L1 GABAergic neurones in the frontal cortex and L6 glutamatergic neurones in the prefrontal cortex use RHO GTPase to differentiate between unconsciousness states

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Abstract

Unconsciousness is caused by sleep and general anaesthesia; however, to what extent they are similar is not well-studied. In this study, we aimed to determine the similarities and differences between the unconsciousness states caused by sleep and that caused by anaesthesia, and to investigate gene expression profiles by applying tensor decomposition-based unsupervised feature extraction to the two gene expression profiles. One of the two expression profiles was composed of mice treated with sevoflurane, a type of inhaled anaesthesia, whereas the other two expression profiles were composed of sleeping and awake mice. We selected two sets of genes (507 and 1048 genes), which are distinctly expressed between sleep or anaesthesia-induced unconsciousness and the awake or control groups. Both sets of genes include various distinct genes which are common to the state of unconsciousness between sleep and anaesthesia. The former is enriched in GABAergic synapses and layer one of the frontal cortex, whereas the latter is enriched in glutamatergic synapses and layer six of the prefroncal cortes. Additionally, both sets of genes were enriched in the RHO GTPase pathway. Based on these results, we hypothesized that L1 GABAergic neurones in the frontal cortex and L6 glutamatergic neurones in the prefrontal cortex use RHO GTPase to differentiate states of unconsciousness induced with general anaesthesia and sleep.

Keywords: anaesthesia, sleep, tensor decomposition, feature selection, AI consciousness studies

1 Introduction

Although sleep and general anaesthetics can cause unconsciousness, the unconsciousness states are not necessarily equivalent [1, 2]. Unconscious but arousable sedation is closely related to sleep-wake circuits, whereas unconscious and unarousable anaesthesia are independent of them. General anaesthesia is notable for its ability to decrease sleep propensity. Conversely, increased sleep propensity due to insufficient sleep potentiates the anaesthetic effects [3].

Natural sleep is an endogenously generated state involving the active suppression of consciousness by specific brain regions, including the nuclei in the brainstem, diencephalon, and basal forebrain. Sleep cycles between the rapid eye movement (REM) and non-rapid eye movement (NREM) stages. In contrast, anaesthesia-induced unconsciousness is a pharmacologically induced and reversible state of unconsciousness characterised by hypnosis, analgesia, amnesia, and akinesia. Unconsciousness is achieved through the systemic administration of anaesthetics that disrupt neural communication, leading to a more widespread suppression of brain activity than that observed in natural sleep [4, 5].

From a neurophysiological perspective, the brain exhibits slow-wave activity (SWA) with specific patterns of connectivity during NREM sleep. In contrast, general anaesthesia often induces a state of burst suppression, indicating a profound disruption of thalamocortical connectivity and impaired information processing [4]. In addition, anaesthesia disrupts thalamocortical connectivity more than natural sleep, resulting in impaired information integration and processing [4, 6–8].

Unlike natural sleep, which is essential for physiological restoration and memory consolidation, anaesthesia-induced unconsciousness does not provide restorative benefits. Moreover, anaesthesia can suppress REM sleep, leading to a deficit in which the body may attempt to recover post-anaesthesia.

In summary, although anaesthesia-induced unconsciousness and natural sleep may appear superficially similar, they differ significantly in their underlying mechanisms, neurophysiological characteristics, and functional outcomes.

This study aimed to understand the differences and similarities between sleep and anaesthesia from a genomic perspective. To this end, we applied the previously proposed tensor-decomposition-based unsupervised feature extraction to sleep/anaesthesia gene expression profiles and compared them with previously reported ones in [9]. We identified several hundred genes whose expression profiles were shared between sleep and anaesthesia. These genes are sleep- and anaesthesia-associated biological processes.

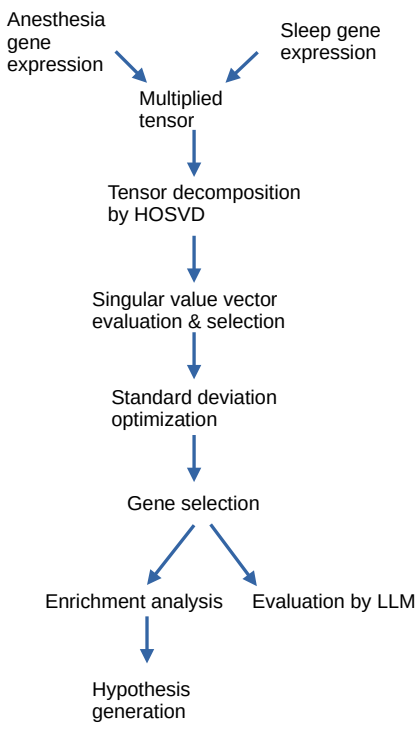

Fig. 1 The schematic figure of analyses performed in this study. First, two gene expression profiles were multiplied to generate a tensor. Tensor decomposition is applied to the generated tensor and singular value vectors were evaluated. After the selection of the singular value vectors attributed to various experimental conditions (such as tissue specificity and sleep vs awake), the singular value vectors attributed to genes and used to select genes are decided. After optimizing the standard deviation, we attributed P-values to genes and genes associated with adjusted P-values less than the threshold value (< 0.01) are selected. The selected genes were evaluated using large language model (LLM) and enrichment analysis, based on which we generated the hypothesis.

2 Results

2.1 Gene selection

After applying HOSVD [10] to $x_{ijkk'mm'}$, we obtained singular value vectors. Because we wanted to find genes whose expression is distinct between sleep or anaesthesia and wake or control without any other dependencies, we selected the singular value vectors shown in Fig. 2. We also investigated which $G(\ell_1, 2, 1, 1, 1, 1)$ had the largest absolute value and selected $\ell_1 = 3$ (Fig. 2).

Next, we attempted to optimise σ_{ℓ_1} such that P-values computed by eq. (5) obeys the null hypothesis that $u_{\ell_1 i}$ follows a Gaussian distribution as much as possible (Fig.

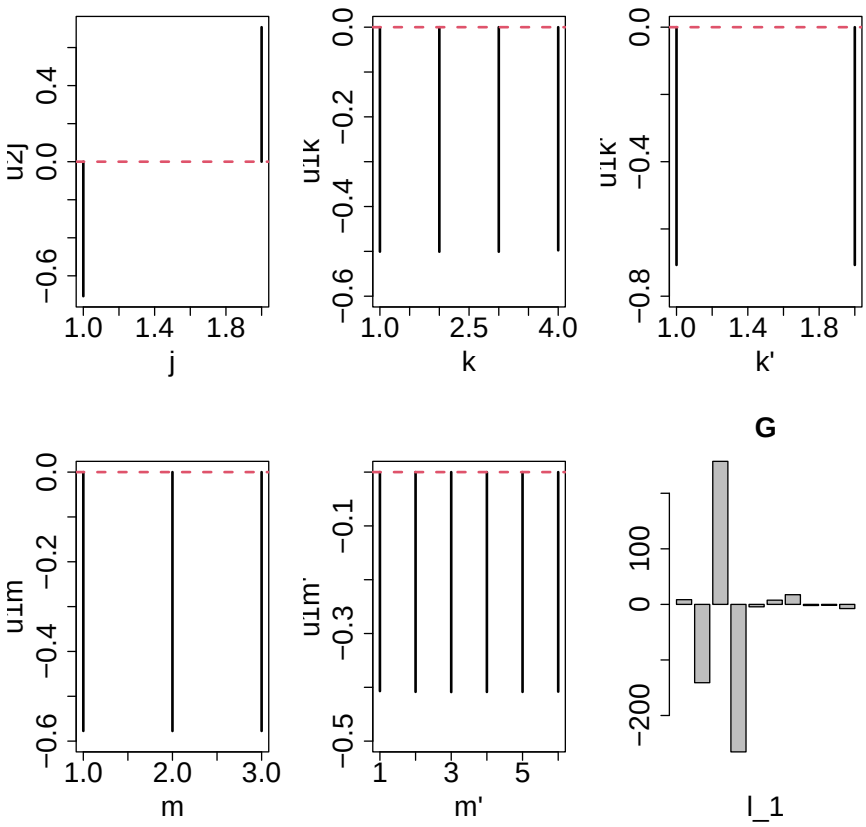

Fig. 2 Singular value vectors and core tensor G. Top left: u_{2j}, top center: u_{1k}, top right:$u_{1k'}$, bottom left right: u_{1m}, bottom center: $u_{1m'}$, bottom right: $G(\ell_1, 2, 1, 1, 1, 1)$

