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Insight into molecular and neural mechanisms of general anesthesia from the invertebrate model *Drosophila melanogaster*

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ABSTRACT

General anesthesia (GA) is a pharmacologically induced, reversible state characterized by unconsciousness, amnesia, analgesia, and immobility in response to noxious stimuli. Accumulating evidence from animal models has elucidated diverse mechanisms of the action underlying GA, including disruption of large-scale brain network connectivity, regulation of multiple neural pathways, and modulation of specific receptors and ion channels. Despite advances in dissecting the neurobiological basis of anesthetic action, the precise cellular and circuit-level processes remain incompletely understood, limiting the development of safer and more effective strategies. Recent studies in *Drosophila melanogaster*, a genetically tractable model organism offering robust genetic analysis, advanced imaging capabilities, and compact neural architecture, have yielded critical insights into the conserved neurobiological mechanisms of GA, offering translational value for mammalian systems. This review outlines: 1) experimental paradigms used to evaluate anesthetic sensitivity and behavioral responses in *Drosophila*; 2) molecular targets and their mechanistic roles in mediating GA; and 3) neural circuit architectures and activity patterns shared by GA and sleep. Cross-species comparisons are integrated to highlight conserved mechanisms that may guide the development of more refined anesthetic strategies.

Keywords: General anesthesia; Molecular mechanism; Neural circuits; Synaptic release; Sleep; *Drosophila*

INTRODUCTION

The serendipitous discovery of general anesthesia (GA) marked a monumental achievement in the evolution of medicine, enabling the widespread application of surgical and diagnostic procedures by eliminating pain and minimizing physiological distress. GA is a drug-induced, reversible state characterized by unconsciousness, analgesia, immobility, and broad suppression of neural activity (Thiele & Nemergut, 2020). Continued exploration of its mechanisms reflects both its clinical significance and biological complexity.

Early hypotheses attributed anesthetic effects to nonspecific interactions with cell membranes, positing that anesthetics interfere with neuronal signaling by dissolving membrane lipids —a concept encapsulated in the Meyer-Overton rule (Meyer, 1899). However, this lipid-based doctrine has since been challenged by molecular and cellular data and is now considered an oversimplified explanation for the multifaceted mechanisms underlying GA agents.

Recent studies have identified a diverse array of molecular targets of anesthetics, including neurotransmitters, ion channels (Barber et al., 2012; Luethy et al., 2017; Ouyang et al., 2007) and receptors (Qiu et al., 2024; Woll et al., 2018; Yip et al., 2013), suggesting that anesthetics exert their effects through precise modulation of synaptic and intrinsic neuronal signaling. In addition to their molecular pharmacological properties, anesthetics also influence distinct anatomical regions of the nervous system, including the spinal cord (Antognini & Schwartz, 1993), brainstem, and limbic system, leading to the development of neural circuit-based frameworks for understanding anesthetic-induced unconsciousness. However, the intrinsic complexity of mammalian neural networks continues to obscure the neural mechanisms underlying GA.

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To overcome these challenges, *Drosophila melanogaster* has emerged as a powerful model organism for the dissection of anesthesia-related neural mechanisms. Its advantages include cost-effective maintenance, compatibility with high-throughput screening across extensive libraries of targeted gene mutations, conservation of neural and molecular pathways with mammalian systems, precise genetic tools for manipulating defined molecular targets, and a fully mapped brain connectome (Adams et al., 2000; Axelrod et al., 2023; Dissel, 2020; Dorkenwald et al., 2024; Duffy, 2002; Franco et al., 2018; Garner et al., 2024; Golic & Lindquist, 1989; Lai & Lee, 2006; Matsliah et al., 2024; Ni, 2021a, 2021b; Pospisil et al., 2024; Sapkal et al., 2024; Scheffer et al., 2020; Schlegel et al., 2024; Zheng et al., 2018).

Both volatile and intravenous anesthetics have been utilized in *Drosophila* studies, although volatile agents remain the predominant focus due to the technical limitations associated with systemic delivery of intravenous anesthetics. Techniques such as drug-feeding or dorsal vessel injection have been explored (Gardner et al., 2016; Howlett & Tanouye, 2013), but variable absorption and poor dosage control have constrained their utility. This review synthesizes recent advances in *Drosophila*-based GA research, with an emphasis on: 1) behavioral and neurophysiological responses and associated paradigms; 2) conserved molecular targets and their mechanistic roles; 3) emerging insights into the neural circuitry underlying anesthetic states; and 4) the functional convergence between GA and natural sleep.

BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO GA

Behavioral paradigms

In clinical settings, GA is primarily evaluated by assessing immobility and loss of responsiveness, particularly the induction of unconsciousness (Thiele & Nemergut, 2020). Comparative studies grounded in behavioral, neurological, and evolutionary continuity suggest that certain animals, including flies (Van Swinderen, 2005, 2007), may possess a rudimentary form of consciousness. Although direct assessment of subjective experience in non-human organisms remains unattainable, the anesthetic-induced immobility and unresponsiveness in *Drosophila* mirror key features of GA in humans, providing a relevant framework for mechanistic study. Various behavioral paradigms have been developed to investigate the molecular and neural mechanisms of GA, each designed to quantify characteristic endpoints such as loss of muscle tone, reduced or absent locomotor ability, and diminished responsiveness to external stimuli (Karunanithi et al., 2018). These paradigms also capture the depressant effects of anesthetics on the central nervous system (Karunanithi et al., 2018).

One widely applied behavioral assay exploits the negative geotropism of flies. In this paradigm, flies are placed in a geotaxis testing chamber (GTC) and exposed to a specific concentration of anesthetic gas. As anesthesia progresses, flies lose coordination and fall to the bottom of the chamber, with the percentage of incapacitated individuals over time serving as a quantitative index of anesthetic depth (Figure 1A) (Allada & Nash, 1993; Leibovitch et al., 1995). A second paradigm evaluates the visual escape response (VER), a rapid, stereotyped jumping reflex triggered by sudden darkness. Suppression of this reflex by anesthetics serves as a sensitive indicator of neural inactivation (Figure 1B) (Thomas & Wyman, 1984; Campbell & Nash, 1998), with motor instability, loss of upright posture, and failure to execute the stereotyped escape pattern used as behavioral endpoints. A third paradigm assesses the avoidance reflex, in which flies are gently stimulated with a fine brush to elicit a defensive reaction. The absence or attenuation of this reflex after anesthetic exposure serves as an index of anesthetic depth (Figure 1C) (Gamo et al., 2003). Another paradigm, known as the distribution test, involves monitoring freely moving flies in a small chamber. Postural orientation, such as upright positioning, and spatial distribution are recorded to determine anesthetic responsiveness (Alone et al., 2009; Guan et al., 2000; Olufs et al., 2018). An advanced variant of this test introduces a startle-evoked locomotor assay in which mechanical stimuli are delivered to elicit movement, and flight trajectories are captured with a camera to track anesthetic-induced changes in motility with fine resolution (Figure 1D) (Zalucki et al., 2015a, 2015b). Each paradigm can calculate the EC_{50}—the concentration of an anesthetic required to achieve 50% of the maximum biological effect after a defined exposure period —which serves as a quantitative index for evaluating anesthetic sensitivity or resistance in *Drosophila*.

Similar to humans, *Drosophila* experience a dynamic sequence of physiological and behavioral transitions during anesthesia exposure. Throughout the induction phase, flies progressively lose motor coordination, accompanied by marked changes in neural activity, including reductions in neuronal firing and shifts in the frequency and amplitude of electroencephalogram-like signals (Van Swinderen, 2006). During the maintenance phase, flies remain immobile and unresponsive to external stimuli. Although physiological indices such as heart rate and respiration have been utilized to monitor changes in physiological function in *Drosophila* (Brophy & Luong, 2021; Choma et al., 2010; Lee et al., 2019; Tsai et al., 2011), these parameters have not yet been integrated into anesthesia-related research. Incorporating such measurements could offer novel avenues for evaluating anesthetic depth and recovery dynamics. During the emergence stage, flies gradually regain their capacity to move and respond to external stimuli, alongside normalization of neural activity. The time required for a fly to become anesthetized and then recover provides a crucial measure of anesthetic induction and emergence dynamics (Troup et al., 2019).

