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Alteration of Consciousness by Anesthetics: a multi-scale modulation from the molecular to the systems level

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Author Contributions

MAD, MC, SD, JAT and EAZ designed the work, supervised the study and contributed to the writing of the manuscript. EAZ, MC, SD collected and rationalized literature data. MAD and JT were responsible for critical revision of the manuscript. All authors read and approved the final manuscript.

Abstract

General anesthesia has been used in medical practice since mid-nineteenth century but its pharmacological mechanism of action is still not entirely clear. In a top-down approach, investigations probe smaller and smaller scales to shed light on how anesthetics disrupt or alter neural activity, ultimately resulting in loss of consciousness. In the past few decades, advances in neuroscience and molecular biology allowed for investigations on the brain structure and function from the behavioral level to the systems, cellular and molecular level. This multiscale approach implicates the molecular domain as a fundamental link between general anesthesia and consciousness, which also sheds light on how anesthetic agents affect the pathogenesis of postoperative behavioral changes. This review discusses the current state of knowledge about the relationship between consciousness and General Anesthesia determined using pharmacokinetics, molecular biology, and advanced medical imaging, including EEG, fMRI, PET, MEG. It is hoped that the mechanisms of action of anesthetic gases may help solve the mystery of consciousness. Conversely, understanding the cellular and molecular mechanisms of consciousness could lead to the design and development of new anesthetic agents and technologies to controllably turn off and on consciousness. It could also generate new concepts in neurocognitive pathophysiology disorders.

Keywords: General Anesthesia, Consciousness, Cytoskeleton, Orch OR, Quantum mobility theory, POCD

Introduction

The very definition of human consciousness has baffled philosophers, spiritualists, and modern scientific scholars alike for a long time, but is yet to be found with consensus. The underlying issue is the lack of understanding of where and how consciousness arises in the brain. A first, pragmatic step toward understanding consciousness is to inspect the role of general anesthesia (GA), whose mechanism is still an enigma in spite of the fact that its discovery dates back to 1860. Indeed, GA is routinely used for reversibly extinguishing consciousness prior to and during surgery, but without a clear knowledge of its mechanisms of action, especially at the cellular and molecular levels. Scientific inquiries aimed at defining the neural correlates of

consciousness, and hence the susceptibility thereof to general anesthetics, have been conducted at all investigative levels, from molecular/genetic to systems biology, but with inconclusive results. From a molecular point of view, this type of investigation is complicated by the fact that in spite of their diverse molecular structures and different putative molecular targets, all general anesthetics consistently induce reversible loss of consciousness and memory (Craddock *et al.*, 2015). Indeed, GA represents an extraordinary clinical procedure that allows for a temporary and reversible manipulation of the person's consciousness level in a dose-dependent manner and with only minor variations among subjects and a similar mode of action in humans as well as animals. Thus, exploring the relationship between general anesthesia and the associated fields of neuroscience becomes intriguing. Understanding the mechanisms underlying anesthesia may help to resolve the mysteries surrounding consciousness. At the same time, understanding the cellular and molecular mechanisms involved in consciousness could help the design and development of new anesthetic agents and psychoactive mood-altering drugs. It may also explain what mechanism of neuronal processing mediates the qualitative feeling associated with subjective experience. What exactly is measured or monitored during general anesthesia? Could there be a more reliable measure of consciousness when patients are under the influence of general anesthesia? The so-called Hard Problem in answering any question related to consciousness is the nature of reality that one experiences (Chalmers, 2007). The complexity of brain structure, function, and behavior further explains the broad spectrum of neuroscience research, ranging from philosophy to quantum physics (Baijpai, 2015). While consciousness at present is not known to be directly measurable or even observed, modern science continues to seek quantitative measurements of its possible neural correlates. Current neuroscience research related to the investigations of general anesthetics, is being conducted at different biological levels, from the whole organism (to determine clinical effects such as changes in the cardiac and respiratory rhythms, blood pressure, sweat production, tears or pupil dilation), to the cellular level (to identify cortical/neural mechanisms and pathways), and deeper to the molecular level (in order to identify and characterize both the sites and microscopic mechanisms of action of anesthetic molecules). Thus, the present work provides a review of the current state of knowledge regarding the targets, mechanisms and effects of general anesthesia at different time- and length-scales, and its contribution so far to the overall understanding of the biology of human consciousness.

This review is organized as follows. After a brief introduction of the different classes and uses of general anesthetics, as well as their specific pharmacokinetic properties, we focus on the

experimental and computational evidence regarding known and putative targets of general anesthetics at the molecular scale. Subsequently, an in-depth review of higher-scale studies is provided, bridging the gap between molecular knowledge and clinical practice. Lastly, we will discuss current hypotheses regarding the basis of human consciousness stemming from such multi-scale evidence of the mechanisms of action of GA.

General Anesthetics

General anesthetics are divided into two major classes: (1) intravenous anesthetic agents, which induce anesthesia and are usually administered in combination with sedatives or narcotics; and (2) volatile anesthetic agents, which are typically utilized to maintain anesthesia (Alkire, Hudetz and Tononi, 2008).

Interestingly, inhalational anesthetics act on suppressing bodily functions in a systemic way allowing for loss of awareness, yet physiological functions are preserved, explaining further why this area of science is so challenging to study. General anesthetics are stereoisomers, a-polar, hydrophobic (lipophilic) molecules, with atomic structures ranging from steroids to the elemental atom Xenon. Their anesthetic concentrations in the phospholipid neural membranes during general anesthesia are high (10–100 mM), without any apparent relationship between their molecular structure and physiological effect they elicit (Herold *et al.*, 2017). This suggests that there might be several possible molecular mechanisms of action of anesthetics on different targets rather than a single unitary theory (Franks, 2008). In the case of volatile agents, their ability to selectively prevent consciousness while sparing non-conscious brain activities depends on achieving therapeutic tissue concentrations in the central nervous system (CNS). These compounds are unique compared to other pharmacological agents, having their main route of administration through the lungs and multiple physical properties determining the bioavailability.