3). As expected, the standard deviation of the histogram of P_i has minimum values at a specific σ_{ℓ_1} and the histogram of $1 - P_i$ follows a uniform distribution, meaning that the null hypothesis stands as well as possible, excluding the peak near $P_i = 0$, which corresponds to the genes to be selected. In particular, σ_{ℓ_1} which has the minimum standard deviation of the histogram of P_i can be uniquely defined because only one minimum exists (left panel of Fig. 3). Setting the threshold value to 0.01, we selected 948 gene symbols associated with adjusted P-values of < 0.01.

Genes associated with $u_{\ell_1 i} > 0$ were distinct from those associated with $u_{\ell_1 i} < 0$ and the latter were not associated with any enriched terms. In the following section, we consider only the 507 genes associated with $u_{\ell_1 i} > 0$.

We also consider $\ell_1 = 4$ to be associated with the second-largest G. We attempt to optimise σ_{ℓ_1} (Fig. 4). In contrast to $\ell_1 = 3$, which σ_{ℓ_1} is associated with the smallest standard deviation of the histogram of P_i is unclear, because many local minima shared nearly the same standard deviation values. We then decided to use the smallest

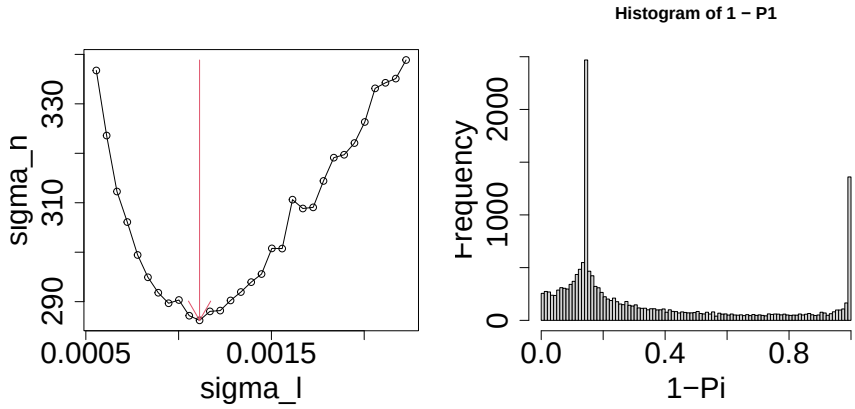

Fig. 3 Left: The standard deviation of histogram of P_i as a function of σ_{ℓ_1} for $\ell_1 = 3$. The vertical red arrow suggests the selected value of σ_{ℓ_1}. Right: The histogram of $1 - P_i$ for the optimal σ_{ℓ_1}

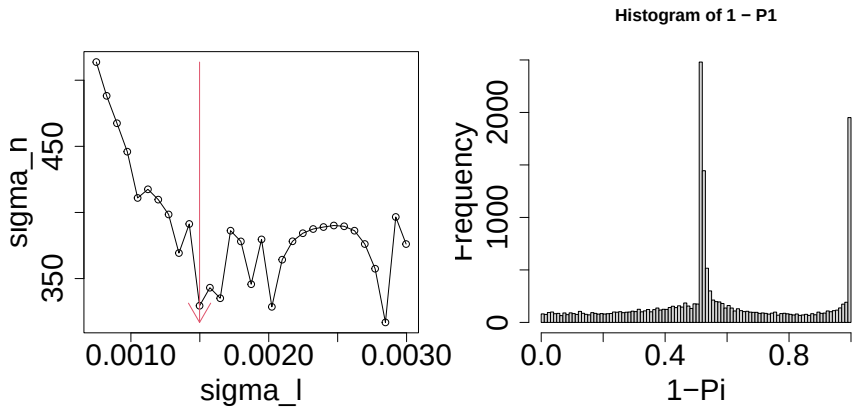

Fig. 4 Left: The standard deviation of histogram of P_i as a function of σ_{ℓ_1} for $\ell_1 = 4$. The vertical red arrow suggests the selected value of σ_{ℓ_1}. Right: The histogram of $1 - P_i$ for the optimal σ_{ℓ_1}

possible σ_{ℓ_1} which resulted in the largest number of selected genes. A total of 1,512 genes were selected. If we divide the set of genes by the sign of u_{ℓ_1}, then only 1,048 genes associated with $u_{\ell_1 i} < 0$ were enriched in various biological terms. Thus, we consider these 1,048 genes.

For $\ell_1 = 3$ genes with $u_{\ell_1 i} > 0$ and for $\ell_1 = 4$ genes with $u_{\ell_1 i} < 0$ are valid because the sign of G is inverted between $\ell_1 = 3$ and $\ell_1 = 4$ (Fig. 2). As can be seen in eq.(4), not individuals $u_{\ell_1 i}$ or G but their products $Gu_{\ell_1 i}$ are important. Thus, for an oppositely signed G, $u_{\ell_1 i}$ must have opposite signs.

Figure 5 shows a Venn diagram of 507 genes and 1,048 genes. Although there was some overlap, the majority of genes were distinct between the two sets of genes.

Thus, it is worthwhile to consider two sets of genes (as for the intersection, we can hypothesise the origin later. See below).

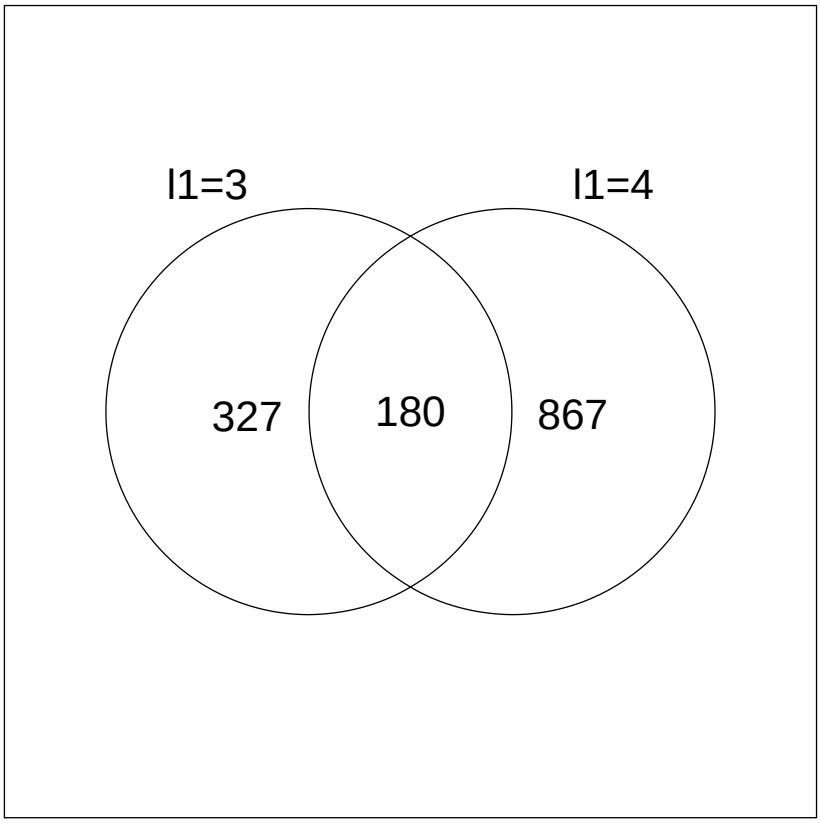

Fig. 5 Left: The standard deviation of histogram of P_i as a function of σ_{ℓ_1} for $\ell_1 = 4$. The vertical red arrow suggests the selected value of σ_{ℓ_1}. Right: The histogram of $1 - P_i$ for the optimal σ_{ℓ_1}