In addition to measuring behavioral incapacitation, these paradigms are instrumental in uncovering neuronal connections in the central brain. The disappearance of specific responses under anesthesia likely reflects functional changes in corresponding neurons, ion channels, or receptors during signal transmission. For instance, loss of the VER suggests disruption of normal signal transmission from visual input to motor output, while absence of the brush avoidance reflex indicates involvement of signaling pathways related to touch perception or mechanosensation. Distribution-based assays delineate arousal-related neural circuits in response to mechanical stimulation under an anesthetized state. However, each paradigm offers distinct advantages and disadvantages. The negative geotaxis and distribution tests are particularly effective for assessing anesthetic depth due to their simplicity, objectivity, and scalability for high-throughput screening, enabling systematic genetic identification of molecular targets and neural circuits involved in the response to and modulation by volatile anesthetics. In contrast, the VER and brush

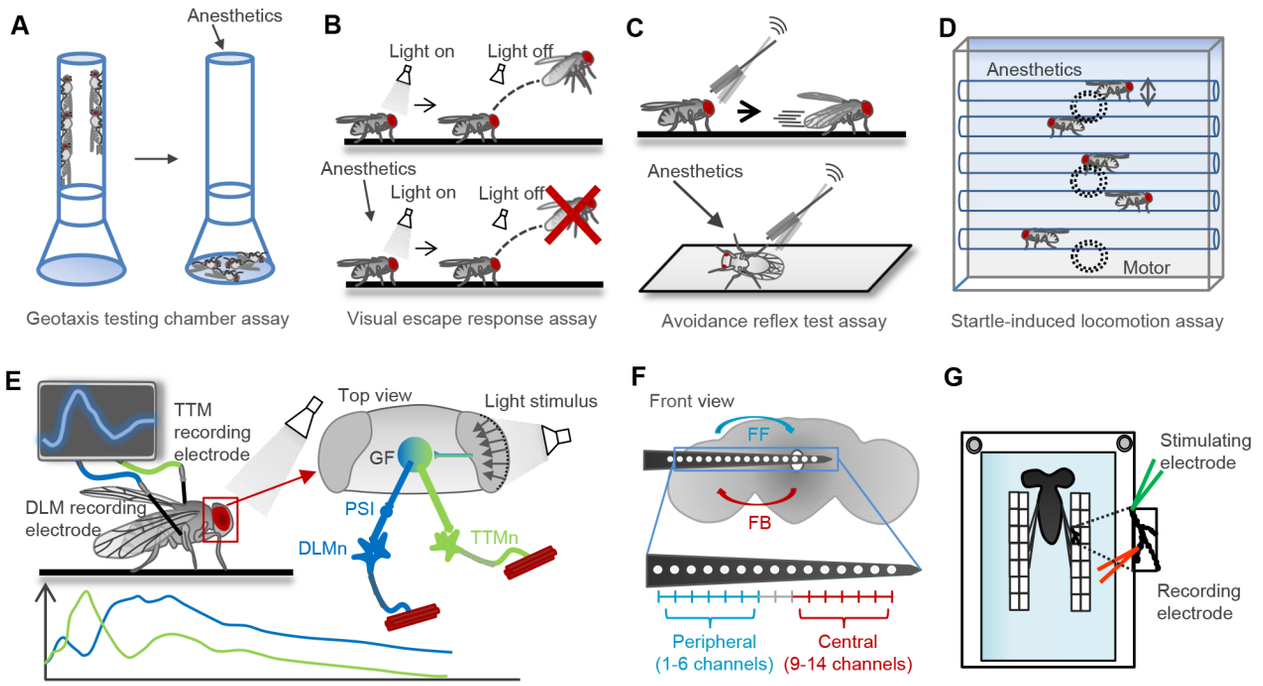

Figure 1 Schematic overview of behavioral assays and electrophysiological recordings used to assess general anesthesia in *Drosophila*

A: Geotaxis testing chamber (GTC) assay. Flies climb upward within vertical tubes but fall to the bottom upon anesthetic exposure. B: Visual escape response assay. In response to light-off stimulation, flies execute an escape jump characterized by a stereotyped posture involving middle leg extension and partial wing unfolding (top). This response is abolished following anesthetic exposure (bottom). C: Avoidance reflex assay. Tactile stimulation with a fine brush triggers an avoidance reflex under normal conditions (top), which is eliminated under anesthesia (bottom). D: Startle-induced locomotion assay. Individual flies are housed in glass tubes, and locomotor activity is recorded from above. A mechanical stimulus (dotted circle) is applied, and anesthesia depth is evaluated based on flight activity fluctuations (black double arrow). E: Electrophysiological responses of giant fibers (GF) to light stimulation. Electrodes are inserted into the tergotrochanteral muscle (TTM) and dorsal longitudinal muscle (DLM) to record motor neuron activity. The motor neuron (TTMn) innervates the jump TTM, while the motor neuron (DLMn) innervates the flight DLM. The peripherally synapsing interneuron PSI connects the GF and DLMn. Response latencies are shown with green (TTMn), and blue (DLMn). F: Multi-channel local field potential (LFP) recordings in the fly brain. A 16-channel silicon probe is inserted from the compound eye towards the central brain. Channels 1–6 represent peripheral regions, and channels 9–14 represent central regions. Information flow is classified as feedforward (FF, blue arrow; peripheral to central) or feedback (FB, red arrow; central to peripheral). G: Intracellular recordings at the larval neuromuscular junction. White squares represent body wall muscles. Red electrode denotes recording site; green electrode denotes stimulation site.

avoidance assays, while limited by lower throughput and subjective interpretation, enable more precise resolution of neuronal control mechanisms linking peripheral sensory input, visual processing, and motor output. These paradigms facilitate the genetic identification of molecular targets and neural circuits involved in the response to and modulation by volatile anesthetics.

Electrophysiological measurements

GA exerts profound inhibitory effects on both behavior and central nervous system activity. In clinical settings, cortical electroencephalography (EEG) and its derivative, the bispectral index (BIS), are routinely employed to monitor anesthetic depth (Myles et al., 2004; Purdon et al., 2015). However, their reliability in providing real-time, continuous monitoring during the entire GA process—including transitions between induction, maintenance, and emergence—remains a matter of debate, particularly in relation to dose-response precision and prognostic value (Avidan et al., 2008; Whitlock et al., 2011; Wildes et al., 2019). Electrophysiological recording techniques, which measure electrical activity at the levels of neuronal firing and synaptic transmission, enable mechanistic investigation into the processes governing anesthetic-induced loss and recovery of consciousness, as well as the neural pathways and physiological changes associated with these stages. These recordings offer critical insight into how anesthetics alter neural circuits and brain function across defined stages of GA and provide a foundational basis for the refinement of anesthetic protocols.

In *Drosophila*, electrophysiological recordings have complemented behavioral paradigms by revealing neural correlates of anesthetic modulation. Early studies focused on the giant fiber system, a bilateral pair of large interneurons descending from the brain to the thoracic ganglion, where they innervate motor neurons controlling the tergotrochateral muscle (TTM) and dorsal longitudinal flight muscle (DLM). Electrical stimulation of these fibers elicits a stereotyped escape response, closely resembling the light-off-induced jump reflex (King & Wyman, 1980; Koto et al., 1981; Tanouye & Wyman, 1980). Microelectrode recordings from the brain and musculature have characterized the neuronal properties associated with this visual escape response (Figure 1E) (Thomas & Wyman, 1984; Walcourt & Nash, 2000). Volatile anesthetics differentially influence response latency in this circuit (Lin & Nash, 1996). Specifically, latency is classified into short-latency (S-L) and long-latency (L-L) responses, depending on whether stimulation directly excites the giant fibers or indirectly activates them via upstream visual ganglion neurons. Agents such as halothane, methoxyflurane, enflurane, and desflurane preferentially eliminate the L-L

response at behaviorally relevant concentrations, while 10-fold higher concentrations are necessary to partially inhibit the S-L response. In addition to response latency, electrophysiological responses at the retinal level have also been studied. Anesthetics such as halothane, enflurane, isoflurane, and desflurane have been shown to reduce the transient component of electroretinogram (ERG) signals evoked by light offset, indicating anesthetic interference with visual processing pathways (Rajaram & Nash, 2004). Local field potential (LFP) recordings, obtained from the central brain, provide a broader spatial overview of anesthetic effects across multiple regions. With the advancement of multi-channel silicon probes inserted laterally through the eye, simultaneous LFP recordings can be obtained across multiple loci in the fly brain (Figure 1F) (Paulk et al., 2013b; Yap et al., 2017). As isoflurane concentration increases, average LFP activity declines, consistent with global neural suppression (Van Swinderen, 2006). This technique not only provides an overview of changes in neural activity across different brain regions but also reflects responses evoked by various modalities (Van Swinderen, 2006; Van Swinderen & Andretic, 2003).

Intracellular recordings have also been used to understand the effects of anesthetics on synaptic transmission at the neuromuscular junction (NMJ) (Figure 1G), demonstrating inhibition of glutamatergic excitatory junction currents by halothane, isoflurane, and propofol (Bademosi et al., 2018; Nishikawa & Kidokoro, 1999; Sandstrom, 2004). Although such measurements are more commonly carried out in mammalian systems due to the challenges associated with performing such recordings in the minuscule fly brain, their application in *Drosophila* has grown. Recent advances have enabled *ex vivo* patch-clamp recordings, including single, dual, and quadruple recordings, from dissected fly brains to elucidate circuit dynamics and signal transduction (Li et al., 2018a; Tang et al., 2022; Xiao et al., 2023). Despite the inherent technical barriers that impede widespread application of these techniques in flies, these studies demonstrate the feasibility and utility of high-resolution electrophysiological approaches in flies, offering translational insight into conserved GA-associated mechanisms across species.