The Meyer-Overton correlation between GA lipid solubility and their minimum alveolar concentration (MAC) suggests that inhalational anesthetic agents have one or more hydrophobic sites of action and may bind to both membrane lipids and to hydrophobic pockets in proteins. To quantify the GA potencies, Eger et al. (1965) introduced the concept of MAC, which defines the alveolar fraction of an anesthetic gas that prevents movement in response to a surgical skin incision in 50% of patients (half-maximal effective concentration values, EC_{50}) (Urban and Bleckwenn, 2002). The MAC concept negated the variability by measuring a single

quantitative endpoint: immobility. Moreover, MAC expresses the end-tidal anesthetic as a measure of the anesthetic level within the alveoli and, in turn, assumed as the level in the CNS (Aranake, Mashour and Avidan, 2013). In other words, the required efficacious site concentrations depend on the partial pressure of these agents in the CNS and at equilibrium (between alveoli, blood, and brain) are given in terms of the alveolar concentration ~~(5)~~. This is a multi-factorial process that principally depends on factors that:

i) determine the alveolar concentration of the drug, such as the inspired concentration, the alveolar ventilation and the lung functional residual capacity,

ii) influence the uptake in the lungs, including the compound's solubility, the pulmonary blood flow and the alveolar-venous partial pressure gradient.

Overall, the biophysical properties of inhaled anesthetics influence both uptake from the lungs and distribution through the body. Inhaled anesthetics enter the body *via* trans-alveolar exchange from gas to blood and their clearance is *via* the same route. Thus, inhaled anesthetic delivery is dependent on pulmonary ventilation, whereas uptake and clearance are dependent on pulmonary perfusion.

Molecular targets and driving mechanisms of anesthesia

The concept of general anesthetic action involving specific neuronal pathways implies they might behave selectively at the molecular level. The exposure to general anesthetics shares a common response from bacteria to human neurons, namely the abolishment of cytoplasmatic motility (Goldstein and van de Meent, 2015; Kelz and Mashour, 2019). The organized movement of cytoplasm within all cells is mediated by cytoskeletal proteins, which have recently been implicated as an invariant anesthetic target. The first attempt to explain the effect of general anesthetics on the neuronal membrane came more than a century ago when Meyer and Overton correlated the hydrophobicity of an anesthetic molecule to its efficacy (Urban and Bleckwenn, 2002). Neuronal cell membranes are mainly composed of phospholipids. Since neuronal signals are governed by the structural organization of these membranes, it was concluded that the Meyer-Overton effect implies a unitary action of anesthetics in lipid regions of the neuronal membranes of the brain. Decades later, Johnson and Flagler (1950) discovered that when ambient pressure is increased to 130 atm, the anesthetic effect of ethanol can be reversed. Although the site where pressure reversal occurred appeared to be the membrane, it was not clear which location in the body was involved in this phenomenon (Miller and Wilson, 1978).

Despite the persistent presence of some still-unresolved issues, such theories imply that a profound physical basis of anesthesia must exist, explaining the effects of anesthetics on cellular and subcellular targets, i.e. membranes and proteins. In fact, it is widely known that the tertiary structures of proteins, fundamental for their functional activity, are easily affected by physical factors (Baluška *et al.*, 2016). Indeed, later the molecular mechanisms responsible for neuronal membrane excitability were found to depend on ion channels and membrane receptor proteins. In the 1980s, Franks and Lieb discovered that anesthetic molecules act directly within hydrophobic regions of these proteins (Franks and Lieb, 1994). These findings provided evidence that the effect of general anesthetics on membranes was also stereospecific (Harris, Moody and Skolnick, 1992). Recently, numerous diverse groups of ion channels, receptors, enzymes, and even cytoplasmic proteins have been identified as putative targets of anesthetic agents. However, there is still no direct experimental evidence linking these findings to a mechanism of action. More recently, Herold (2014) investigated a wide range of chemically diverse general anesthetics and structurally related non-anesthetics with the aim of characterizing their neuronal properties employing a standard fluorescence assay, which detects drug-induced changes in the affected lipid bilayer. These investigators established that the lipid bilayers exposed to general anesthetics exhibit only an insignificant change in their properties at clinically relevant concentrations. This would indicate that the effects of anesthetic agents on the function of ion channels are not lipid bilayer-mediated but rather surprisingly directly engage in protein interactions (Herold *et al.*, 2017). Thus, it may be agreed that general anesthetics interact with many proteins through non-specific activity (binding to more than one receptor site) with half-maximal effective concentration (EC_{50}) values, most of which are the order of 1 mM (Chau, 2010). Thus far, sufficiently strong evidence is only available for two ligand-gated ion channels, namely the γ-aminobutyric acid (GABA) type A receptor and the *N*- methyl-D-aspartic acid (NMDA) receptor, as the most probable sites of action (Solt, Johansson and Raines, 2006) (see Figure 1).