2.2 Evaluation by LLM

First, we separately uploaded the selected 507 genes and 1,048 genes to various LLM to determine whether there were genes related to both sleep and anaesthesia. The prompt used is "This list of genes has been listed as being related to sleep and anaesthesia. Please review these lists, select those that you think are relevant to both sleep and anaesthesia, and summarise them with citations to supporting articles" or its Japanese translation (Table 1). Multiple genes were reported by multiple LLMs. Thus, the results were trustworthy. Notably, the selected genes are quite distinct between $\ell_1 = 3$ and $\ell_1 = 4$; considering $\ell_1 = 3, 4$ separately is quite informative despite spite of

Table 1 Sleep and anaesthesia-related genes were reported by various LLMs (bold).

$\ell_1 = 3$	
Gemini	1700001K19Rik,Actb,**Avp,Gabbr1,Ntrk2,Oxt**,Sox2
Perplexity	**Gad2**,Slc32a1,Slc6a1,Vamp1,Vamp2,**Atp1a2**,Atp1b2, Kcnj10,Kcnk2,**Avp,Oxt**,Vgf,Nrxn2
Grok	**Atp1a2**,Atp1b1,**Gad2,Slc6a1,Ntrk2**
DeepSeek	**Gabbr1,Slc6a1,Nr1d1**,Bace1,Cul3,Csnk1a1
AI Scientist	**Nr1d1, Ntrk2**, SCN9A, **GABBR1**, Shank3, DNM1, **GAD2**
$\ell_1 = 4$	
Gemini	**Gabrg2**, **Gabra1**, **Gabrb2**, **Gabrb3**, Adora1, **Clock**, **Cry2**
Perplexity	**Clock**, **Cry2**, Per2, **Gabbr1**, **Gabra1**, **Gabrb2**, **Gabrb3**, **Gabrg2**
Grok	**Gabra1**, **Gabrb2**, **Gabrb3**, **Gabrg2**, **Grin1**, Grin2a, Grin2b, Nr4a1, Ptprd, Kcna1
DeepSeek	**GABRA1**, SIK3, **CLOCK**, CACNA1C, **GRIN1**,
AI Scientist	**Gabra1**, **Gabrb2 Gabrb3**, **Gabrg2**, **Gabbr1**, Prnp, Snca

hundred genes shared between the two (Fig. 5). In the following section, we summarise the references based on which LLM insisted to select genes reported by multiple LLMs. Thus, we did not simply trust the results of LLM but evaluated them based on the papers cited by LLM.

2.2.1 Avp ($\ell_1 = 3$)

AVP controls the circadian rhythm, which is a key mechanism in wake-sleep control. AVP is an important neuronal peptide in suprachiasmatic nucleus (SCN) and acts as a master clock. The AVP signal controls the network characteristics of the SCN. AVP neurones in the SCN exhibit rhythmic calcium activity[11]. The arginine vasopressin neurones in the paraventricular nucleus of the hypothalamus (PVH^{AVP}) are involved in promoting arousal [12]. AVP participates in REM sleep regulation under normal physiological conditions [13]. Avp plays an important role in maintaining circulatory stability, especially in situations where the sympathetic and renin-angiotensin systems are suppressed (e.g. during anaesthesia or in shock) [14].

2.2.2 Gabbr1($\ell_1 = 3, 4$), Gabra1, Gabrb2, Gabrb3, Gabrg2 ($\ell_1 = 4$)

GABA_B receptor signalling is involved in sleep regulation, particularly slow wave sleep (SWS), and GABA_B agonists such as baclofen and γ-hydroxybutyrate (GHB)/sodium oxybate increase SWS and delta wave power in animals and humans, with minimal effects on REM sleep, whereas GABA_B antagonists reduce SWS. In preclinical studies, GABA_B agonists have been associated with the increased expression of genes related to SWS production and circadian function [15].

The GABAergic pathway is central to the action of many general anaesthetics (e.g. propofol, isoflurane, sevoflurane, etomidate, and barbiturates), which often target GABA_A receptors [16]. GABA_B receptors may also be involved, and the GABA_B agonist baclofen has shown potential therapeutic effects in animal models of alcohol dependence (reduced self-administration behaviour) [17]. In a study using GABAB flox mice and astrocyte-specific ablation using a viral vector (AAV-Cre) during surgery under isoflurane anaesthesia, Gabbr1 was ablated, demonstrating the utility of these

models for in vivo GABA_B function studies. This technique avoids the developmental problems associated with constitutive KO [18]. Although many anaesthetic agents act primarily on GABA_A receptors, GABA_B receptors modulate the overall network inhibition and may contribute to anaesthetic states, particularly specific elements such as sedation and muscle relaxation, as suggested by their functions and agonist/antagonist effects. However, GABA_B receptors are the primary targets of common anaesthetic agents such as propofol and isoflurane, and are not as prominent in these snippets as the GABA_A receptor, although there is evidence for the direct involvement of the GABA_A receptor in these snippets.

Gabbr1 is a member of the gamma-aminobutyric acid (GABA) receptor gene family, which encodes receptors for GABA, the principal inhibitory neurotransmitter in the central nervous system. GABAergic neurotransmission is a well-established mediator of sleep induction and of the sedative effects of many anaesthetic agents, including propofol. Variants in GABA receptor genes have been associated with altered anaesthetic sensitivity and modulation of sleep-promoting circuits, underscoring the dual role of Gabbr1 in these processes [2, 19].

2.2.3 Ntrk2 ($\ell_1 = 3$)

In the rat brain, Ntrk2 shows diurnal variation in expression levels and use of selective polyadenylation (APA) sites, specific APA isoforms are altered by sleep deprivation and subsequent restorative sleep [20], suggesting that Ntrk2 regulation at the mRNA processing level is associated with circadian and sleep homeostasis mechanisms. Ntrk2 has been identified as one of 46 genes in which sleep/circadian-related APAs overlap with APAs associated with brain disease susceptibility genes [20]. In studies using yak testes, lncRNAs (MSTRG.19083.1) were identified as miR-429-y and were shown to function as sponges for Ntrk2 to regulate circadian rhythms via the cAMP pathway, suggesting the existence of a complex regulatory network in circadian processes involving Ntrk2 (even outside the brain) [21].

Genetic polymorphisms in human NTRK2 are associated with symptom burden in patients with breast cancer undergoing chemotherapy. Specifically, the T allele of SNP rs1212171 is associated with increased sleep disruption and fatigue interference during treatment [22]. This suggests that individual genetic variants in the TrkB pathway may influence susceptibility to common treatment side effects such as sleep interruption and fatigue, suggesting that they may affect susceptibility to common treatment side effects such as fatigue, possibly via alterations in neurotrophic factor signalling and interactions with inflammatory responses [22]. From the data provided, Ntrk2 function is directly involved in the anaesthetic mechanism (e.g. LORR). Although there is no evidence, its role in neuroplasticity, survival, and regulation of sleep/circadian rhythms suggests that it may be a modulator of anaesthetic sensitivity and recovery. The most direct link is its association [22] with sleep disorders.