Electrophysiological recordings in *Drosophila* thus offer a comprehensive view of the impacts of anesthetics across multiple biological scales, from individual neurons and signaling pathways to large-scale neural networks. This multilevel approach offers a powerful platform for dissecting the molecular and neural mechanisms underlying GA.

MOLECULAR MECHANISMS OF GA

Membrane-associated effect: Lipid theory

Lipids constitute fundamental structural and functional components of cell membranes, contributing not only to membrane integrity but also to dynamic biological processes such as signaling, ion regulation, and molecular transport. The initial mechanistic link between lipids and GA was established through the seminal observation that anesthetic potency exhibited a strong correlation with solubility in olive oil (Meyer, 1899; Overton, 1899). This finding gave rise to the classical lipid theory, which proposed that anesthetics act by incorporating into the lipid bilayer, thereby disrupting membrane structure by expanding the total volume and enhancing membrane fluidity (Mori et al., 1984; Ueda et al., 1986).

Subsequent studies involving diverse anesthetics increasingly challenged the monolithic theory. For example, despite being lipophilic, various structurally related analogs and optical isomers were shown to exhibit little or no anesthetic efficacy compared to clinical agents (Koblin et al., 1994; Tomlin et al., 1998). Furthermore, several anesthetics, such as halothane, methoxyflurane, and chloroform, as well as the optical isomers of isoflurane, have been shown to bind directly to lipid-free luciferase and proteins rather than lipids (Dickinson et al., 1994; Franks & Lieb, 1984).

However, the lipid theory remains controversial. Current perspectives have shifted to more nuanced views in which membrane-associated effects act in concert with target proteins, such as disrupting the structure and affecting the function of ion channels. Recent research has shown that several anesthetics, including chloroform, isoflurane, ether, xenon, and propofol, disrupt lipid rafts, membrane microdomains critical for protein localization and signaling (Pavel et al., 2020). Notably, chloroform and isoflurane interfere with phospholipase D2 (PLD2) localization in these rafts, activating its catalytic function. Activated PLD2 hydrolyzes phosphatidylcholine (PC), producing phosphatidic acid (PA), a signaling lipid that binds to the gating helix of the K2P channel TREK-1, ultimately inducing anesthesia (Pavel et al., 2020) (Figure 2A). In *Drosophila*, phospholipase-deficient mutants (PLD^{null}) show resistance to chloroform-induced anesthesia (Pavel et al., 2020), providing evidence that membrane disruption, lipid raft disorganization, and PA signaling contribute to anesthetic effects in flies (Figure 2A). These findings strengthen the case for the membrane as a functional target of inhaled anesthetics and highlight the need for further investigation to refine the lipid-based framework of GA.

Acting on microscopic organelles: Mitochondrial theory

An alternative mechanistic framework for GA centers on mitochondrial dysfunction, with the mitochondrial theory positing that volatile anesthetics inhibit mitochondrial activity, thereby inducing loss of consciousness. Early studies in vertebrate systems demonstrated that agents such as halothane, methoxyflurane, and chloroform suppress mitochondrial respiration at or near the rotenone-sensitive site of complex I —NADH-ubiquinone oxidoreductase —of the mitochondrial electron transport chain (mETC) (Harris et al., 1971; Hoech et al., 1966). This suppression is believed to interfere with substrate oxidation and modulate the sensitivity of key enzymatic steps. Complex I, the largest component of the respiratory chain, is composed of 44 subunits and plays a central role in cellular metabolism by oxidizing NADH to NAD+ and providing reductase for key biochemical processes (Sousa et al., 2018) (Figure 2B). This mitochondrial mechanism is conserved in *Drosophila* and has been further clarified using high-resolution, tissue-specific fluorescence-based assays. Notably, recent research has shown that isoflurane and sevoflurane directly impair electron transport through complex I, leading to the reversal of the ATP synthase by increasing mitochondrial membrane potential and reactive oxygen species (ROS) flux (Rodriguez et al., 2025). The findings suggest that complex I plays a critical role in the action of volatile anesthetics.

In eukaryotes, complex I consists of 44 subunits encoded either by mitochondrial or nuclear DNA (Vinothkumar et al., 2014), including 13 conserved nuclear-encoded genes such

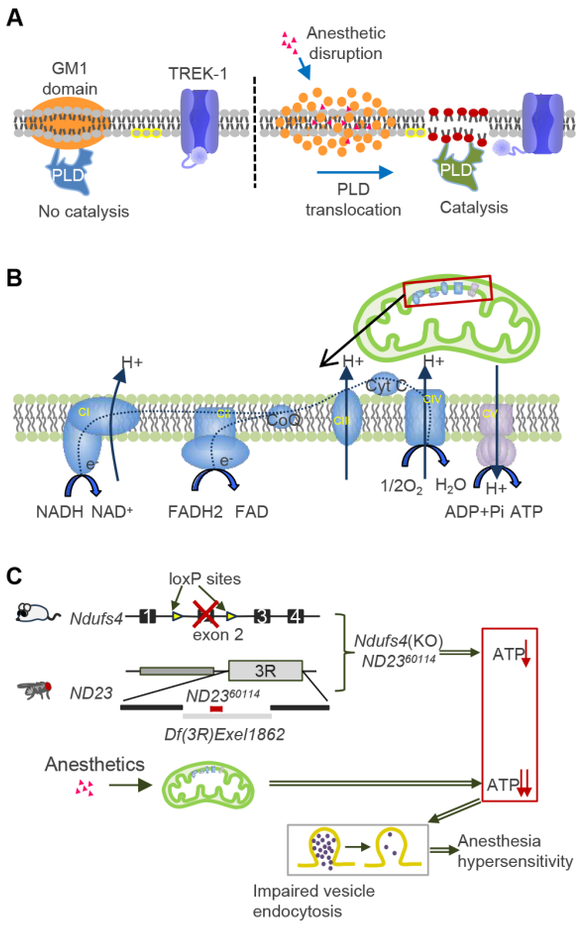

Figure 2 Schematic representation of anesthetic mechanisms involving lipid raft disruption and mitochondrial interference

A: Left: Monosialotetrahexosylganglioside1 (GM1), a glycosphingolipid enriched in the outer leaflet of the plasma membrane lipid rafts, anchors phospholipase D2 (PLD2) within these specialized membrane domains. The unique lipid composition of GM1-rich rafts influences PLD2 activity and localization. Right: Anesthetics disrupt GM1 lipid rafts, triggering PLD2 translocation and leading to disordered membrane. PLD2 catalysis results in phosphatidylcholine (PC) hydrolysis (yellow circles) and phosphatidic acid (PA) signaling (red dots). This activates the two-pore-domain potassium (K2P) channel TREK-1, inducing anesthesia. B: Mitochondrial respiratory chain components. Red box is magnified region. This system comprises five protein complexes (Complexes I–V) that coordinate electron transfer from NADH and $FADH_2$ via redox reactions. Electrons pass through two electron transporters, coenzyme Q and cytochrome c, ultimately leading to proton translocation across the inner mitochondrial membrane. The resulting electrochemical gradient drives ATP synthase (complex V) to generate ATP, the main energy source for cellular processes. CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; CV, complex V; CoQ, coenzyme Q; Cyt C, cytochrome C. C: Two genes encoding subunits of mitochondrial Complex I—*Ndufs4* in mammals and *ND23* in *Drosophila*—are critical for ATP production. Loss-of-function mutations in either gene reduce mitochondrial ATP synthesis. *Ndufs4(KO)* mutant is a deletion of exon 2 between two loxP sites, while $ND23^{60114}$ mutant (*Df(3R) Exel1862*) corresponds to a defined chromosomal deletion on the right arm of the third chromosome. General anesthetics further suppresses mitochondrial function by targeting the respiratory chain, leading to reduced ATP output. This energy deficiency impairs vesicular endocytosis, contributing to increased anesthetic sensitivity.

as NDUFS1–8 (Rodenburg, 2016). In *Drosophila*, *ND23* encodes a homolog of the mammalian NDUFS8 subunit (Porcelli et al., 2007). Mutations in *ND23* result in profound mitochondrial abnormalities, including disrupted morphology, reduced presynaptic ATP availability, impaired vesicular endocytosis, and increased neurotoxicity (Hang et al., 2024; Jung et al., 2022; Olufs et al., 2020). $ND23^{60114}$ mutant flies, carrying a point mutation in the core subunits of complex I, exhibit hypersensitivity to isoflurane exposure, although their sensitivity to sevoflurane is inconsistent (Borchardt et al., 2023; Loewen & Ganetzky, 2018; Olufs et al., 2018, 2020, 2024; Scharenbrock et al., 2024). A similar phenotype has been observed in the $ND2^{del1}$ mutant, which carries a 9-bp deletion in the coding region of ND2. This mutation also produces hypersensitivity to isoflurane and enhanced susceptibility to halothane-induced toxicity, mirroring the effects seen in $ND23^{60114}$ (Borchardt et al., 2023). These results reinforce the role of mitochondrial complex I in mediating anesthetic responses. Supporting evidence from other species —including nematodes, mice, and humans —further implicates NDUFS4 in altered anesthetic sensitivity and mitochondrial dysfunction (Figure 2C) (Hsieh et al., 2021; Kayser et al., 1999; Morgan et al., 2002; Perouansky et al., 2023; Quintana et al., 2012). These findings provide a strong mechanistic link between loss-of-function mutations in complex I subunits, impairment of mitochondrial activity, and altered sensitivity to GA, offering critical insight into the molecular mechanisms of anesthetic action (Figure 2C).