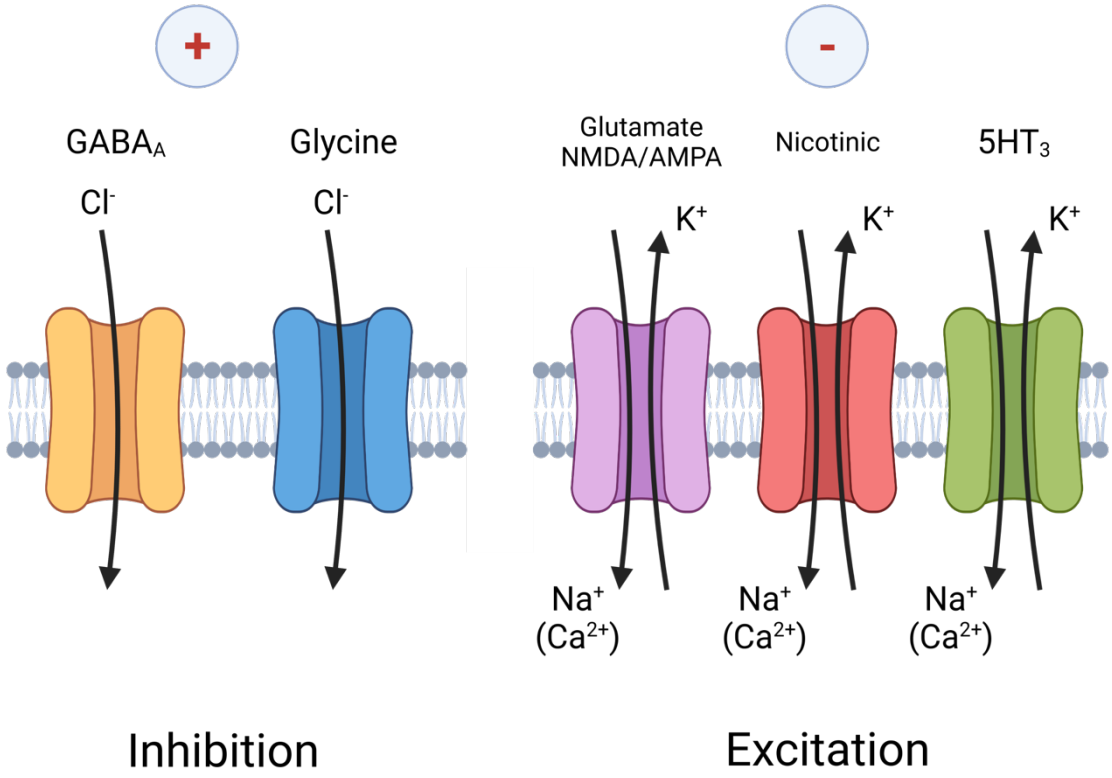


Figure 1. Inhalational anesthetics modulatory activity on transmitter-gated ion channels. Anesthetics can modulate both pre- and postsynaptic synaptic activity. Presynaptic, by interacting with a variety of proteins, that regulate resting membrane potential and calcium dynamics, thus modulating the neurotransmitter release. Postsynaptic, by binding to targets such as extra-synpatic $GABA_A$ receptors will also affect signaling. Anesthetic agents alter synaptic function through their inhibition of excitatory synapses and through enhancement of the activities of inhibitory synapses

The family of $GABA_A$ receptors has been dissected for each subunit composition. Interestingly $GABA_A$ receptor subunits are differently distributed among brain regions, according to their pharmacology, cell-type expression, and subcellular localization (Baldassarre, Scarpati and Piazza, 2020a). The $GABA_A$ receptor activation is responsible for evoking two forms of inhibitory currents (modulatory inhibition). Understanding how the different $GABA_A$ subunit modulates the inhibitory transmission under clinical conditions could be essential for the recognition of disease conditions (Herold *et al.*, 2017). There is evidence that the $GABA_A$ receptor may be involved in GA effects since haloalkanes, etomidate, and the barbiturates interact with different domains of the α- and β-subunits, while propofol binds to the β-subunit (Tang and Eckenhoff, 2018). Propofol has not been shown to bind to the α-subunit of the $GABA_A$ receptor.

Glutamate is an excitatory neurotransmitter found in the CNS whose effects have been seen on three classes of receptors: NMDA, AMPA, and kainate. NMDA receptor seems to reduce

activated current in the presence of nitrous oxide and haloalkanes. However, their effects are not as significant as those exerted on the $GABA_A$ receptor (Hollmann, 1999; Baldassarre, Scarpati and Piazza, 2020b). Many excitatory or inhibitory postsynaptic receptors, once activated, initiate a cascade of metabolic steps that involve cytoskeletal protein conformational changes to modulate cell activity.

To act as a general anesthetic agent in the CNS, a drug must be able to cross the blood-brain barrier (BBB) and diffuse through the brain parenchyma in order to interact with its designated target. Therefore, anesthetic agents must have apolar characteristics. The interactions between two apolar gas molecules are referred to as van der Waals forces and are relatively weak since they are due to induced polarization in the electron cloud around the nucleus of each of the interacting molecules. For the same reason, GAs typically interact with their targets through weak polarization forces (mostly London dispersion forces) and to a lesser degree by hydrogen bonding (Abraham, Lieb and Franks, 1991). This explains the absence of large conformational changes in the target protein following anesthetic binding. Consequently, anesthetics do not possess the common functional groups that endow most pharmacological agents with their specificity.

Furthermore, the binding sites of anesthetic agents on protein surfaces are also expected to be non-polar, pre-formed cavities where the anesthetic displaces water (Bertaccini, 2010). These non-polar intra-protein regions were found to be composed of highly polarizable aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine, and can provide a chiral environment. As a result, the orientation of the anesthetic molecule in its binding pocket is determined by its chemical structure. These findings suggest that anesthetics do not act by inducing new conformational states of proteins instead they bind to pre-existing conformational states. At present, there are only two viable methods to identify the general anesthetic's binding site at this biological level of investigation by direct measurements. The first method involves a mutation of an amino acid at the putative binding site. The second method uses a photo-affinity analog of the anesthetic in order to be able to observe and image its binding location. Eckenhoff et al. used radiolabeled halothane in mice and was able to show that at clinically relevant concentrations, anesthetics bind to roughly 70 different neuronal proteins. About half of them are cellular membrane components, and the other half are cytoplasmatic proteins, particularly tubulin. Proteomic analysis conducted after halothane, isoflurane, desflurane, and sevoflurane exposure shows that tubulin expression levels change even several days after treatment while the membrane protein expression does not modify

(Kalenka *et al.*, 2007; Elsevier, 2014). More recently some authors presented a new model for the anesthetic mechanism of action based on experimental findings, which may involve some form of electron transfer processes in protein. Experiments showed (Turin, Skoulakis and Horsfield, 2014) that *Drosophila* exposed to an external magnetic field generates spontaneous radio frequency emission that is abolished by chloroform (Cukras and Sadlej, 2021). Similar results were found using Xe, N_2O, CH_3Cl, further corroborating the hypothesis that anesthetics may affect electron spin degrees of freedom as shown in specific electron spin resonance signals (ESR) (Pavel *et al.*, 2020). These findings appear to open a new avenue for the study of the physical mechanism of anesthesia and consciousness since they include electronic properties of proteins, possibly embedded in the membranes of excitable cells resulting in free-electron transfer and possibly interactions with nuclear spin. The involvement of anesthetics in the electronic properties of a neurological systems also allows important progress in understanding how quantum effects contribute to information transfer processes in biological systems (Smith *et al.*, 2021). In this regard, low light intensity pulses have been demonstrated to trigger neuronal action potential in vivo showing the neurons are susceptible to light and thus hypothesizing a possible role in information transfer (Bouche *et al.*, 2001) including quantum electromagnetic interaction (Burdick *et al.*, 2019).