Ntrk2 encodes the neurotrophic receptor, tyrosine kinase B (TrkB), which is involved in neuroplasticity, synaptic regulation, and learning. Ntrk2 expression is modulated following isoflurane anaesthesia, indicating that Ntrk2 may influence the neural circuits that underlie both sleep and anaesthetic states. Given that proper synaptic function and plasticity are essential for maintaining stable sleep patterns as well as

for the emergence of anaesthetic-induced unconsciousness, Ntrk2 has emerged as an important candidate in the shared biology of these processes [23].

2.2.4 Oxt ($\ell_1 = 3$)

Oxt signalling via the paraventricular nucleus (PVN) to the prefrontal cortex (PrL) pathway modulates the inhibitory balance of PrL (PV/VIP interneurons) during REM sleep and supports social memory fixation; OXT release occurs during REM sleep; lack of OXT signalling (e.g. due to chronic sleep deprivation) disrupts this balance and leads to social memory impairment. The activation of the REM sleep-specific PVN^{OCT} $\rightarrow$PrL pathway or intranasal OXT administration rescues these deficits in sleep-deprived mice [24]. This suggests that it does not act universally on social memory, but by regulating specific cortical circuits during a particular offline brain state (REM sleep), which is important for memory fixation. Polymorphisms in the human OXTR gene (rs2254298 A allele) are associated with symptoms of obstructive sleep apnoea (OSA), particularly shortness of breath, snoring, and reduced subjective sleep efficiency [25]. This suggests that endogenous OXT signalling may be involved in breathing regulation during sleep. oXT levels, social support, and sleep quality in HIV-positive women, with some studies finding an association.

OXT reduces ethanol consumption and motivation in mice; however, this is not due to a general sedative effect [26]. Oxtr KO mice do not exhibit ethanol-induced conditioned social preferences [27]. OXT administration (intranasal or IP) is a potential treatment for ASD-related behaviour used in animal models (VPA rats) to study its effects. However, this usually involves behavioural testing under baseline or manipulated conditions rather than under anaesthesia [28]. These findings suggest a clinical context in which OXT-related systems may interact with anaesthetics, but they do not investigate the interaction itself.

2.2.5 GAD2 ($\ell_1 = 3$)

GAD2 is a promising candidate to trigger the consciousness mechanism of action. This gene encodes glutamate decarboxylase 2, an enzyme that is crucial for the synthesis of GABA, the primary inhibitory neurotransmitter in the central nervous system. Because both natural sleep and various anaesthetic agents rely on the enhancement of inhibitory neurotransmission to induce loss of consciousness, GAD2 is functionally important in these processes. Increased GABA synthesis due to GAD2 activity can modulate the inhibitory tone in key sleep-regulating circuits and contribute to the depth of anaesthetic states [2, 29]

GAD2^{LPO} neuronal stimulation did not merely trigger wakefulness; the awake state produced by this stimulation is characterised by increased EEG theta activity. In contrast, unilateral inhibition of GAD2^{LPO} neurones decreased the drive for arousal, as reflected by the persistent increase in NREM EEG SWA throughout the day. In summary, our experiments demonstrated the important role of GAD2^{LPO} neurones not only in the control of state transitions but also in linking arousal to sleep homeostasis [30].

2.2.6 Alpha2 ($\ell_1 = 3$)

Evidence for Atp1a2 is indirect. This gene encodes the catalytic and regulatory subunits of the sodium potassium pump. This pump maintains ionic gradients across the cell membrane and is essential for neuronal excitability and transmission. alpha2 agonist anaesthetics hyperpolarise neurones and inhibit action potential generation, for example, through the activation of inwardly rectifying potassium channels [31]. alpha2 is also involved in ion regulation processes and contribute to sleep and anaesthesia regulation.

Rapid eye movement (REM) sleep deprivation increases Na-K ATPase activity. This finding suggests the importance of ion balance in sleep regulation [32]. In general anaesthesia, alpha2 may be responsible for altered neural activity under anaesthetic conditions [33].

2.2.7 Slc6a1 ($\ell_1 = 3$)

Evidence for Slc6a1 is also indirect. Slc6a1 encodes GABA transporter 1 (GAT-1), which reuptakes GABA from synapses and regulates its levels. Anaesthetic drugs exert neuroinhibitory effects via GABA transporters. GABA system dysfunction has been observed in models of sleep disorders, and mutations in this transporter affect sleep regulation [34].

2.2.8 Nr1d1 ($\ell_1 = 3$)

Nr1d1 encodes a nuclear receptor that is a key transcriptional regulator of circadian clock machinery. Its expression and central position in gene networks are significantly affected by isoflurane anaesthesia, suggesting that changes in Nr1d1 levels can alter circadian timing, mood, and memory. Its dual role of maintaining normal sleep–wake cycles and modulating anaesthetic sensitivity supports its relevance as a molecular bridge between sleep and anaesthesia regulation [23].

2.2.9 Clock ($\ell_1 = 4$)

Closks are fundamentally involved in daily sleep timing and circadian rhythm regulation [31]. General anaesthesia (isoflurane, sevoflurane, propofol, and dexmedetomidine) may disrupt circadian rhythms and alter clock gene expression (e.g. Per2 and Cry) in the master clock, SCN [35].

2.2.10 Cry2 ($\ell_1 = 4$)

The Cry2 mutation is associated with familial advanced sleep phase syndrome (FASP), which is characterised by early sleep and wake times, indicating its role in sleep-wake timing in humans [36]. General anaesthetics (such as isoflurane) alter clock gene expression (such as Cry-m in honeybees and Per2), which is often studied together with Cry. This indicates that isoflurane delays Cry-m mRNA oscillations [35].

2.3 Enrichr

By uploading the selected 507 genes to Enrichr, we identified several enriched terms.

2.3.1 Reactome Pathways 2024

GABA Synthesis, Release, Reuptake, and Degradation

GABA synthesis plays a critical role in consciousness [37–39]; it is the third highest ranked term in this category for $\ell_1 = 3$.

RHO GTPases

"RHO GTPases Activate Formins" and "RHO GTPase Effectors" are the sixth and seventh terms in this category for $\ell_1 = 3$, respectively, and "Signaling by Rho GTPases" and "Signaling by Rho GTPases, Miro GTPases and RHOBTB3" are the fifth and seventh terms in this category for $\ell_1 = 4$, respectively. Recently, the RHO GTPases pathway was shown to control sleep-wake rhythm. For example, SIK3, a kinase that controls mammalian sleep demand, can target the small RhoA protein, whose activity regulates sleep-wake controls [40]. Similarly, some general anaesthesia can bind to Rho GTPase regulators and affect Rho GTPase. Inhaled anaesthetics, such as halothane, can bind to RhoGDI and alter its functionality [41]. These results suggest that RHO GTPases are involved in consciousness.

Regarding the relationship with sleep, Rho family small GTPases (RhoA, Rac1, and Cdc42) act as molecular switches that govern synaptic structure and function in the brain and are deeply involved in plastic changes in neuronal circuits during sleep and wakefulness. During wakefulness, these pathways contribute to synaptic strengthening and maintain neuronal excitability in response to experience, whereas during sleep, they play a dynamic role in promoting synapse reduction and reorganisation to stabilise brain networks. Animal model studies have shown that the disruption of the Rho GTPase pathway leads to reduced sleep duration, abnormal sleep homeostasis, and cognitive dysfunction [42, 43], suggesting its involvement in human sleep, developmental, and neurological disorders.