Affecting synaptic transmission: Specific protein targets

Synaptic transmission, a critical component of neuronal communication, is significantly affected by GA. The release of neurotransmitters from presynaptic to postsynaptic neurons involves a tightly regulated sequence of events, including the storage, mobilization, fusion, and release of synaptic vesicles, followed by the subsequent release, activation, and clearance of neurotransmitters. Each of these steps is mediated by specific protein complexes that serve as potential molecular targets for anesthetic action across the entire synaptic release process.

Presynaptic molecules: Anesthetics exert significant effects on synaptic transmission, particularly by disrupting the presynaptic machinery responsible for neurotransmitter release (Baumgart et al., 2015; Krick et al., 2021). One key regulator is dynamin, a GTPase encoded by the *shibire* gene in *Drosophila*, which plays a crucial role in synaptic vesicle endocytosis, facilitating the scission of budding vesicles and their recycling and refilling with neurotransmitters for successive rounds of release (Ferguson & De Camilli, 2012). The temperature-sensitive *shibire* mutant shi^{ts1}, bearing a missense mutation in the GTPase domain, exhibits complete synaptic vesicle depletion at restrictive temperatures (~29°C) while maintaining normal endocytosis function at permissive temperature (<25°C) (Koenig & Ikeda, 1983; Van Der Bliek & Meyerowitz, 1991). Wild-type flies display reduced sensitivity to repeated exposure to benzyl alcohol, a local anesthetic, indicative of adaptive resistance. However, this resistance is abolished in shi^{ts1} mutants or in flies exposed to N-ethylmaleimide, which also inhibits vesicle recycling (Al-Hasan et al., 2011), highlighting the necessity of functional endocytosis in modulating anesthetic responsiveness.

Another important protein involved in the vesicle cycle is

syntaxin1A, a membrane-anchored component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex essential for neurotransmitter release. Syntaxin1A contains an N-terminal H_{abc} domain involved in molecular transport, localization, and conformational change, and a highly conserved C-terminal H3 domain implicated in anesthetic sensitivity via promotion of vesicle and plasma membrane fusion (Dawidowski & Cafiso, 2013; Lagow et al., 2007; Rizo, 2022). In *Drosophila*, two mutations within the H3 domain—*syxH3-C* (a 14-amino-acid deletion) and *syxKARRAA* (a double substitution of two lysines) (Khuong et al., 2013; Wu et al., 1999)—produce opposing phenotypes, conferring resistance and hypersensitivity to isoflurane, respectively. The hypersensitivity of *SyxKARRAA* may be attributable to the elimination of the ability of phosphatidylinositol 3,4,5 trisphosphate ($PI(3,4,5)P_3$) to aggregate syntaxin1A (Khuong et al., 2013). Interestingly, flies carrying both mutations exhibit the resistance phenotype, suggesting a dominant protective role of *syxH3-C* against isoflurane-induced transmission deficits (Zalucki et al., 2015b).

A recent study demonstrated that deletion of the syntaxin1A H3 structural domain accelerates recovery from isoflurane anesthesia in *syxH3-N* mutants, as measured by behavioral activity assessed at 10 minute intervals post-exposure (Troup et al., 2019). To further investigate the underlying mechanisms, a truncated syntaxin1A construct (syx^{227}) lacking most of the H3 domain was expressed in specific neuronal populations under upstream activation sequence (UAS) control. Electrophysiological recordings at the larval neuromuscular junction revealed that isoflurane perfusion reduced excitatory junction potential (EJP) amplitudes in wild-type larvae, whereas syx^{227} mutants maintained baseline EJP amplitudes, suggesting that this truncated component specifically contributes to the development of isoflurane resistance. This resistance phenotype was also observed with propofol exposure (Bademosi et al., 2018), suggesting a shared molecular mechanism underlying anesthetic response. Together, these findings demonstrate that the H3 domain of syntaxin1A plays a critical role in both the induction and recovery phases of GA.

In addition to syntaxin1A, other core components of the SNARE complex, including synaptic vesicle-associated membrane protein (VAMP/synaptobrevin) and synaptosome-associated protein 25 (SNAP-25), also contribute to neurotransmitter release-dependent membrane fusion and presynaptic sensitivity to anesthetics (Söllner et al., 1993). The SNARE complex is composed of four α-helices: one from VAMP2, one from syntaxin1A, and two from SNAP-25 (Chen et al., 2002). Initially, SNAP-25 forms a stable dimer with syntaxin1A at the plasma membrane, which subsequently assembles into an intermediate t-SNARE complex (Rickman et al., 2004; Weninger et al., 2008) and upon engaging vesicle-bound VAMP2, completes SNARE complex assembly through the establishment of a four-helix bundle (Xiao et al., 2001). Recent imaging studies have shown that anesthetics such as propofol and etomidate limit the migration of *Drosophila* syntaxin1A by affecting its fixation or aggregation, with disruption of the syntaxin1A and SNAP-25 interaction found to rescue this restricted migration (Bademosi et al., 2018). The syx^{227} construct also rescues propofol-induced defects in syntaxin1A immobility and exocytosis (Bademosi et al., 2018). Together, these findings suggest that anesthetics target syntaxin1A, VAMP, and SNAP-25 through dynamic interactions during the assembly process, rather than acting solely on the fully formed SNARE complex (Figure 3A), highlighting a key presynaptic mechanism of action.

Receptors: Anesthetics modulate neural communication by disrupting key components of synaptic transmission, including postsynaptic receptors that respond to neurotransmitters released into the synaptic cleft. Among these, γ-aminobutyric acid (GABA) and dopamine receptors (DARs) have emerged as significant targets implicated in anesthetic-induced behavioral and physiological alterations (Mihic et al., 1997; Taylor et al., 2013).

In *Drosophila*, GABA serves as the primary inhibitory neurotransmitter, establishing a connection between anesthetic-induced suppression of neuronal activity and GABAergic signaling. Two main types of GABA receptors have been identified: inotropic $GABA_A$ receptors and

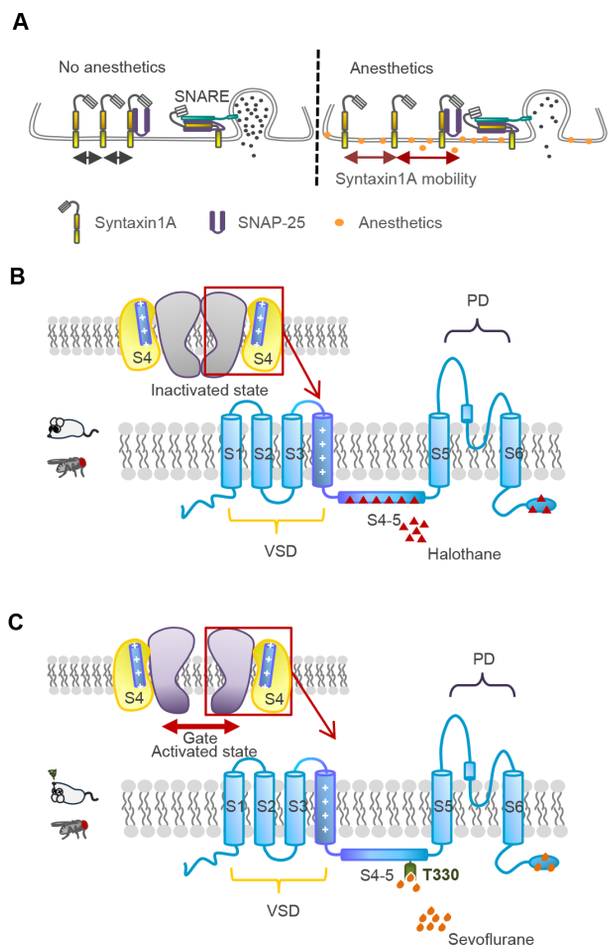

Figure 3 Schematics illustrating anesthetic effects on syntaxin1A and K-shaw2 channels