The link between anesthesia, consciousness and biophysical phenomena involving neuronal tubulin was already proposed in the mid- and late 1990s by Hameroff and Penrose (Hameroff, 1998). They formulated the Orchestrated Objective Reduction (Orch OR) theory, a paradigm linking together biology and quantum physics, still a matter of lively debate and controversy to this day. The theory states that the site of action of anesthetics is a distributed phase of non-polar, hydrophobic medium composed of discrete pockets inside microtubules throughout the brain. In the hydrophobic regions, the theory states that quantum mechanical forces direct the functions responsible for consciousness, which is postulated to occur due to 'orchestrated' ('Orch') objective reduction ('OR'), i.e. quantum collapse of quantum states in microtubules, which resonate through a multiscale vibrational hierarchy to the membrane and synaptic activities in the low frequency range (below 100 Hz) (Hameroff and Penrose, 2014). Others have also investigated the relationship between microtubule activity and cognitive function. It has been shown how during brain development, a period associated with the formation of synapses and visual learning at the highest rates, the visual cortex of the brain produces extremely large quantities of tubulin (see The Human Protein Atlas https://www.proteinatlas.org/).

What is interesting in the context of quantum consciousness is that neurons contain structures such as microtubules, mitochondria and dendritic spines that might support coherent energy transfer. Furthermore, living organisms tend to be favorably affected by coherent patterns of electromagnetic (EM) waves, which may induce a "biological order". Pribram pioneered the notion that brain activity at the level of neural web patches involves EM interactions in terms of local field potentials leading to holographic phenomena (Xu et al. 2018). These would represent interference patterns that form images due to constructive and destructive interference effects of the overlapping waves. This hypothesis is referred to as the Holonomic Brain Theory (Pribram, 1986; Pribram *et al.*, 2013). Holonomic processes have subsequently been termed "Quantum Holography" by Schempp (Schempp, 1992) and applied to image processing in brain tomography including PET scans and functional Magnetic Resonance . Dolgoff instead of looking at the effects of EM waves on biological structures, hypothesized that biological structures such as synapses generate electromagnetic waves for ultra-efficient storage, processing, retrieval of data and performing biological functions (Dolgoff, 1973). The study of the brain's EM properties has added valuable information on neuronal physiology and could shed light on the role of anesthetics in the interference patterns of electromagnetic waves. The idea is that if holographic images are formed due to conscious activities in the brain, then anesthetics may act to extinguish or scramble such images. Obviously, experimental studies are needed to validate the holographic brain hypothesis and its consequences for anesthesia.

In summary, it is clear now that anesthetics at their MAC values preferentially bind to membrane receptors and ion channels as well as phospholipids. However, we now know that they also bind to tubulin in microtubules. Although the molecular mechanisms and locations of the action of anesthetic molecules are unknown, it is generally accepted that the potency of the effects of anesthetics is correlated to their hydrophobicity, electrostatic polarity, polarizability, and ability to affect hydrogen-bonds (Davies, Bagnall and Jones, 1974; Urban, 2008). As mentioned above, the Meyer-Overton relationship most directly exemplifies the link between the potency of an anesthetic and its lipid solubility (Urban, 2008) and hence represents a quantitative structure-activity relationship (QSAR). Craddock et al. further proposed that anesthetics can alter London force-mediated oscillations acting in non-polar, hydrophobic regions of tubulin dimers in neuronal microtubules, particularly in dendrites, since they exhibit a high level of polarizability (Craddock *et al.*, 2015). These hydrophobic regions are mainly composed of the aromatic amino acid residues, namely tryptophan, tyrosine and phenylalanine. They all are highly polarizable and hence susceptible to interactions with anesthetics *via* their

induced dipoles. However, without more accurate and precise information, the proportions of anesthetic binding to these different cellular locations listed above and the strengths of these interactions, it is not possible to determine the molecular mechanism of action at the subcellular level. As this requires the most precise biological resolution (nano-level) of investigation to date, the development of new technologies (i.e., X-ray free-electron laser and crystallography) could provide a better determination of membrane protein structures in general anesthesia.

Insights from in silico modelling

In recent years, thanks to the dramatic increase of computational power and to the refinement of the underlying methodologies, the computational investigation of biomolecules, including lipids and proteins, has proven a valuable tool for shedding light on molecular events taking place at the sub-nanometer and sub-millisecond time- and length scales (Lu *et al.*, 2006; Apicella *et al.*, 2013; Deriu *et al.*, 2014; Bernardi, Melo and Schulten, 2015; Grasso *et al.*, 2015, 2016, 2018; Hollingsworth and Dror, 2018). As a matter of fact, thanks to the computing power available in current days, atomistic Molecular Dynamics simulations of protein-ligand complexes embedded in lipid membranes can be performed, with simulated systems reaching sizes in the order of hundreds of thousands of atoms and simulation timescales close to or even beyond the microsecond (Priel, Tuszynski and Woolf, 2010).

In the context of anesthesia, a significant obstacle towards its further understanding is the lack of conclusive evidence for a single, putative receptor at the atomic level, so different putative functional mechanisms have been investigated computationally to this date, including (a) the structural effects of anesthetics on the cell membrane and (b) direct effects of anesthetics on ion channels and cytoskeleton proteins. As for the effects on the lipid bilayer, anesthetics have been shown to alter bilayer properties through Molecular Dynamics simulations in 1994 by Huang and coworkers (Huang and Bertaccini, 1995), who reported among others an increase in membrane fluidity and lipid diffusion caused by the presence of trichloroethylene. Early computational predictions carried out by Tu and colleagues (Tu *et al.*, 1998) suggested no significant effects of halothane on local membrane structure, but later investigations by Koubi et al. highlighted how the anesthetic induced significant alterations of membrane electrostatics (Koubi *et al.*, 2001). A similar effect has been predicted more recently by Mojumdar and Lyubartsev (Mojumdar and Lyubartsev, 2010), who highlighted how the local anesthetic articaine behaves differently depending on its charge, with the neutral form altering the local electrostatic properties of the membrane. This behavior was later confirmed by the findings of