With regard to anaesthesia, phenomena at the cellular level induced by anaesthetic drugs, such as isoflurane, sevoflurane, and propofol, cause neuronal dendritic spine loss and growth cone collapse, delayed astrocyte maturation, altered vascular endothelial barrier, and accelerated endothelial vesicle release, with small GTPase activity fluctuations in the Rho family. Particularly, RhoA and its downstream Rho kinase (ROCK) are hub molecules involved in anaesthetic-induced cytoskeletal changes, indicating that manipulating the RhoA/ROCK pathway may mitigate the adverse effects of anaesthetic drugs [44, 45].

2.3.2 KEGG 2021 Human

GABAergic synapse

GABAergic synapses play a critical role in consciousness [37–39]and is the highest-ranked term in this category for $\ell_1 = 3$ and the fourth top-ranked term for $\ell_1 = 4$.

Glutamatergic synapse

In addition, the glutamatergi synapse plays a critical role in consciousness [46] and is the highest-ranked term in this category for $\ell_1 = 4$.

Dopaminergic synapse

Dopaminergic synapses are related to both sleep and anaesthesia [47], and are the second top-ranked term in this category for $\ell_1 = 4$.

Circadian entrainment

Circadian rhythms are known to play critical roles in sleep [48] and anaesthesia [49], and are the eighth top-ranked term in this category for $\ell_1 = 4$.

Thyroid hormone signaling pathway

"Thyroid hormone signaling pathway" is the third top ranked term in this category for $\ell_1 = 3$. The thyroid gland produces two primary hormones [50].

- Thyroxine (T4): A prohormone.
- Triiodothyronine (T3): The active form.

T4 is converted to T3 by deiodinases (especially type 2 Deiodinase (DIO2) in the brain). T3 binds to thyroid hormone receptors (TRα and TRβ), which are nuclear receptors that regulate gene expression. These receptors are highly expressed in brain regions critical for consciousness, including the cortex, thalamus, and reticular formation of the brainstem [51]. Neurophysiologically, the thyroid hormone signaling pathway neurophysiologically affects consciousness. T3 increases neuronal excitability by regulating Na^+/K^+ ATPase expression (maintaining membrane potential) [52] and ion channel functions (e.g. K^+ channels) [53]. T3 also enhances synaptic plasticity in areas like the prefrontal cortex [54] and hippocampus [55], supporting higher-order functions tied to consciousness (e.g., attention, memory, and awareness). Thalamocortical circuits regulate arousal states and sleep-wake transitions [56–58]. Thyroid hormones modulate the neurotransmitter systems (e.g. glutamate, GABA, and acetylcholine) involved in the thalamocortical oscillations that underlie different states of consciousness [59, 60]. These observations strongly suggest that the thyroid hormone signaling pathway regulates consciousness, although the thyroid hormone signaling pathway is not likely to play a critical role.

2.3.3 Allen Brain Atlas down

This category listed genes whose expression was lower in the treatment group than in the control group. Since the control and treatments vary between experiments, the distinction between "Allen Brain Atlas down" and "Allen Brain Atlas up" is not critical. In this category, several important regions were identified during sleep. The top-ranked regions not listed here were also expected to be important during sleep.

Hindbrain

Hindbrain neurones flip the switch among REM sleep, non-REM sleep, and wakefulness [61]. The hind brain also plays a critical role in anaesthesia [62]. "Hindbrain" is the top ranked term for $\ell_1 = 4$.

layer 1 of FCx

"layer 1 of FCx" is the top ranked term for $\ell_1 = 3$. Layer 1 (L1) is also known as bimolecular layer, and there are a few neurones in L1. Instead, dendrites and axon terminals accumulated in L1. In particular, the apical tufts of pyramidal neurones were concentrated. There are few interneurons, for example neurogliaform cells, in layer 1, which is GABAergic. L1 is a hub of synaptic connections, such as corticocortical and thalamic inputs. L1 is the control centre of the pyramidal neurones in the lower layer based on incoming information. Attention control and conscious judgment are controlled through L1 of the frontal cortex (Fcx). For example, incoming signals from fronto-parietal network affect the activity of pyramidal neuron in layer 5 (L5). NMDA receptor-dependent synaptic plasticity is thought to be related to L1 of the FCx [63].

As mentioned above, the L1 governs wide-ranging cortical regulation, especially top-down control, and changes in the state of consciousness. Regarding the relationship with sleep, the L1 of the FCx is the source of delta waves of brain waves, whose occurrence and propagation are important in slow-wave sleep (SWS) [64–66]. In contrast, general anaesthesia strongly suppresses corticocortical inputs and dendritic spikes. Dendritic spikes in pyramidal neurones are reduced, and the activities of lower-layer neurones are reduced. This results in global consciousness suppression. For example, isoflurane and other anaesthetics can suppress the coupling of L1 dendrites and the soma in cortical pyramidal neurones [67]. This process is related to unconsciousness. Ketamine affects the L1 of the FCx differently from that of deep layers [68]. In short, during deep sleep or anaesthesia, neuromodulatory tone drops and L1 inputs diminish, which, along with strong L1 inhibition, contribute to the hyperpolarization of apical dendrites and synchronisation of slow oscillations.

FCx layer 2

"layer 2 of FCx" is the second top ranked term for $\ell_1 = 3$. In contrast to L1 which included a few cell bodies, Layer 2 (L2) mainly included cell bodies. Thus, L2 was expected to work in the collaboration with L1. L2 receives thalamic inputs through L1, especially from higher-order thalamic nuclei, acts as a relay hub like L1, and plays a role in associative learning, memory encoding, and modulation of cognitive function. Neurones in L2 tend to have a high input resistance and low firing thresholds, making them sensitive to synaptic inputs from L1 and other superficial layers [69]. Layer 2 of the frontal cortex contains pyramidal cells and GABAergic interneurons. GABAergic interneurons are crucial for local circuit processing in the frontal cortex.

In NREM, especially in SWS, small pyramidal neurones and astrocytes (also known as interneurons which are missing in L1) in L2 of FCx exhibit large up/down states, and their occurrence and propagation through L3 and L5 generate slow waves. Thalamocortical inputs toward L4 in FCx propagated with the help of L2 and L3 (L3 of FCx is the fifth top-ranked term) of FCx and generated slow eave activity and sleep spindles in NREM. Sleep spindles in the L2/L3 of the FCx also play critical roles in memory consolidation [70].

General anaesthesia drastically altered the frequency of synaptic firing and synchronisation patterns in L2/L3. Especially, slow oscillation (0.1-1 Hz) is enhanced, which is analogous to slow-wave activity in NREM. Nevertheless, the propagation

speed and interaction between layers can differ from SWS. Slow wave propagation under anaesthesia from L1 to L5 through L2/L3 is relatively slower and more unstable than during sleep [71]. Pyramidal cells in the L2/L3 phase are affected by the anaesthetic-induced overactivation of GABA receptors, inhibition of excitatory synaptic transmission, and intermittent firing. This causes large-scale disruption of cortical networks, which leads to a loss of consciousness [72].

Primary motor area, Layer 1/Secondary motor area/Secondary motor area, layer 2/3

These are the eighth–tenth top-ranked terms in this category for $\ell_1 = 3$. In contrast to L1–L3 of FCx which are important in NREM, the motor area is important in REM. In NREM, the primary motor cortex (M1) and premotor areas show reduced activity compared with wakefulness [73]. However, sleep spindles and slow oscillations in motor areas contribute to motor memory consolidation (e.g. learning new motor skills) [74]. During REM, the primary motor cortex and premotor cortex are reactivated, often showing activity levels similar to those during wakefulness [73]. Despite this cortical reactivation, motor output is suppressed by the brainstem (specifically, the pontine reticular formation), which induces muscle atonia (paralysis) to prevent dreams [75]. This paradox (active motor areas but paralyzed muscles) is critical for dreaming without movement.