A: Under baseline conditions, syntaxin1A exhibits normal membrane mobility (left). Following propofol exposure, syntaxin1A mobility becomes restricted (right). SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor. B: In the inactivated state, each K-shaw2 channel subunit is composed of six transmembrane helices (S1–S6), as indicated by the red box. S1–S4 form the voltage-sensing domain (VSD), and S5–S6 form the pore-forming domain (PD). Halothane (red triangles) binds to sites within the S4–S5 junction or distal region of S6, producing an inhibitory effect (gray channel). C: In the activated state, sevoflurane (orange teardrops) binds to the T330 site at the S4–S5 junction, leading to channel activation (purple with bidirectional red arrow).

metabotropic $GABA_B$ receptors (Kaupmann et al., 1997; Mezler et al., 2001). $GABA_A$ receptors, in particular, have been extensively characterized as crucial targets for various anesthetics (Macdonald & Barker, 1978), including halothane, isoflurane, trichloroethanol, pentobarbitone, propofol, etomidate, and alphaxalone, all of which potentiate GABA-induced chloride influx and neuronal inhibition (Belelli et al., 1999; Franks & Lieb, 1984). In *Drosophila*, the pentameric GABA receptor complex comprises subunits encoded by genes such as resistance to dieldrin (*Rdl*), ligand-gated chloride channel homolog 3 (*Lcch3*), and GABA and glycine-like receptor (*Grd*) (Hosie et al., 1997; Manev & Dzitoyeva, 2010; McGonigle & Lummis, 2009). Among these, RDL receptors have been most strongly associated with the actions of general anesthetics (GAs) and are subject to modulation through allosteric regulatory sites. Notably, propofol and pentobarbital enhance GABA-evoked currents through RDL without directly activating the channel, contrasting with their ability to directly activate mammalian $GABA_A$ receptors (Belelli et al., 1996). This observation suggests that anesthetics potentiate RDL activity through positive allosteric modulation rather than agonist binding. Furthermore, transmembrane amino acid residues within the TM2 structural domain serve as a common regulatory site for both indirect potentiation (as seen in RDL receptors) and direct activation (characteristic of mammalian $GABA_A$ receptors). Substituting the transmembrane methionine residue of the RDL receptor with the corresponding asparagine from mammalian β2 or β3 subunits, or serine from the β1 subunit, transforms the receptor into one that responds directly to propofol even in the absence of GABA (Pistis et al., 1999). However, additional evidence has also revealed the pharmacological diversity and clinical necessity of $GABA_A$ receptors in the context of GA. These receptors potentiate inhibitory signaling in the brain via distinct mechanisms (Kim et al., 2020). The presence of similar or different binding sites on $GABA_A$ receptors, resulting in varied responses to specific anesthetics across species, implies intricate mechanisms that require further investigation.

In parallel, dopaminergic signaling pathways have also been implicated in anesthetic responses (Kottler et al., 2013). DARs are widely expressed in the central nervous system and modulate arousal and sleep-wake states (Beaulieu et al., 2015). In *Drosophila*, genetic tools have facilitated the characterization of downstream effectors, such as enzymes and ion channels, and signaling cascades initiated by DARs in response to anesthetics (Martinez et al., 2020; Zhang et al., 2022). In *Drosophila*, DARs are categorized into D1-like and D2-like receptors based on their downstream signaling properties. The D1-like group comprises Dop1R1 and Dop1R2 (also known as DAMB), collectively referred to as DopR, while D2R represents the sole *Drosophila* homolog of mammalian D2-like receptors (Feng et al., 1996; Han et al., 1996; Hearn et al., 2002). DopR has been the primary focus of anesthesia-related studies in *Drosophila*. The *dumb*^{2} allele, a DopR mutant associated with reduced dopamine levels, exhibits hypersensitivity to isoflurane (Kottler et al., 2013). RNAi-mediated knockdown of DopR in neurons recapitulates the same hypersensitive phenotype to isoflurane (Kottler et al., 2013), further supporting the involvement of DARs in regulating the effects of GA.

Dopamine binding to DopR activates adenylate cyclase (AC), increasing intracellular cyclic adenosine monophosphate (cAMP) levels and activating cAMP-dependent protein kinase (PKA), ultimately modulating various downstream targets (Boyd & Mailman, 2012). Anesthetic hypersensitivity in DopR-deficient flies is likely attributable to reduced cAMP signaling. This hypothesis is supported by the opposite phenotypes observed in cAMP pathway mutants: *rutabaga* (*rut*) mutants carrying a loss-of-function mutation in the gene encoding AC result in reduced cAMP (Levin et al., 1992; Zhong et al., 1992) and heightened anesthetic sensitivity (Tanaka et al., 2007), whereas *dunce* (*dnc*) mutants harboring a phosphodiesterase mutation with elevated cAMP levels (Chen et al., 1986; Zhong et al., 1992) confer partial resistance to diethyl ether (Tanaka et al., 2007). Together, these findings demonstrate that DAR-mediated cAMP signaling modulates anesthetic sensitivity, although the precise role of DARs in the induction and maintenance phases of anesthesia remains incompletely defined.

Ion channels: Anesthetics modulate neuronal excitability in part through direct actions on K^+ channels, which are critical determinants of membrane potential and synaptic responsiveness (Li et al., 2018b; Nicoll & Madison, 1982). The voltage-gated potassium (Kv) channel family is highly conserved across taxa and displays considerable molecular diversity. In *Drosophila*, the first link between potassium channel function and anesthetic response emerged from the observation of ether-induced leg tremors, leading to the identification of the *shaker* gene encoding the prototypical Kv1 channel (Jan et al., 1977; Kaplan & Trout, 1969). This discovery facilitated the subsequent identification of homologous Kv channels, including Shab (Kv2), Shaw (Kv3), and Shal (Kv4), through targeted genetic screening (Butler et al., 1989; Covarrubias et al., 1991; Tsunoda & Salkoff, 1995). Extensive sequence conservation across species enabled cloning and characterization of additional Kv-related genes in *Drosophila*, mammals, and even humans (Roberds & Tamkun, 1991; Swanson et al., 1990). Eight major Kv channel types have been described in *Drosophila*, including seven α-subunit families (Kv1, Kv2, Kv3, Kv4, Kv10, Kv11, and Kv12) and the calcium-activated K^+ channel K^+Ca1.1 (Atkinson et al., 1991; Drysdale et al., 1991; Warmke & Ganetzky, 1994).

Among these, the Kv1 and Kv3 families have been functionally implicated in GA. Kv1 channels exhibit a conserved six-transmembrane topology (S1–S6), with the S4 region acting as the voltage-sensing domain and the S5–S6 regions forming the ion-conducting pore (Figure 3B) (Guy & Seetharamulu, 1986). In *Drosophila*, Kv1 channels encoded by *shaker* contribute to the regulation of the action potentials of neurons, lymphocytes, heart cells, muscle cells, and kidney cells via transient K^+ currents (type A currents, IA), thus participating in synaptic plasticity and broader physiological processes such as sleep, memory, and cardiac protection (Cirelli et al., 2005; Elkins & Ganetzky, 1988; Guan et al., 2011; Liu et al., 2021; Mahdavi & Kuyucak, 2013; Ueda & Wu, 2006; Wu et al., 1983). Functional studies have demonstrated that Kv1 channels modulate anesthetic sensitivity. Notably, *shaker* mutants lacking Kv1 exhibit resistance to volatile anesthetics including isoflurane and sevoflurane, mirroring observation in mammalian models (Weber et al., 2009). In contrast, these mutant remain a normal sensitivity to xenon (Schaper et al., 2012), suggesting that different anesthetics may target specific Kv1 channel sites, a phenomenon that remains to be elucidated.

The *Drosophila* K-shaw2 channel, encoded by *shaw* and homologous to the mammalian Kv3 channel, has provided

critical mechanistic insights into anesthetic modulation of voltage-gated Kv channels. Despite functional distinctions, K-shaw2 shares remarkable structural similarity to Kv1 channels, enabling comparative analyses of anesthetic action. Halothane is capable of exerting inhibitory effects on the K-shaw2, and this inhibition is conserved as mammalian Kv1.2 channels. Further mechanism was found in *Drosophila* that they achieve this by binding to the S4–S5 linker or to the site located within the distal region of S6 (Bhattacharji et al., 2010) (Figure 3B). In contrast, sevoflurane exerts an activating effect on K-shaw2, a phenomenon attributed to regulation at residue T330 within the S4–S5 junction, structurally equivalent to the G329 site in mammals (Liang et al., 2015) (Figure 3C). In the classical model of Kv channel activation, the coupling of S4 voltage sensor displacement to pore opening is highly associated with the interaction between the S4–S5 linker and S6 distal region. Consequently, anesthetics may interfere with the S4–S5 linker and S6 distal region to achieve activation or inhibition (Figure 3B) (Lu et al., 2002). However, how the same anesthetic state induced by sevoflurane and other inhaled anesthetics is achieved yet mediated by distinct mechanisms remains incompletely investigated. The complex combined effects require further research for elucidation.