Saeedi and coworkers (Saeedi, Lyubartsev and Jalili, 2017), who also provided quantitative estimates of the binding free energies of arcticaine and lidocaine within the membrane. The pressure reversal phenomenon has also been the focus of computational predictions: Yamamoto et al. (Yamamoto *et al.*, 2012) employed molecular dynamics simulations to investigate the pressure reversal mechanism for Xenon at the atomistic scale, providing computational evidence for the alteration of Xenon diffusivity inside the membrane caused by the increase in pressure. With similar methodologies, different computational studies have investigated the interaction between anesthetics and ion channels: in 2002, Tang and Xu provided early evidence supporting the theory that volatile anesthetics may significantly alter the dynamics of ion channels at the nanosecond time scale (Tang and Xu, 2002). More importantly, these findings underlined the physical validity of the assumption that anesthetics might defy the lock-and-key paradigm of drug-target interaction, and might instead exhibit global effects on protein functional motions even without the need of a single, well-defined binding site. In 2008, the work of Vemparala and colleagues (Vemparala, Domene and Klein, 2008) employed Molecular Dynamics to directly investigate the effects of halothane on the open and closed states of the KirBac1.1 channel, and later simulations carried out by Cheng et al. in 2010 (Cheng, Coalson and Tang, 2010) suggested an effect of halothane on the GLIC channel, in the form of a destabilization of the open conformation.

The subsequent availability of experimentally solved atomic structures of anesthetic-bound GLICs (Nury *et al.*, 2011; Pan *et al.*, 2012) further supported this idea. Interestingly, other protein targets have also been considered by atomistic simulations, as a consequence of a progressive shift of interest from ion channels and membrane proteins alone towards other classes of proteins. An example is represented by cytoskeleton proteins, in the light of genomic and proteomic studies pointing specifically to microtubules as a putative functional target of anesthetics (Pan *et al.*, 2008). Indeed, there is increasing experimental evidence of the interaction of tubulin, the main building block of microtubules, with anesthetics (Livingston and Vergara, 1979; Eckenhoff and Johansson, 1997; Pan *et al.*, 2006; Sahni, 2016), but the exact consequences of said interaction remain to be elucidated. In this regard, some works have suggested a pivotal role of the specific microtubule architecture and topology, both in axons and in dendrites, in the signaling process across the neuronal network (Kapitein and Hoogenraad, 2015). Further, the clinical relevance of microtubules in the context of anesthesia has been underlined in the light of the already discussed role of cytoskeleton reorganization in learning, cognition and memory, resulting in a selective tuning of both the activity and the firing intensity of synapses (Janke and Kneussel, 2010). Moreover, extended exposure to

anesthetics has been related, together with low temperature, to depolymerization of neuronal cytoskeletal proteins, which is thought to mediate postoperative cognitive dysfunction (Hameroff, 2018). Thus, the role of tubulin might also be of significance in the light of potential side effects of anesthesia such as POCD, as well as in the context of anesthesia with simultaneous chemotherapy or neurodegenerative disorder. In this context, the investigations of Craddock et al. predicted putative binding sites of anesthetics on tubulin and postulate the ability of anesthetic gases to alter dipole oscillations along the tubulin dimer (Craddock *et al.*, 2017). More specifically, they found that the presence of an anesthetic agent (acting as a potential alteration on the network of aromatic residues within microtubules) creates a shift of the normal oscillatory modes of the dipoles and resulting in the addition of a further normal mode of the dipolar oscillations, according to their anesthetic potency. The addition of an anesthetic slows down the normal oscillatory modes of the dipoles by orders of 1 to 100 GHz, whereas non-anesthetics did not. A recent computational investigation of ours further mapped the interaction between volatile anesthetics and human tubulin, finding transient hydrophobic binding sites on the dimer surface and quantitative estimates of tubulin-anesthetic binding energies (Zizzi *et al.*, 2021). These results obtained through computational modeling demonstrated that binding of anesthetics to microtubules may be involved in the mode of action of anesthetics, or at least in some of their side effects, by altering the polymerization and function of microtubules.

From Molecules to Organs

Experimental data point to GA action on synaptic transmission at both pre-and postsynaptic nerve terminals. Also, direct interaction with plasma membranes seems possible, and also indirect action through second messenger systems. Clinical signs of anesthetic effectiveness (i.e., depth) have been reported since early 1900 and have been associated with specific targeted neural pathways. ~~Arthur Ernest~~ Guedel (1937) described a classification of clinical anesthetic states using the inhaled anesthetic agent, diethyl ether. The classical signs of Guedel's classification were based upon clinical observations such as eyelash reflex, respiration, blood pressure, heart rate, eyeball movements, pupillary size, and muscular movements, among others. This clinical evidence suggests that anesthetics might act on different brain regions (i.e., cortex, midbrain, and spinal cord) at different times and durations (early/acute vs. late/sustainable) (Devika Rani and Harsoor, 2012). This heterogeneity of neurophysiologic effects, shown by general anesthetics, in extinguishing awareness, supports the consideration

that consciousness is intrinsically a *multiscale* phenomenon, emerging through very complex interactions of both spatially and temporally localized functions of the human brain.

a) Pathway and Cell Level

Moving from the molecular to the cellular scale, elegant electrophysiology and connectivity studies support the hypothesis of feedforward and feedback processing in the brain. Thalamo-cortical projections are shown to terminate in granular layer IV. Feedforward cortico-cortical projections arise mostly from upper layers II-III, containing the cellular bodies of large pyramidal neurons, and terminate primarily in deep layers V-VI and some in layer III. Feedback cortico-cortical projections originate mostly from deep layers V-VI (fewer from layer III) and end in the upper layers II-II (and some in layers I and IV) (Mejias *et al.*, 2016). Collectively, low field potentials (LFP), which are generated by the apical dendrites within the cortex, are the primary source of electroencephalogram (EEG) signals (Koht, Sloan and Toleikis, 2017; Phillips, Bachmann and Storm, 2018). EEG recording during general anesthesia shows a transition from wakefulness high-frequency pattern to the low-frequency EEG of deep NREM sleep, and finally to a burst-suppression pattern(Voss and Sleigh, 2007). EEG recording during the normal transition from the state of wakefulness to sleep, shows characteristic 'spindle waves' (7 to 14 Hz occurring every few seconds), produced by the thalamus. Spindling reveals the deactivation of pathway between the thalamus the cortex and the inhibitory GABAergic reticular neurons. The deactivation of sensory input and output to the cortex during the progression from the state of wakefulness to deep sleep in human, has been observed during brain imaging studies and it correlates strongly with the EEG spindle (Le Masson *et al.*, 2002).

