低下を伴う。橋核のPB 核を損傷すると運動失調および昏睡状態に陥り、その後死亡する。本研究では覚醒を促進するメカニズムを明らかにするため、昏睡状態のモデルマウスを作成し、覚醒に関わる脳神経細胞や神経サーキットを人為的にコントロールする。IVM-GLu-Cl を用いてPB 核の働きを抑制すると運動失調および昏睡状態に陥る。PB 核損傷マウスにおいてLC ノルアドレナリン細胞あるいはvPAG ドーパミン細胞の活性が昏睡状態からの回復、すなわち覚醒促進に重要であるという仮説を立て、生化学的手法を用いてこれらの核の活性化を行い仮説を検証した。

研究成果の概要（英文）：Substantial insights into understanding the nature of arousal can be gained by considering the relationship between coma state, sleep and wakefulness. This study is focuses on the fine localization of neuronal regions in the brain and identifying arousal promoting cells and pathways in case of recovery from coma. We first lesion PB nuclei in the pons to produce coma-state mouse. This resulted in ataxia state, followed by coma and death of mice. Inhibition of PB nuclei using IVM-Glu-Cl produced no change to the state. We predicted that noradrenergic LC or dopaminergic vPAG are potential centers for reverse of coma state and chemogenetic activation would produce reverse of coma state. We are currently investigating whether other arousal centers or combined activation may produce arousal from coma in a coma state mouse.

研究分野：　Neuroscience

キーワード：　coma　arousal　parabrachial nucleus　lesion　DTA-AAV　IVM-Glu-Cl　pons　DREADD

１．研究開始当初の背景

Sleep disturbances have a high worldwide prevalence and cause significant loss of quality of life either by itself or through secondary effects such as metabolic disturbances or fatigue related accidents. Almost all neuropsychiatric diseases are comorbid with sleep disorders – in many cases disturbed sleep is one of the early symptoms of the disease.

Loss of consciousness is an obligatory and defining feature of sleep, as is the rapid reversibility of the unconscious state. Thus we physiologically switch between an alert and conscious waking state and an unconscious sleep state in a regular and daily pattern. The control circuitry that underlies this is poorly understood. In particular the link between loss of consciousness in sleep and during other forms of unconsciousness such as coma is not understood. There is a gradual decrease in ‘wakeability’ between sleep, vegetative state and coma, but we do not know whether this implies differences in the function of the same control circuits or involvement of additional circuits or a mixture of both.

As with most mental processes that occur in the brain, the biology of arousal is very complicated and not well understood. The ascending arousal system begins in the upper pons and contains two branches, one on the thalamus and the other through the hypothalamus and basal forebrain, both of which activate the cerebral cortex and are responsible for wakefulness. On the other hand, neurons in the preoptic area, posterior lateral hypothalamus, and possibly the lower brainstem are promoting sleep. In general, two brain systems with opposite outcomes are competing each other in order to regulate sleep and wakefulness. Coma is a condition of unarousability with a complete absence of wakefulness and awareness, whereas VS is characterized by a lack of awareness despite a preserved wakefulness. Various physiological characteristics indicate similarities and functional connections between coma and sleep, however exact relationship remains completely unknown. It was shown, in rodents, cell-specific lesions of parabrachial (PB) nuclei caused a coma-like state, suggesting that this nucleus may be particularly important in maintaining wakefulness[1]. Combination of coma-mouse model with the advent of novel pharmoco- and optogenetic tools enables precise and specific way of manipulating neuronal activity and understanding relationship between arousal, coma and sleep.

２．研究の目的

This project aimed at understanding circuits that underlie the transition between unconscious and conscious states and their involvement in sleep/wake transitions as well as coma/waking transitions.
Understanding this circuitry has obvious implications for our understanding of the regulation of sleep, but will also improve our knowledge of different states of unconsciousness. This knowledge has direct implications of the well-being of a large number of patients and may provide pathways for the rational developments of treatments aimed at reversing unconscious states, such as coma or vegetative state. We hypothesize, that coma state can be reversed by induction of arousal centers. We aim to investigate the role of arousal centers (brain regions: LC, vPAG, TMN, DR, PC and LH) and their sub-nuclei in the control of wakefulness and their ability to “wake up” the brain from coma states or sleep.

３．研究の方法

To induce experimental coma-state in mouse we used pharmocogenetic silencing of PB nucleus by means of ivermectine (IVM). This advanced genetic technique is being used in our work in conjunction with adeno-associated virus (AAV) vectors, for stereotaxic brain microinjections and allows regionally restricted transduction of neuronal cell inhibition for a period from hours to days.
Further, pharmocogenetic activation of arousal-promoting nuclei used to reverse an experimental coma state. Certain arousal-involved brain regions transfected using stereotaxic brain injection of adeno-associated virus (AAV) that drive the expression of mutant G protein coupled receptors (AAV-DREADD), which respond to otherwise inert compounds CNO and activates those neurons (hM3Dq). All-in-all these advanced tools provide a unique opportunity to decipher the molecular mechanisms behind the arousal from unconsciousness.
Finally, EEG electrodes were placed on the surface of the skull (1.5 µm from bregma and lambda, 1 µm lateral) and mounted them with the dental cement. EMG electrodes were placed into neck muscles to record

muscle activity.