In addition to K^+ channels, other ion channels may also serve as critical targets for GA action. The *Drosophila har38* mutant, induced by P element insertion, exhibits three distinct phenotypes: abdominal constriction, footfall cessation, and halothane resistance, linked to disruption of the narrow abdomen (*na*) gene, which encodes a sodium channel (Lu et al., 2007; Mir et al., 1997; Weber et al., 2009). This mutant also demonstrates resistance to methoxyflurane, chloroform, trichloroethylene, and xenon, but sensitive to isoflurane and sevoflurane (Lu et al., 2007; Mir et al., 1997; Schaper et al., 2012; Weber et al., 2009). Notably, *har38* mutants exhibit reduced expression of the sodium leak channel (NALCN), a selective cation channel that, consistent with its mammalian homolog, may undergo activation or desensitization in response to anesthetic exposure, depending on its regional expression in the brain (Ou et al., 2020; Wu et al., 2024). However, the specific allosteric mechanisms underlying NALCN activation and the anesthetic-specific divergence in phenotypic outcomes remain unresolved.

Drosophila has proven to be a powerful model for identifying molecular targets of GAs due to its rapid screening capacity, well-characterized anesthetic responses, and extensive library of genetic mutants (Table 1). Multiple mutants exhibit distinct anesthetic sensitivity or resistance phenotypes, providing key insights into the underlying mechanisms of anesthetic action. Resistance to anesthesia has been observed in mutants of functional phospholipase D (PLD^{null}), synaptic protein syntaxin1A (*syxH3-C*), PDE (*dnc*), voltage-gated potassium channel (*shaker*), and sodium channel (*har38*). In contrast, heightened sensitivity to anesthetics has been reported in mutants of ETC complex I ($ND23^{60114}$), syntaxin1A synaptic protein (*syxKARRAA*), AC (*rut*), and sodium channel (*har38*). During post-anesthetic recovery and arousal regulation, syntaxin1A synaptic protein (*syxH3-N* and syx^{227}) and DopR mutants ($dumb^2$) exhibit resistance to anesthesia. These phenotypic divergences, particularly the contrasting responses to different anesthetics underscore the complexity of anesthetic mechanisms and provide a foundation for dissecting the molecular basis of anesthetic action, maintenance and recovery.

Collectively, findings from *Drosophila* models highlight the multifaceted nature of GA action, involving a broad spectrum of molecular targets and intricate interactions within neural circuits (Table 2). Anesthetics act on these targets via affecting neuronal excitability and the overall state of anesthesia through several principal mechanisms: (1) altering membrane biophysics by disrupting lipid bilayers and modulating membrane fluidity; (2) directly binding to specific sites on proteins, including receptors and ion channels, to induce conformational changes that shift membrane potential and synaptic signaling probability; and (3) indirectly modulating intracellular signaling through second messenger pathways. Despite significant advances, the full scope of GA mechanisms remains incompletely understood, necessitating further investigation to refine anesthetic pharmacology and improve clinical outcomes.

NEURAL MECHANISMS OF GA

Neuronal responses to anesthetics

GAs eliminate behavioral reactivity primarily through the inhibition of neuronal excitability. In addition to modulating molecular targets, such as ion channels involved in synaptic transmission, anesthetics also disrupt neural circuits and impair network-level dynamics. Exposure to anesthetics reduces rhythmic neural firing and desynchronizes activity across different brain regions (Troup et al., 2023).

Simultaneous recordings of LFPs using multi-electrode arrays in *Drosophila* have enabled quantification of neural activity from peripheral to central brain regions under both normal and anesthetized states. Under normal conditions, low-frequency LFP power predominates in both central and peripheral regions, with central signals exhibiting significantly higher power. Under isoflurane anesthesia, central power is markedly reduced across all frequency bands, while peripheral power decreases only at certain frequencies. The more pronounced suppression in central regions at low frequencies suggests that isoflurane preferentially attenuates integrative cortical activity and impairs interregional coordination (Cohen

Table 1 Summary of molecular targets responding to anesthetics and those affected by mutations

Mutation	Phenotype	Anesthetic	Mechanism	Reference
$ND23^{60114}$	Hypersensitivity	Isoflurane Sevoflurane	Mitochondria	Olufs et al., 2018
syxKARRAA	Hypersensitivity	Isoflurane	Synaptic protein	Zalucki et al., 2015b
$dumb^2$	Hypersensitivity	Isoflurane	Dopamine receptor	Kottler et al., 2013
har38	Hypersensitivity	Isoflurane Sevoflurane	NALCN	Weber et al., 2009
	Resistance	Methoxyflurane chloroform trichloroethylene Xenon	NALCN	Mir et al., 1997
PLD^{null}	Resistance	Chloroform	Lipid	Pavel et al., 2020
syxH3-C	Resistance	Isoflurane	Synaptic protein	Troup et al., 2019; Zalucki et al., 2015b
syx^{227}	Resistance	Isoflurane Propofol	Synaptic protein	Bademosi et al., 2018; Troup et al., 2019

Table 2 Anesthetics used to understand general anesthesia-related mechanisms

Anesthetic	Phenotype	Mechanism	Reference
Isoflurane	Hypersensitivity	Mitochondria Synaptic protein Dopamine receptor NALCN	Olufs et al., 2018; Zalucki et al., 2015b; Kottler et al., 2013; Weber et al., 2009;
	Resistance	Synaptic protein	Zalucki et al., 2015b;
Sevoflurane	Hypersensitivity	Mitochondria K-shaw2 channel NALCN	Olufs et al., 2018; Weber et al., 2009; Liang et al., 2015;
Propofol	Hypersensitivity	$GABA_A$ receptor activity	Belelli et al., 1996; Pistis et al., 1999;
	Resistance	Synaptic protein	Bademosi et al., 2018;
Pentobarbital ketone	Hypersensitivity	$GABA_A$ receptor activity	Belelli et al., 1996; Pistis et al., 1999;
Chloroform	Resistance	Lipid NALCN	Pavel et al., 2020; Mir et al., 1997;
Halothane	Resistance	K-shaw2 channel inhibition	Bhattacharji et al., 2010
Methoxyflurane	Resistance	NALCN	Mir et al., 1997
Trichloroethylene	Resistance	NALCN	Mir et al., 1997
Xenon	Resistance	NALCN	Schaper et al., 2012

GABA, γ-aminobutyric acid.

et al., 2018). Flashing visual stimuli can evoke periodic LFPs, enabling assessment of steady-state visual evoked potentials (SSVEPs) (Figure 1F) (Norcia et al., 2015; Paulk et al., 2013b). Isoflurane reduces SSVEPs in central regions while enhancing them peripherally, accompanied by loss of visual responsiveness (Cohen et al., 2016). These opposing effects reflect differential modulation of neural activity across brain regions, indicating region-specific sensitivity to anesthetic perturbation. Neural oscillations in different frequency bands are crucial for neural communication and coordination, underpinning various functions such as sensory perception, motor control, and cognitive processing. Anesthetics interrupt these coordinated rhythms, disrupting long-range functional brain networks and impairing sensorimotor integration. Gaining insight into the electrophysiological properties of various brain regions can unlock the mysteries of brain function, inspire innovative diagnostic and treatment approaches, and drive the development of next-generation brain-computer interfaces.

Neural networks under GA

Drosophila has proven to be an excellent model for pioneering studies on neural network dynamics under GA, owing to the relative simplicity of its brain, containing only approximately 100 000 neurons, and the tractability of its neural circuits. Despite its simplicity, *Drosophila* exhibits conserved mechanisms of neural information encoding and transmission, facilitating high-resolution dissection of anesthetic effects on circuit function.

In the *Drosophila* brain, visual information transmission is a complex and highly organized process involving multiple neural circuits and cell types. Visual stimuli are detected by photoreceptors and relayed through a series of optical lobe structures, including the lamina, medulla, lobula, and lobula plate, before integration with higher brain regions to generate appropriate behavioral responses. This anatomically layered system enables clear delineation of feedforward (FF) and feedback (FB) signaling, which correspond to ascending transmission from peripheral to central structures and descending modulation from higher-order to lower-order regions, respectively, mirroring principles observed in the mammalian system (Felleman & Van Essen, 1991). Using this model, electrophysiological analyses have revealed that FF signaling predominates in high-frequency bands, while FB regulation dominates at low frequencies (Otsuna & Ito, 2006; Paulk et al., 2013a; Shih et al., 2015; Cohen et al., 2018). Interestingly, isoflurane selectively attenuates low-frequency FB regulation, consistent with observations in mammals (Papadopoulou et al., 2019). These observations suggest that GA acts in a systemic regulatory manner (Lee et al., 2013; Ranft et al., 2016). However, simultaneous LFP recordings across multiple *Drosophila* brain regions offer only a preliminary understanding of how GAs affect neural networks. Comprehensive understanding remains elusive and will require the integration of innovative techniques and methodologies.