During both sleep and general anesthesia, the disruption of cortical connectivity is likely to be related to loss of consciousness. It is still not known to what extent cortical inhibition causes anesthetic-induced loss of consciousness, which remains an open research issue (Franks, 2008).

Investigations on the anesthesia effects have often involved the brain's connectome, specifically, interactions among functionally distinct neural networks, which may be suppressed by general anesthetic agents. However, the molecular mechanism responsible for these effects may have a more integrated spatio-temporal dimension that extends beyond current neuroimaging or neurorecording resolution limitations. The activity received or transmitted by neurons is generated from a cellular electrical potential that can be recorded

441 locally, by microelectrodes, in experimental conditions, or during selected brain surgery
442 procedures (Buzsáki, Anastassiou and Koch, 2012). Charges in brain cells, including neurons
443 and glial cells, generate detectable electric currents (i.e., Local Field Potential, LFP) (Einevoll
444 *et al.*, 2013). LFPs have attracted renewed interest in the past decade as a potentially useful
445 signal, which lends itself to studying the aggregate activity behavior of small populations of
446 neurons.
447 LFP represents a continuous voltage signal with a variation in its amplitude and frequency.
448 Similarly, to the EEG, LFP can be decomposed into their power spectrum (Fourier
449 transformation) containing a wide range of frequencies shifting from delta waves (<1 Hz), theta
450 waves (4–8 Hz) and alpha (8–12 Hz), beta (12–30 Hz), gamma (30–80 Hz), and high-gamma
451 or high-frequency activity (>80 Hz) (Jia and Kohn, 2011).

452 Importantly, neural circuits often exhibit patterns of oscillatory activity, and temporally
453 coordinated input can enable both the transmission and integration of information within
454 neurons. This is consistent with the functional significance of synchronized oscillations
455 (Pereda *et al.*, 2013). Investigations of gamma oscillations are aimed at establishing a link
456 between changes in synchrony and/or coherence across different brain regions between signals
457 in the frequency range centered at 40 Hz with perceptual, behavioral, and cognitive processes.
458 These processes are hypothesized to involve long-distance network coordination and reflect
459 the LFP gamma power in a neural population of two or more connected interneurons (Hu and
460 Agmon, 2015). Gap junctions have been reported in thalamocortical neurons in the thalamus
461 and cortical interneurons' dendrites, which might be responsible for gamma synchrony
462 oscillations in the 30-80 Hz band (Traub *et al.*, 2001).

463 In general, gamma oscillation periodicity reflects the integrative excitatory and inhibitory
464 synaptic processes at the level of neural population activity. The LFP is sensitive to
465 subthreshold dendritic processes therefore serving as an indicator of engaged cortical networks
466 (Buzsáki, Anastassiou and Koch, 2012). Gamma synchrony has been observed in cortex areas
467 and in subcortical structures, and it is associated to a broad range of cognitive phenomena (Liu
468 and Newsome, 2006). In the higher cortex, gamma signals have been found to be elevated
469 during functional activation cortex such as working memory and learning activities (Bauer,
470 Paz and Paré, 2007). The range of time scales available in LFPs offers the possibility to
471 investigate whether or not the neuronal information processing is spread throughout the many
472 dimensions. Indeed, recent studies determined that bundles of brain microtubules are

electrically active and can generate electrical oscillations that might correlate with the oscillatory neuronal activity (Cantero and Cantiello, 2020). It is worth noting that irregular gamma signature has been associated with such neurodegenerative disorders as Alzheimer's disease, Parkinson's disease, schizophrenia, and epilepsy (Uhlhaas and Singer, 2006).

It has been also demonstrated that gamma activity correlates with activated neuronal networks, but it is much less clear whether it is just a byproduct of fast-spiking GABAergic inhibitory interneurons or has an important functional role. In this context, literature studies have provided a metaphorical description of the synaptic neurocomputational activity as the "conscious pilot," expressed as a mobile gamma synchronized gap junctions dendritic web which prevails over the otherwise non-conscious autopilot (Hameroff, 2010). Further investigations are still needed to address the underlying molecular mechanism of these signals and how they modulate spiking activity to comprehend the correlation between the frequencies and patterns of these rhythms and any corresponding brain states.

b) Tissue and System Level

Neuroimaging enables the extension of the investigations of the brain from the cellular to the system level, which allows a better understanding of how the brain network dynamics works. Through advanced imaging and electrical signaling technologies, we are now able to interrogate the brain for different temporal and spatial patterns: examples include positron emission tomography (PET), functional magnetic resonance imaging (fMRI); magnetoencephalography (MEG); electroencephalography (EEG) (see Figure 2).