With regard to anaesthesia, motor cortex activity is strongly suppressed, but not identical to sleep [76]. Similar to NREM sleep, motor output is abolished, but cortical dynamics differ depending on the anaesthetic agent; propofol, sevoflurane, and isoflurane tend to produce strong, slow oscillations (0.1–1 Hz) in the motor cortex [77–79], reflecting deep cortical suppression. Some anaesthetics (e.g. ketamine) show preserved or increased gamma activity (30–80 Hz), even in motor areas, despite the loss of consciousness [80]. Motor evoked potentials (MEPs): Under anaesthesia, transcranial magnetic stimulation-evoked (TMS)-evoked responses from the motor cortex are markedly reduced [81]. This indicated that the motor output pathways (cortex to spinal cord) were functionally disconnected.

However, unlike sleep, anaesthesia does not consolidate motor memory. Studies comparing motor sequence learning before and after anaesthesia showed no performance gains (opposite to sleep effects) [82]. This is likely because anaesthesia globally suppresses plasticity mechanisms in motor circuits.

Supraoptic nucleus/episupraoptic nucleus

Although "Supraoptic nucleus" and "episupraoptic nucleus" are not highly ranked terms but only 145th and 185th ranked terms with significant adjusted P-values (6.28×10^{-4} and 2.80×10^{-4}, respectively) for $\ell_1 = 3$, a recent study found a novel group of GABAergic and glutamatergic neurones in the supraoptic nucleus (SON) of the hypothalamus that was activated by multiple anaesthetics and that promoted NREM sleep [38] and termed them anaesthesia-activated neurones (AAN). Notably, AAN was discovered by investigating AVP and OXT expression, which were also identified using LLM (Table 1). Although they were also associated with significant Pvalues for $\ell_1 = 4$, they were not highly tank (as low as 42th and 72th, respectively).

2.3.4 Allen Brain Atlas up

This category lists the genes whose expression increased in the treatment groups compared with that in the control group. Since the control and treatments vary between experiments, the distinction between "Allen Brain Atlas down" and "Allen Brain Atlas up" is not critical.

layer 6 of PCx

Multiple layer 6 of the PCX-related subregions were ranked. "sublayer 6b of PCx" (2nd),"layer 6 of PCx" (3rd), and "sublayer 6a of PCx" (4th) for $\ell_1 = 4$ It is heterogeneous and is composed of various types of neurones, mostly glutamate [83]: Corticothalamic (CT) Pyramidal (Glutamate), Corticocortical (CC), pyramidal (glutamate), L6b/Drd1a-Cre+ Pyramidal (glutamate), Orexin-Sensitive L6 Pyramidal (glutamate) and GABAergic Interneurons (GABA).

Cortical layer 6b mediates state-dependent changes in brain activity and the effects of orexin on waking and sleeping [84]. The presence of orexin-sensitive neurones, almost exclusively within the mPFC L6 [85] adds another layer of complexity and significance. Orexin is a neuropeptide critical for maintaining arousal and wakefulness. Anaesthetics suppress arousal [86]. Thus, interference by anaesthesia with orexin signaling or direct effects on these orexin-sensitive L6 neurones could represent a specific mechanism by which anaesthesia dampen arousal-related modulation of PFC activity at this laminar level, contributing to the hypnotic component of anaesthesia.

In contrast, general anaesthesia affects layer 6 of PCx in various ways.

- Propofol: This widely used GABAergic anaesthesia has marked effects on deep PFC neurons. In layer 5 of the medial PFC (mPFC), which shares functional characteristics and connectivity relevant to L6, propofol predominantly inhibits the firing of regular-spiking (RS) neurons (putative pyramidal cells) and causes a significant suppression of fast-spiking (FS) interneurons. While the majority of RS neurons are inhibited, a small subset may exhibit excitation [87]. Propofol also leads to a general reduction in neuronal excitability, an effect that can be particularly pronounced in the lateral PFC compared to sensory areas like the auditory cortex [88]. In in vitro cortical slice preparations, a clinically relevant concentration of propofol (3μM) has been shown to reduce muscarine-induced persistent potentials and associated spiking activity [89].
- Sevoflurane: Another commonly used inhaled GABAergic anaesthetic, sevoflurane, exerts effects on mPFC L5 neurons that are largely similar to those of propofol. Sevoflurane primarily inhibits RS neurons and suppresses FS interneuron activity [87]. Compared to isoflurane, sevoflurane tends to produce a uniform suppression of single-unit activity across the cortex. This potent suppression may be related to its effects on glutamatergic transmission; sevoflurane more effectively reduces calcium-dependent glutamate release than isoflurane, especially at higher concentrations [90].
- Ketamine (NMDA Antagonist): In stark contrast to GABAergic anaesthetics, ketamine induces predominantly excitatory effects on both RS and FS neurons in mPFC L5, although a subpopulation of neurons can still be inhibited [87]. A striking

effect of ketamine is its ability to cause a rapid and widespread switch in spontaneously active pyramidal neuron populations across all cortical layers, including deep layers L5 (and by extension, L6), in various cortical regions (somatosensory, visual, motor, retrosplenial cortices). This phenomenon involves the suppression of neurons that are normally active during wakefulness and the concurrent activation of previously silent neurons [91]. This profound reorganization of active neuronal ensembles occurs with both systemic administration and local cortical application of ketamine, suggesting a fundamental alteration in cortical state rather than simple suppression. At low, therapeutically relevant concentrations (e.g., 1μM) in mPFC L5 pyramidal neurons, ketamine has been shown to decrease the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) while increasing the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs). This is consistent with a preferential blockade of NMDARs located on GABAergic interneurons, leading to pyramidal cell disinhibition. High concentrations of ketamine (e.g., 10 µM) tend to decrease the rates of both sIPSCs and sEPSCs [92]. In cortical slices, 20μM ketamine appeared to enhance muscarine-induced persistent potentials and spiking (though non-significantly), whereas a much higher dose (100μM) suppressed them [89]. This dichotomy between the general suppressive effects of GABAergic anaesthetics and the complex, often excitatory and state-switching effects of ketamine on L6/deep layer neurons suggests fundamentally different mechanisms by which these agents disrupt L6 processing and, consequently, consciousness.
- Isoflurane: This inhaled anaesthetic also modulates activity in deep cortical layers, including PFC infragranular layers, in a complex, frequency-specific manner (detailed in section 3.2). Compared to sevoflurane, isoflurane causes a less uniform suppression of neuronal firing [90].

Regarding firing patterns, anaesthetics differentially affect RS and FS neurones. For GABAergic agents such as, sevoflurane and propofol, the activity of inhibited RS neurones in mPFC L5 is closely correlated with the transitions between loss of righting reflex (LORR) and recovery of righting reflex (RORR), highlighting their potential role in tracking anaesthetic depth. FS interneurons are generally significantly suppressed under these GABAergic anaesthetics. However, ketamine has mixed (inhibitory or excitatory) effects on FS neurones. Anaesthetics also influence bursting activity. For example, urethane can increase burst duration and the number of spikes within a burst in cortical neurones. The arousal-promoting peptide orexin, which acts on specific L6 neurones, increase burst frequency in the mPFC even under isoflurane anaesthesia, suggesting that some intrinsic circuit dynamics can be modulated regardless of the anaesthesia state.