INTERACTIONS BETWEEN GA AND SLEEP

GA shares overlapping behavioral features with sleep, such as reduced responsiveness and reversible unconsciousness, and EEG patterns resembling slow-wave activity. These similarities in flies and mammals imply a shared evolutionary origin for the neural processes and molecular bases underlying altered states.

Sleep is endogenously governed by two principal regulatory systems: sleep homeostasis and circadian rhythm. Sleep homeostasis accumulates sleep pressure during wakefulness, determines sleep duration, enables compensatory responses to sleep loss, and interacts with circadian timing. This regulatory architecture is conserved across species, including *Drosophila* and mammals (Huber et al., 2004). Similar to observations in rats, flies exhibit a propofol dose-dependent reduction in locomotion, increased arousal threshold, and delayed recovery from anesthesia (Gardner et al., 2016; Tung et al., 2002), suggesting an interaction between anesthesia exposure and sleep homeostatic regulation. Unlike in mammals, rebound sleep after sleep deprivation in *Drosophila* closely resembles that of non-deprived flies recovering from anesthesia, but is significantly greater than in deprived flies without anesthesia, which also exhibit accelerated lethality (Gardner et al., 2016). This suggest that propofol may suppress homeostatic regulation and impair sleep recovery, or alternatively, that the additional sleep induced by propofol compensates for prior sleep loss and overrides homeostatic drive. In either case, these results imply that anesthetics and sleep deprivation may interact at molecular or circuit levels to influence pharmacokinetics and drug sensitivity, requiring further investigation.

Post-anesthetic sleep disturbances observed in clinical settings have been partly attributed to circadian dysregulation, a hypothesis supported by *Drosophila* studies. Several key

genes, including *period* (*per*), *timeless* (*tim*), *clock* (*clk*), and *cycle (cyc)*, have been identified and characterized as core molecular clock machinery in *Drosophila*, forming a transcription-translation negative feedback loop that regulates behavior in an approximately 24-hour cycle (Anna et al., 2023; Patke et al., 2020). A recent study demonstrated that isoflurane exposure induced phase advances during subjective day and phase delays during the night in *Drosophila*, with shifts corresponding to the timing of administration (Li et al., 2020). Further analysis of the *per* gene expression levels showed a similar pattern of phase shifts, but about four hours earlier than the behavioral shifts (Li et al., 2020). Whether these temporal dynamics extend to other clock genes or anesthetics remains unresolved, as does the broader nature of the interaction between GA and the circadian system. Beyond canonical circadian regulators, GAs may also influence other molecular systems, including membrane lipid organization. As noted in lipid-based theories of GA, anesthetics primarily interfere with the lipid membrane structure by disrupting GM1 lipid rafts and altering their size and distribution (Pavel et al., 2020). However, in sleep regulation, lipids are more closely associated with transport and metabolic pathways. A recent study found that glial cells in the hemolymph-brain barrier of *Drosophila* utilized endocytosis to transport lipids such as acylcarnitine. Blocking endocytosis increased sleep pressure and acylcarnitine accumulation (Li et al., 2023). Additionally, disruptions in lipid metabolism have been linked to sleep deficits, while dietary lipid supplementation has been shown to restore normal sleep duration (Avila et al., 2025). Collectively, these findings highlight, to some degree, the distinct molecular mechanisms underlying GA and sleep.

Although GA overlaps with natural sleep in certain neurophysiological features, its pharmacological hallmarks —such as dose dependency —and widespread disruption of interregional neural networks suggest that the anesthetic state constitutes a distinct physiological condition from natural sleep (Mashour & Hudetz, 2017; Yang et al., 2021).

Recent studies in *Drosophila* have highlighted fundamental discrepancies between the neural mechanisms underlying GA and natural sleep. The dorsal fan-shaped body (dFB) has been identified as a key brain region that promotes sleep and drives sleep pressure, as evidenced by the association between increased dFB activity and increased sleep (Donlea et al., 2011; Pimentel et al., 2016; Troup et al., 2018). However, whole-brain calcium imaging has demonstrated that optogenetic activation of the dFB evoked neural activity patterns characteristic of wakefulness, despite concurrent behavioral unresponsiveness (Tainton-Heap et al., 2021). Similarly, isoflurane exposure failed to activate dFB neurons or ellipsoid body R3m ring neurons, both previously identified as sleep-promoting (Singh et al., 2023; Troup et al., 2023; Yan et al., 2023). Although neuronal activity persisted during GA and dFB-induced sleep, the composition of active neurons differed from that observed during wakefulness (Troup et al., 2023). Temporal mapping revealed ongoing shifts in neuronal recruitment, with the rate of active neuron turnover per minute remaining comparable between anesthetized and awake states. However, the population of neurons engaged under anesthesia was markedly less diverse than that observed during wakefulness or dFB-induced sleep. Additionally, anesthetics selectively disrupted functional correlations among neurons positioned at intermediate distances, while correlations among nearby or widely separated neurons remained largely intact (Troup et al., 2023). These findings advance understanding of dynamic neural signatures associated with GA, underscoring that loss of network-level coordination may represent a pivotal mechanism by which anesthesia induces behavioral unresponsiveness.

GA was previously thought to act on endogenous sleep and wake circuits, as supported by observations of GA-induced activation of sleep-promoting circuits in the mammalian brain (Moore et al., 2012; Zecharia et al., 2009). Interestingly, the discovery of large-scale neural activity monitoring in flies provides a new perspective, suggesting that the brain undergoes dynamic changes during GA and natural sleep. These changes involve the recruitment of different neurons, which assemble into distinct neural networks. Consistent with findings in other animal models and humans (Awal et al., 2018; Hudetz, 2012; Yang et al., 2021), impairment of the brain network, rather than neural activity, by GAs may be a common mechanism inducing loss of coordination across the brain, leading to unresponsiveness or unconsciousness. However, further study is needed to clarify this possibility.

An overview of the current understanding of GA and sleep in *Drosophila* is summarized in Table 3. The observed commonalities and differences between these two states offer a framework for understanding the mechanisms of anesthesia, with far-reaching applications across multiple fields. Leveraging the shared molecular and neural pathways between GA and sleep may facilitate the development of drugs with improved efficacy, faster onset and recovery, and reduced side effects. Furthermore, insights from GA research could inform therapeutic strategies for sleep disorders, including insomnia. In contrast, characterizing the differences between GA and sleep, such as brainwave patterns and physiological responses, may enhance intraoperative monitoring and enable more precise modulation of anesthetic depth. Moreover, as GA has been shown to disrupt postoperative sleep architecture, recognizing these differences may help optimize recovery and improve patient outcomes.

FUTURE PERSPECTIVES

The discovery of GAs has not only transformed clinical practice but also catalyzed the emergence of a distinct field in basic neuroscience. However, challenges such as inter-individual variability, neural network complexity, multi-target effects, and ethical constraints continue to hinder precise mechanistic dissection. *Drosophila melanogaster* has emerged as a powerful model for advancing our understanding of the molecular and neural foundations of GAs (Figure 4). First, flies display evolutionarily conserved behavioral and physiological responses to various GAs, enabling high-throughput screening for agents with improved efficacy and reduced side effects. Second, the availability of well-annotated genomic libraries and extensive mutants facilitates the identification of specific molecular targets and signaling pathways involved in GA sensitivity and resistance. Moreover, the conservation of protein complexes between *Drosophila* and humans allows for detailed investigation into subunit-specific roles in complex assembly, function, and regulation under GA influence. Third, despite their numerically limited neuronal population, *Drosophila* possesses a fully mapped brain connectome (Dorkenwald et al., 2024; Schlegel

Table 3 Current understanding of molecular and neural mechanisms underlying general anesthesia and natural sleep in *Drosophila*