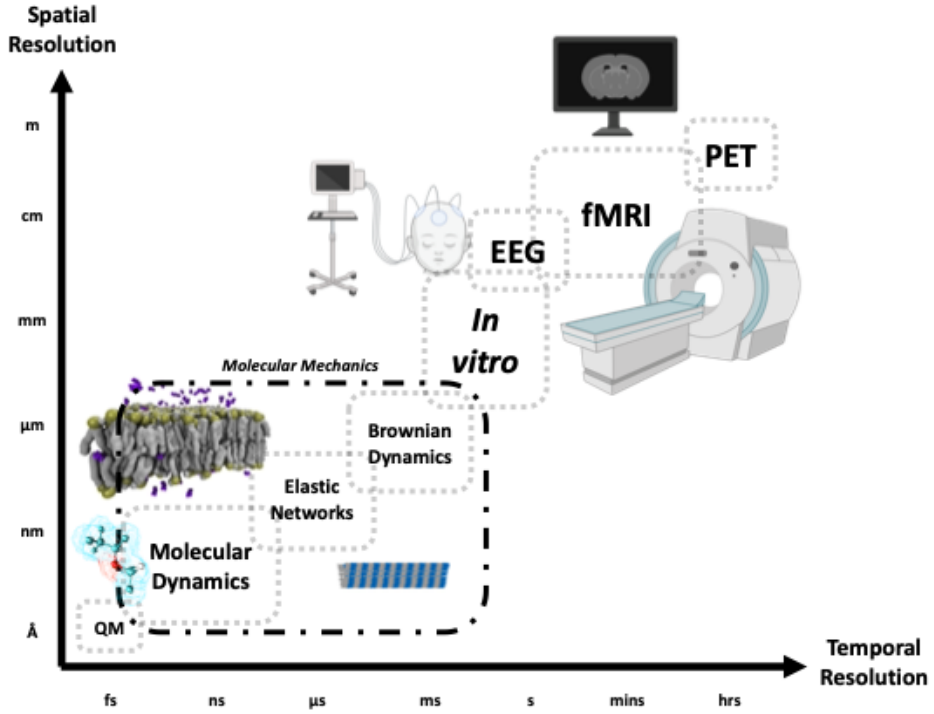


Figure 2. Diagram comparing different techniques to investigate the effect of anesthetics, from the all-atom molecular level (bottom left corner) towards the cellular, tissue and whole organ level (top right corner). The myriad of modalities employed by researchers in exploring the brain functionality are characterised by a specific spatial and temporal resolution. Techniques positioned near the bottom of the figure have a higher spatial resolution than those at the top. Modalities situated at the left show better temporal resolution.

[18 F]-fluorodeoxyglucose positron emission tomography (FDG-PET) allow for neuronal activity quantization since it reveals the cerebral glucose uptake at the synaptic level. Early PET research indicated that both very low and high brain activity patterns are typically associated with unconscious states (Chiaravalloti *et al.*, 2019).

Blood oxygen level-dependent (BOLD)-fMRI provides a hemodynamic reflector of neuronal activity becoming a useful tool to identify structured patterns of slow neuronal oscillations in the human brain at rest (Hermes, Nguyen and Winawer, 2017). However, these neuroimaging relationship findings are far from absolute, and a specific connection between brain metabolism and awareness remains unclear. For consciousness or perception of the external world, sensory inputs (e.g., visual, auditory, etc.) enter the CNS and converge within the thalamus and are then relayed onto primary sensory cortices. From there, secondary feedforward mechanisms project to frontal, temporal, and parietal association areas of the brain before converging onto the pre-frontal 'executive' cortex. There is evidence suggesting that a third wave exists involving feedback from frontal areas to more posterior cortical regions responsible for conscious

experience. This third wave, which represents a higher-order neuro-circuit that integrates sensory information from the first two waves, is specifically sensitive to anesthesia (Craddock, Priel and Tuszynski, 2014). Feedforward projections provide the sensory input about the external world entering the brain/body, while feedback projections are reverberant circuits letting ignition and persistent neuronal firing.

Cortex's areas connected by feedforward projections exhibit an equal or greater density of feedback pathways connecting higher order to lower-order regions, dominant in the awake state. Frontoparietal connectivity interruption due to feedback processing inhibition, has been reported during general anesthesia, while feedforward connectivity may generally persist (Uhrig, Dehaene and Jarraya, 2014).

c) Bridging the scales: Consciousness as an integrated multiscale property

The modulation effect on consciousness states exerted by general anesthesia has been used as an extraordinary means to explore the molecular mechanisms at the root of neural information processing. Indeed, a chemical compound such as an anesthetic may produce clinically different states of consciousness depending on the chemical structure as well as its dose: at low doses anesthetics cause depersonalization, sedation, analgesia, amnesia, and distorted time perception. At meager higher doses, a patient becomes unresponsive to a verbal command and is considered unconscious (Thomas, Martin and Pollard, 2020). This peculiar pharmacology has manifestations which span from the molecular to the whole-organism scale: at higher scales, the ability of anesthetics to modulate consciousness has been highlighted directly by neuroimaging and EEG recordings during general anesthesia, providing quantitative measures of changes in the brain network topology, and spatio-temporal neuronal firing dynamics. Unfortunately, though, it is still impossible to describe a comprehensive and precise cascade of molecular events ultimately leading to these observable phenomena. It is even more difficult to precisely predict the process of recovery from anesthesia. This is crucial since at the end of a surgical procedure an adequate recovery is expected, with an individual being awake again and aware of its surroundings and identity (Bonhomme *et al.*, 2019). Unexpected delayed awakening and failure to arouse are the most common early neurologic problems following general anesthesia (Rabinstein, 2014). These complications have been reported since the early 1900s (Bedford, 1955). These effects are yet another aspect of the interference of general anesthetics with the conscious state and are well worth investigating further. In more general terms, anesthesia-related mental disorders are broadly classified into i) postoperative delirium

(POD) and ii) postoperative cognitive dysfunction (POCD). POD is a clinical situation, sometimes occurring after general anesthesia and lasting a few hours. Patients are disoriented, think, and speak incoherently, and show impairment of memory and attention, not explained by a previous medical history of dementia, and an impaired ability to focus (Jellish and Edelstein, 2014). POCD is defined by a range of changes in mental condition and behavior, such as memory loss and inability to perform complex intellectual tasks. These impairments can last for weeks or even months after general anesthesia and surgery (Pappa *et al.*, 2017), with a number of possible trajectories during that time. Currently, the only univocal risk factors seem to be extremes of age and extent of surgical trauma (Figure 3) (Krenk, Rasmussen and Kehlet, 2010).