4．研究成果

We established an experimental coma-state mouse model by pharmocogenetic silencing of PB nucleus. First, we designed and produced AAV that caries diphtheria-toxin-subunit A (DTA). The DTA-AAV plasmid and virus was created in our laboratory and it allows focal lesion of neurons without undesired side effects, such as inflammation. Then we performed surgery, in brief, we stereotaxically microinjected DTA-AAV to the parabrachial (PB) nucleus of the pons, which is responsible for vigilance state maintenance. Lesion of this area by DTA-AAV produced vestibular-ataxia state on day 3 and developed in to minimally-conscious state following by coma on day 6 (7) and death by the day 7 (Fig.1).

	Days after DTA-AAV microinjection		
	day 3	day 6	day 7
vestibular ataxia	n=5		
minimally-conscious state		n=3	n=2
coma		n=3	n=2
death			n=5

Fig.1. Development of symptoms in mice injected with DTA-AAV in PB nuclei (n=5)

All animals (n=5) displayed very similar phenotype with a small difference in severity of coma, mainly varying in onset of symptoms and lifespan. Electrographic analysis showed low level of muscular activity and locomotion. Electroencephalogram was degrading day-by-day showing the signs of bursting activity suppression by day 6-7 (Fig.2), which is a hallmark of coma state.

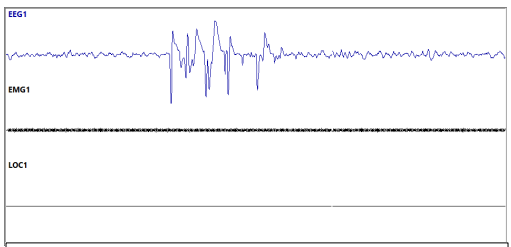

Fig. 2. Typical electrographic characteristics of coma state: burst suppression

On the day 7 EEG resembled isoline (Fig.3), which is the sign of brain death. Soon after, animals stopped breathing and we confirmed their death. The fact that DTA-AAV microinjection resulted in ataxia suggests some dopaminergic neurons are affected which results in Parkinson-like tremor disorder. More careful investigation of this experimental result is necessary.

Next we aimed to produce reversible coma

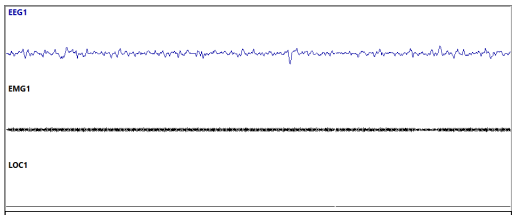

Fig.3 Typical electrographic characteristics of brain death: isoelectric line

state. To do this we investigated whether inhibition of PB by ivermectin-Glu-Cl (IVM-Glu-Cl) system would provide less severe and possibly reversible coma state. Initial stereotaxic AAV microinjections and further activation by IVM did not result in any phenotypical change. Therefore we continued to search for the reversible coma mouse model using chemogenetic inhibition approach.

Furthermore, stereotaxic microinjection of viral vectors of mutant muscarinic (DREADD) receptors resulted in restricted expression at the activation target (LC or vPAG). We found that pharmacogenetic exciting LC or vPAG neurons via hM3Dq by CNO did not produced arousal in a coma mice (Fig.4).

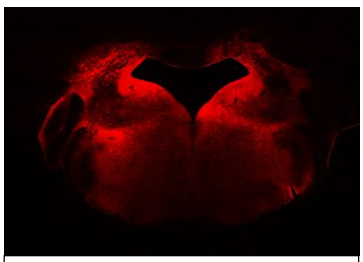

Fig.4. IHC with antibody against mCherry shows LC regions expressing hM_3DQ-mCherry

To critically optogenetically activate LC or vPAG in freely behaving animals, the WT mice were microinjected with AAV-DIO-ChR2-mcherry and stereotaxically implanted with EEG recording electrodes. This approach also did not produce arousal in coma mouse. Therefore we are planning to stimulate both centres at the same time or to include activation of other arousal centres.

1. Fuller P. et al, J. Comp. Neurol. 2011; 519:933-956

5．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 0 件）

〔学会発表〕（計 0 件）

〔図書〕（計 0 件）

〔産業財産権〕

○出願状況（計 0 件）

名称：
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権利者：
種類：
番号：
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国内外の別：

○取得状況（計 0 件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
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〔その他〕
ホームページ等

６．研究組織
(1)研究代表者
マリシェフサカヤ オリガ
(MALYSHEVSKAYA, Olga)
筑波大学・国際統合睡眠医科学研究機構・
研究員
研究者番号：20739429