3 Methods

3.1 Gene expression profile

3.1.1 Gene expression profiles under anaesthesia treatment

Gene expression profiles under anaesthesia were generated using DRA010292 [9]. Three mice treated with sevoflurane, anaesthesia, and three control mice, in total, six

mice were investigated. Fastq files of RNA expression in brain sections (hippocampus, hypothalamus, medial prefrontal cortex, and striatum) were generated. Fast q files were mapped onto the mouse genome (mm10) using bowtie2 [93]. bowtie2 instead of the other transcriptome mappers because the gene expression profiles to be compared were measured by a transcriptome array but DNA array. Thus, mapping short reads to the genome using a transcriptome mapper but a DNA mapper is preferable. In total, we had two conditions (control and anaesthesia treatment), three mice times four brain subsections, whose number was equal to $2 \times 3 \times 4 = 24$.

3.1.2 Gene expression profiles under sleep disruption

Gene expression profiles under sleep deprivation conditions were downloaded from GEO using GEO ID GSE69079 [94]. Gene expression profiles were measured using an Affymetrix Mouse Genome 430 2.0 Array. Mice were in sleep or sleep-deprived conditions, and six biological replicates were used for the bound fraction. For the unbound fraction, there were sleep-deprived mice with two biological replicates. In total, 16 mice were used in the study.

3.2 Tensor decomposition based unsupervised feature extraction [10]

First, the gene expression profiles must be formed as tensors. For anaesthesia treatment, the gene expression profiles are formed as a tensor $x_{ijkm} \in \mathbb{R}^{N\times2\times4\times3}$ which represents the expression of the ith gene at jth condition (anaesthesia treatment and control) in kth brain tissue in mth replicate. For sleep, the gene expression profiles are formed as a tensor $x'_{ijk'm'} \in \mathbb{R}^{N\times2\times2\times6}$ which represents the expression of the ith gene at jth condition (sleeping and waking) at kth condition (bound vs unbound) in mth replicate. For the unbound condition, because only two replicates were used, each replicate was used three times to obtain six replicates.

We generate another tensor by multiplying the two tensors as follows:

$$x_{ijkk'mm'} = x_{ijkm}x'_{ijk'm'} \in \mathbb{R}^{N\times2\times4\times2\times3\times6} \tag{1}$$

where only genes i common between the two profiles (sleep and anaesthesia) were considered. Gene symbols attributed to genes via bowtie2 were converted to ENTREZ GENE ID using org.Mm.eg.db package of Bioconductor. ENTREZ GENE IDs are also attributed to Affymetrix Mouse Genome 430 2.0 Array, thus we can compare ENTREZ GENE IDs between the two gene expression profiles. $j = 1$ corresponds to sleep or anaesthesia and $j = 2$ corresponds to waking or control. This enabled us to find a gene expression that correlates with the similarity between sleep/wake and anaesthesia/control: unconscious/conscious. $x_{ijkk'mm'}$ is scaled as

$$\sum_{i=1}^{N} x_{ijkk'mm'} = 0 \tag{2}$$

$$\sum_{i=1}^{N} x_{ijkk'mm'}^2 = N. \tag{3}$$

Then HOSVD was applied to $x_{ijkk'mm'}$ and we get

$$x_{ijkk'mm'} = \sum_{\ell_1=1}^{N} \sum_{\ell_2=1}^{2} \sum_{\ell_3=1}^{4} \sum_{\ell_4=1}^{2} \sum_{\ell_5=1}^{3} \sum_{\ell_6=1}^{6} G(\ell_1\ell_2\ell_3\ell_4\ell_5\ell_6) u_{\ell_1 i} u_{\ell_2 j} u_{\ell_3 k} u_{\ell_4 k'} u_{\ell_5 m} u_{\ell_6 m'} \tag{4}$$

where $G \in \mathbb{R}^{N\times 2\times 4\times 2\times 3\times 6}$ is a core tensor that represents the contribution of individual product $u_{\ell_1 i} u_{\ell_2 j} u_{\ell_3 k} u_{\ell_4 k'} u_{\ell_5 m} u_{\ell_6 m'}$ toward $x_{ijkk'mm'}$. $u_{\ell_1 i} \in \mathbb{R}^{N\times N}$, $u_{\ell_2 j} \in \mathbb{R}^{2\times 2}$, $u_{\ell_3 k} \in \mathbb{R}^{4\times 4}$, $u_{\ell_4 k'} \in \mathbb{R}^{2\times 2}$, $u_{\ell_5 m} \in \mathbb{R}^{3\times 3}$, and $u_{\ell_6 m'} \in \mathbb{R}^{6\times 6}$, are singular value matrices and orthogonal matrices, which represent the dependence of $x_{ijkk'mm'}$ on i, j, k, k', m and m'.

After selecting $u_{\ell_2 j}, u_{\ell_3 k}, u_{\ell_4 k'}, u_{\ell_5 m}, u_{\ell_6 m'}$ associated with desired property, we investigate $G(\ell_1\ell_2\ell_3\ell_4\ell_5\ell_6)$ to see which $u_{\ell_1 i}$ is maximally associated with the selected $u_{\ell_2 j}, u_{\ell_3 k}, u_{\ell_4 k'}, u_{\ell_5 m}, u_{\ell_6 m'}$. Typically, $u_{\ell_1 i}$ with $G(\ell_1\ell_2\ell_3\ell_4\ell_5\ell_6)$ which has the largest absolute value, is selected.

Next, we selected is with a larger contribution to $u_{\ell_1 i}$. To do this, we attribute Pvalues to i by assuming that $u_{\ell_1 i}$ obeys a Gaussian

$$P_i = P_{\chi^2}\left[> \left(\frac{u_{\ell_1 i}}{\sigma_{\ell_1}}\right)^2\right] \tag{5}$$

where $P_{\chi^2}[> x]$ is the cumulative χ^2 distribution where the argument is larger than x and σ_{ℓ_1} is the standard deviation. P_is are corrected by the Benjamini-Hochberg criterion [10] to consider multiple comparison corrction and is associated with adjusted P-values less than the threshold value, that is, 0.01, are slected.

σ_{ℓ_1} is optimized such that the histogram of P_i becomes as flat as possible, since the histogram of P_i becomes uniform distribution when the null hypothesis, that is, $u_{\ell_1 i}$, obeys Gaussian stands. To seek σ_{ℓ_1} that maximally coincides withe the null hypothesis, we performed the following:

1. Set σ_{ℓ_1}
2. Compute P_i with using σ_{ℓ_1}.
3. Exclude is associated with adjusted P-values less than the threshold value.
4. Re-compute σ_ℓ with remaining σ_{ℓ_1}s.
5. Compute the histogram $h(P_i)$, of P_i.
6. Compute the standard deviation of $h(P_i)$.
7. Update σ_{ℓ_1}.
8. Go to step 2

This process was repeated until the standard deviation of $h(P_i)$ was minimised. This results in σ_{ℓ_1} that maximally satisfies the null hypothesis, because the smallest standard deviation of $h(P_i)$ should correspond to a uniform distribution that coincides with the null hypothsesis as much as possible.

3.3 LLM

LLM [95] is useful for evaluating gene function to some extent. Here is a list of how to use LLM to retrieve significant genes from a set of selected genes.

- Gemini 2.5(experimental) + Deep Research (https://gemini.google.com/)
- perplexity Research (https://www.perplexity.ai/)
- Grok Deep Search (https://grok.com/)
- DeepSeek DeekThink(R1) + Search (https://chat.deepseek.com/)
- AI Agents for Scientific Discovery Crow (https://platform.futurehouse.org/)

The prompt used was "This list of genes has been listed as being related to sleep and anaesthesia. Please review these lists, select those that you think are relevant to both sleep and anaesthesia, and summarise them with citations to supporting articles" or their Japanese translations. The prompt was used to upload a list of the selected genes (one gene per line). LLM sometimes incorrectly listed genes in the list of 507 selected genes or 1,048 genes. These genes were excluded from analysis. Among the genes selected by LLM, those associated with suitable references were included.