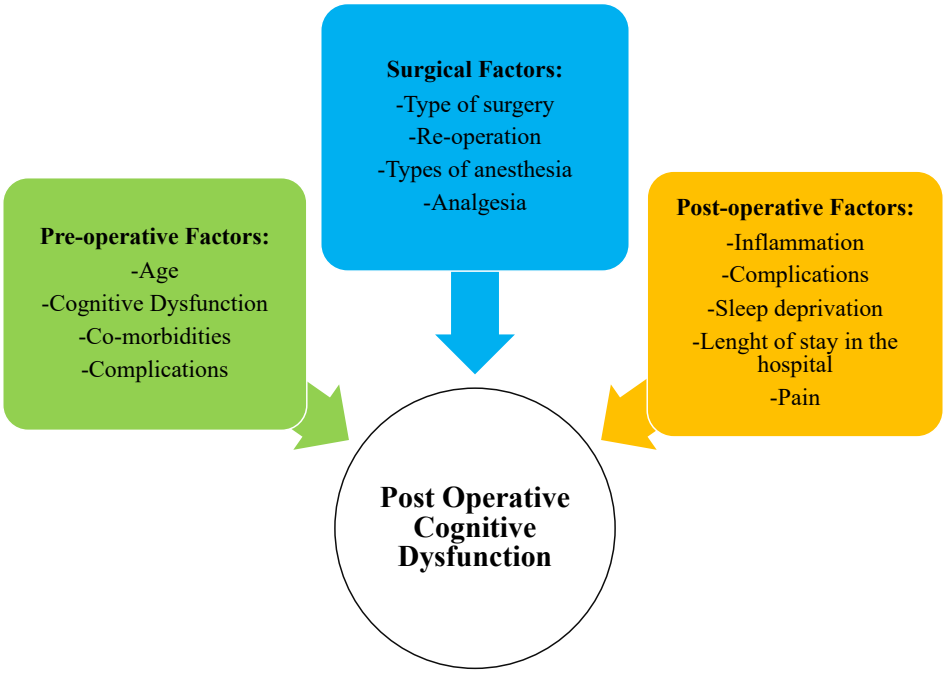


Figure 3. Risk Factors for the Development of POCD. The causes of POCD are multifactorial and may include the preoperative health conditions of the patient, periprocedural accidents related to the surgical set up, possible neurotoxic reactions to anesthetic agents, and environmental factors.

Some concern that general anesthetics may be implicated in POCD has come from animal studies utilizing long exposure to inhalational agents showing neurodegenerative changes such as cell damage and apoptosis (Brambrink *et al.*, 2010). While many studies have attempted to investigate POCD; however, the pathophysiology is not entirely elucidated. There is growing evidence in regard the neuroinflammatory etiology of POCD.

It has been reported that subclinical inflammatory states activate the neuronal tissue, including both microglia and astrocytes that if exposed to noxious stimuli, for example, major surgery

under general anesthesia, release a cascade of inflammatory cytokines and mediators, leading to neuronal damage and blood-brain barrier dysfunction (Ji, Su and Yang, 2017).

Although some experimental findings suggest a possible correlation, the evidence available is sometimes conflicting and may not be readily translated or observed in clinical practice.

In the aging brain the multifactorial pathogenesis of post-anesthetic cognitive dysfunction might modify the phenotypic trajectory of a patient's cognitive decline with age (Avidan and Evers, 2016). For instance, in patients with Alzheimer's disease has been observed an impaired axonal transport (Sahni, 2016). Many neurodegenerative diseases, at molecular level display a common dysfunctional neuronal cytoskeleton (e.g., Alzheimer's, Parkinson's, etc.). Cytoskeletal proteins are responsible for neuron morphology and intracellular transport, and the interaction of anesthetics with microtubules and their constitutive protein, tubulin, might play an important role in the pathophysiology of anesthesia-induced postoperative cognitive dysfunction (POCD) (Craddock *et al.*, 2017).

In this broad context of diverse clinical manifestations, the scientific challenge of elucidating the precise mechanisms of volatile anesthetic-induced unconsciousness (or altered consciousness) has been tackled both by *bottom-up* and *top-down* approaches. Bottom-up investigations are pointing to the modulatory effects exerted by anesthetic gases which selectively depress arousal-promoting nuclei in the brain stem and diencephalon. This can be regarded as a putative action on the *level* of consciousness (Brown, Purdon and Van Dort, 2011; Leung *et al.*, 2014). On the other hand, most current theories of consciousness rely on *top-down* approaches, arguing that anesthetics suppress consciousness by modulating areas of the brain involved in the subjective qualities that define individual experience. In particular anesthetic gases depress brain functions in cortical and thalamocortical circuits involved in the integration of neural information (Franks, 2008; Sleigh, 2016). This can be seen as a putative action on the *content* of consciousness.

Such a multidimensional framework for the mechanisms of anesthesia suggests the hypothesis that consciousness is dual in nature, possessing both a *level* and a *content*. Simultaneous alterations of both aspects, in different forms and strengths, ultimately manifest clinically in a broad spectrum of outcomes, from slight euphoria to unconsciousness and POD/POCD.

Conclusions

General anesthetics are the sole class of drugs routinely used by physicians to manipulate consciousness of their patients. The underlying mechanisms by which anesthetic agents control

606 wakefulness are fundamental questions in both anesthesiology and neuroscience, with links to
607 the neurobiology of consciousness itself. From quantum and molecular modelling to
608 neuroimaging-based approaches, an emerging body of evidence suggests that a complex
609 network of processes occurring at different scales (of both time and space) mediate both
610 consciousness and anesthesia in the brain. The multiscale investigative methods on neural
611 activity point to the molecular domain as fundamental in understanding the link between
612 general anesthesia and consciousness. While a conclusive integrative theory of anesthetic
613 action is still missing, the ever-increasing resolution of both experimental and computational
614 investigative techniques is progressively shedding light on important actors involved in neural
615 information processing, with conclusive evidence of anesthetic action on $GABA_A$ and NDMA
616 receptors, as well as a plethora of theoretical and computational studies suggesting the
617 alteration of membrane properties and protein structure within cells. Also, on a higher level,
618 refined neuroimaging investigations are unveiling the neural connections and topology
619 underlying conscious brain activity, and how anesthetics disturb the integration of information
620 in neuronal circuits during anesthesia. The starting point for understanding the complex
621 phenomenon of anesthesia might reside in acknowledging its multi-scale nature, where actions
622 at the molecular, and possibly even quantum level progressively integrate and unfold at
623 increasingly higher scales, disrupting the complex information exchange network within the
624 brain. It is clear that understanding the effects of general anesthetics on consciousness will help
625 science take a further step towards uncovering one of the greatest mysteries in nature.
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