3.4 Enrichment analysis

The enrichment analysis was performed using Enrichr [96]. The list of gene symbols for the selected genes has been uploaded. Pvalues provided by Enrichr should be corrected by considering multiple comparison corrections and a suitable background set of genes and biological terms.

4 Discussion

In this study, we identified 507 genes that are commonly associated with unconsciousness caused by sleep and anaesthesia. The set of 507 genes included candidate genes commonly related to unconsciousness caused by sleep and anaesthesia and was identified using LLM. In addition, using enrichment analysis, we identified various biological terms and brain subregions that are commonly associated with sleep and anaesthesia-associated unconsciousness. In this analysis, we identified genes whose expression is commonly distinct between sleep or anaesthesia and wakefulness or control. In the conventional analysis, sleep and anaesthesia were studied separately and differentially expressed genes (DEGs) were identified in both groups. An overlap of DEGs between sleep and anaesthesia was identified. In contrast to the conventional analysis, using TD based unsupervised FE, we can identify commonly distinctly expressive genes by analysing $x_{ijkk'mmm'}$ since These are the products of gene expression between sleep and anaesthesia. Only genes whose expression are expressive/suppressive between sleep or anaesthesia and wake and control can have larger/smaller values of $x_{ijkk'mm'}$. No other method could effectively perform this task. Consequently, despite the fully unsupervised nature of this method, TD based unsupervised FE can select a reasonable set of genes. The selected genes were enriched in multiple brain subregions related to sleep and anaesthesia. Biological terms related to both sleep and anaesthesia were

identified. In particular, the identification of RHO GTPases activity is important as it was not identified until recently.

However, in contrast to expectations, gene expression analysis cannot help determine the unconsciousness caused by sleep and anaesthesia. Accordingly, a more sophisticated tool is required for this purpose.

The reason why we generated the tensor, $x_{ijkk'mm'}$ with multiplying two tensors, x_{ijkm} and $x'_{ijk'm'}$ is as follows: By multiplying the two tensors, is that take larger or smaller values for both tensors can have larger or smaller values. In contrast, is that take larger values for one of two tensors whereas smaller values for another one of two tensors have medium values (i.e., neither larger nor smaller values). After standardising $x_{ijkk'mm'}$ as eqs. (2) and (3), respectively. since positive (negative) signed absolutely larger values are attributed to $x_{ijkk'mm'}$ with larger (smaller) values, they have tendency to have smaller P_i defined in eq. (5), and selected. This means that the consideration of $x_{ijkk'mm'}$ enables us to find is associated with smaller or larger values simultaneously for sleep and anaesthesia.

One might wonder if there are justifications for assuming that $u_{\ell_1 i}$ is Gaussian. To address this problem, we attempted to optimise σ_{ℓ_1} such that the histogram of P_i is as uniform as possible when excluding the selected is. As a result, we obtain a relatively uniform distribution of the histogram of $1 - P_i$ as shown in Fig. 3.

Although the trustability of LLM is questionable, we only listed genes supported by published work. Thus, we believe that the trustability is well guaranteed.

Gene expression for sleep is obtained from astrocytes whereas that for anaesthesia is obtained from various brain regions. Although one might wonder whether this is problematic, a comparison of these two methods has already been published [9]. This indicates that a comparison between these two methods has already been approved. In addition, since the purpose of this study was to identify commonly expressed genes, if we can find genes common between various brain subregions and astrocytes, it can reveal the robustness of the selection and cannot be evidence of untrustability.

We identified two sets of genes: $\ell_1 = 3$ and $\ell_1 = 4$. These proteins have distinct functions. Those selected by $\ell_1 = 3$ are mainly GABAergic and enriched in layer 1 of FCx, which primarily includes GABAergic neurones. In contrast, those selected by $\ell_1 = 4$ are mostly glutamatergic and enriched in layer 6 of PCx, which primarily includes glutamatergic neurones. The latter is closely related to orexin. Notably, the RHO GTPases pathway was enriched in both sets of genes despite their distinct nature of two sets of genes. Based on these results, we hypothesized that L1 GABAergic neurones in the frontal cortex and L6 glutamatergic neurones in the prefrontal cortex use RHO GTPase to differentiate between the states of unconsciousness during anaesthesia and sleep. Figure 6 shows the interconnectivity between the biological terms detected by Enrichr (only those with adjusted Pvalues of less than 0.05). As expected, L1 of FCx and L6 of PCx were distinct (no genes were shared). The RHO GTPase pathways share many genes, even between $\ell_1 = 3$ (GABAergic) and $\ell_1 = 4$ (glutamatergic), and are separately connected to L1 of FCx and L6 of PCx. This graph is consistent with the hypotheses above. The intersections shown in Fig. 2 is possibly because of RHO GTPase pathways shared between $\ell_1 = 3$ (GABAergic) and $\ell_1 = 4$ (glutamatergic).

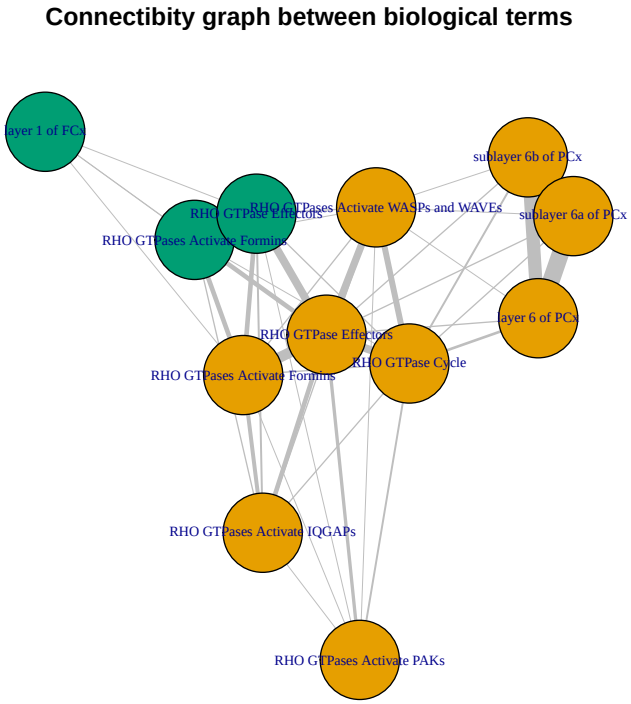

Fig. 6 Connectivity graph between biological terms detected by Enrichr (only those with adjusted P-values less than 0.05). Green: $\ell_1 = 3$ (GABAergic), yellow: $\ell_1 = 4$ (glutamatergic). Width of edges represents the number of genes shared between adjusted biological terms.

5 Conclusion

In this study, we applied TD-based unsupervised FE to the gene expression of sleep and anaesthesia and identified two sets of genes that are commonly expressed distinctly between sleep or anaesthesia and wakefulness or control. Two sets of genes were enriched in GABAergic synapses in L1 of FCx and Glutamatic synapses in L6 of PCx and RHO GTPase pathways were shared by both sets of genes. Then we hypothesized that GABAergic synapses in L1 of FCx and Glutamatic synapses in L6 of PCx differentiated unconsciousness between sleep and anaesthesia through the RHO GTPase pathways.

Declarations

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- Conflict of interest/Competing interests
  We declare that the authors have no conflicts of interest.
- Ethics approval and consent to participate
  Not applicable
- Consent for publication
  Not applicable
- Data availability
  All data analysed in this study are from GEO and are publicly available.
- Materials availability
  Not applicable
- Code availability
  https://github.com/tagtag/TDbasedUFE_deconvolution
- Author contribution
  Y. H. T. planned the study and performed the analyses. Y.-H.T. and T.T. evaluated the results and wrote and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript. The conceptualization, data curation, analysis were performed by Y. H. T.

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