		Mechanism	Effect	Reference
Lipid theory	GA	Chloroform, isoflurane, ether, xenon, and propofol disrupt lipid rafts to activate PLD2 and generate the signaling lipid PA to activate TREK-1 to induce anesthesia.	Induction	Pavel et al., 2020
	Sleep	Lipid substances, such as acylcarnitines, cross the blood-brain barrier through sleep-dependent endocytosis, and accumulation of lipid substances reflects increased sleep drive.	Sleep-promoting	Li et al., 2023
		Apolipoprotein E-dependent lipid transfer from neurons to glial cells protects neurons from oxidative damage during wakefulness and removes lipids from glial cells during sleep.		Haynes et al., 2024
		Decreased carnitine transporter *CG6126* activity in the blood-brain barrier leads to lipid accumulation, reducing sleep. Supplementation with fatty acids restores sleep. Transporting carnitine through *CG6126* is essential for promoting brain fatty acid metabolism to maintain sleep.	Sleep-promoting	Avila et al., 2025
Mitochondria	GA	*ND23* mutant flies exhibit hypersensitivity to isoflurane and sevoflurane.	Induction	Olufs et al., 2018
	Sleep	Oxidative byproducts of mitochondrial electron transfer regulate sleep by binding to NADPH cofactor.	Sleep-promoting	Kempf et al., 2019
		Electron transport chain complex I and V RNAi in D42 glutamate neurons leads to increased sleep.	Sleep-promoting	Landis et al., 2023
Presynaptic molecule	GA	*Drosophila* develops resistance to benzyl alcohol, which disappears when vesicular cycle is inhibited.	Induction	Al-Hasan et al., 2011
		Syntaxin1A is involved in induction and recovery of general anesthesia.	Recovery & Induction	Troup et al., 2019; Zalucki et al., 2015b
		Propofol and etomidate limit migration rate of *Drosophila* syntaxin1A by affecting its fixation or aggregation. Eliminating syntaxin1A and SNAP-25 interaction rescues migration rate.	Induction	Bademosi et al., 2018
	Sleep	Increase in core scaffold protein BRP in the active region of the ELKS family enhances synaptic strength, augments plasticity of presynaptic active region, and promotes sleep.	Sleep-promoting	Huang et al., 2020
		Ric, a special GTPase, participates in presynaptic DAT endocytosis and enhances DAT function, with Ric overexpression leading to sleep fragmentation.	Wake-promoting	Fagan et al., 2021
Neurotransmitter	Sleep	Glutamate, a wakeful-regulating neurotransmitter, is the main neurotransmitter of nocturnal wakefulness activity.	Wake-promoting	Zimmerman et al., 2017
Ion channel	Potassium channels			
	GA	*Shaker* mutants lack Kv1 channel and exhibit resistance to isoflurane and sevoflurane, but show normal sensitivity to xenon.	Induction	Schaper et al., 2012; Weber et al., 2009
		Halothane inhibits K-shaw2 channels at S4–S5 linker or distal S6 region; sevoflurane shows activation at T330 site.	Induction	Barber et al., 2012; Bhattacharji et al., 2010
	Sleep	Kv (Shaker and Shab) and K2P channels mediate DA regulation of dFB neurons. dFB-restricted interference with Shaker or two-hole domain potassium pass (Sandman) expression decreases and increases sleep, respectively, by slowing repetitive discharge of dFB neurons in ON state or blocking their entry in OFF state.	Wake-promoting & Sleep-promoting	Pimentel et al., 2016
		ORK1 (TREK-1 homolog in *Drosophila*) shows partial loss of channel function and reduced sleep, indicating involvement in sleep regulation.	Sleep-promoting	Zhang et al., 2017
		Shal/Kv4, a voltage-gated K^+ channel, plays a controlling role in dusk wakeful-sleep transition in circadian rhythm neurons of *Drosophila*.	Sleep-promoting	Feng et al., 2018
	Other ion channels			
	GA	Mutation in *har38* reduces sensitivity to halothane, increases resistance to fluoroalkanes, methoxyflurane, chloroform, trichloroethylene, and xenon, but maintains sensitivity to isoflurane and sevoflurane.	Induction	Lu et al., 2007; Schaper et al., 2012; Weber et al., 2009
	Sleep	T-type Ca^{2+} channel Ca-α1T promotes arousal.	Wake-promoting	Jeong et al., 2015
Receptor	DA/GABA receptors			
	GA	Propofol and pentobarbitone enhance efficacy of RDL receptors and directly activate RDL receptors by replacing transmembrane methionine residues of the RDL receptor with equivalent asparagine residues of the mammalian β2 and β3 subunits or serine residues of the β1 subunit.	Induction	Belelli et al., 1996; Pistis et al., 1999
		dumb2, an allele mutation of DopR, reduces dopamine levels and induces hypersensitivity to isoflurane.	Recovery	Kottler et al., 2013
	Sleep	DopR of dFB mediates wake-promoting effects of dopamine.	Wake-promoting	Ueno et al., 2012
		PAM DANs projecting to γ5, γ4, and β'2 MB compartments are GABA responsive and regulate sleep via ionotropic $GABA_A$-RDL receptor. γ4 and β'2 MB compartments are GABA responsive and regulate sleep via ionotropic $GABA_A$-RDL receptor. Arousal-promoting effects of PAM-DANs projecting to γ5 and β'2 MB compartments are mediated by dopamine receptors, DopR1 and DopR2, which play a role in KCs and MBONs, and regulate arousal of MB compartments.	Wake-promoting & Sleep-promoting	Driscoll et al., 2021
		DopR regulates daytime sleep within mushroom body, independent of the known role of DopR in nighttime sleep.	Sleep-promoting	Jiang et al., 2016

Continued

			Mechanism	Effect	Reference
			CLOCK-dependent Fbxl4 expression rhythmically down-regulates $GABA_A$ receptor level to increase activity of pacemaker neurons and promote wakefulness.	Wake-promoting	Li et al., 2017
		NMDA receptor			
	Sleep		NMDAR promotes sleep regulation.	Sleep-promoting	Tomita et al., 2015
			Ellipsoid body R5 network shows complex delta oscillations, which increase the power of slow-wave oscillations with increasing sleep demand. Oscillatory synchronization depends on NMDAR-mediated coincidence detection.	Sleep-promoting	Raccuglia et al., 2019
		5HT receptor			
	Sleep		5HT2b receptors are sleep-promoting and are involved in regulation of sleep homeostasis in a small subset of dFB neurons.	Sleep-promoting	Qian et al., 2017
		PDF receptor			
	Sleep		DANs expressing PDF receptors act as downstream PDF signals to promote arousal during the day.	Wake-promoting	Potdar & Sheeba, 2018
Neural circuit	FB				
	GA		Isoflurane does not activate sleep-promoting R23E10-Gal4 circuit.	Induction	Troup et al., 2023
	Sleep		Activation of some dFB neurons induces increased sleep duration during the day and night, while inhibition of dFB neurons leads to insomnia. dFB neurons act as a control switch for homeostatic sleep.	Sleep-promoting	Donlea et al., 2011, 2014
			Pair of DA neurons located in the PPL1 cluster promotes arousal by projecting dFB neurons.	Wake-promoting	Liu et al., 2012
			dFB neuron R23E10-Gal4 contains external dFB and VNC. Two VNC-SP cholinergic neurons promote sleep when activated and reduce sleep when suppressed.	Wake-promoting & Sleep-promoting	Jones et al., 2023
			Various drivers were used to determine the role of dFBs in promoting sleep.	Sleep-promoting	De et al., 2023
	EB				
	GA		Isoflurane does not activate R28D01-Gal4-labeled EB R3m ring neurons associated with sleep	Induction	Troup et al., 2023
	Sleep		Ellipsoid body R2 neurons play an important role in sleep drive. After prolonged wakefulness, R2 neurons show an increase in IP3R-dependent cytoplasmic Ca^{2+} levels and an increase in NMDA receptor levels, resulting in increased conductance of R2 neurons, increased synaptic strength, and increased sleep.	Sleep-promoting	Liu et al., 2016
			EB E3m neuron activation increases sleep, and ER 3d neuron activation promotes arousal.	Wake-promoting & Sleep-promoting	Singh et al., 2023
			Screening of 34 ellipsoid body drivers revealed several EB drivers that significantly regulate sleep phenotypes. Among sleep-associated cell types, R3p promotes daytime sleep, whereas R4m enhances nighttime wakefulness.	Wake-promoting & Sleep-promoting	Yan et al., 2023
			EPG neurons promote sleep, with their inhibition reducing homeostatic sleep rebound. R5 neurons act upstream of EPG neurons to promote sleep.	Sleep-promoting	Ho et al., 2022
	MB				
	Sleep		Five sleep suppressors express glutaminergic proteins (MBON-γ5β '2a, MBON-β'2mp, MBON-β'2a_bilateral and MBON-γ4>γ1γ2), while sleep-promoting factors express GABAergic proteins (MBon-γ3β'1 and MBON-γ3) or cholinergic proteins (MBON-γ2α'1).	Wake-promoting & Sleep-promoting	Aso et al., 2014
			DPM neurons increase sleep via release of GABA onto wake-promoting mushroom body (MB) α'/β' neurons. Down-regulation of α'/β' $GABA_A$ and $GABA_BR3$ receptors results in sleep loss, suggesting possible sleep-relevant targets of DPM-mediated inhibition.	Sleep-promoting	Haynes et al., 2015
			α′/β′ and γm KCs are wake-promoting and γd KCs are sleep-promoting. α′/β′ and γm KCs promote wakefulness by activating MBON-γ5β′2a/β′2mp/β′2mp_bilateral and γd KCs promote sleep by activating MBON-γ2α′1.	Wake-promoting & Sleep-promoting	Sitaraman et al., 2015a
			DANs projecting to lobe compartments containing dendrites of wake-promoting MBONs promote wakefulness. Activation of DANs projecting to γ5 and β′2 compartments induces greater activation of MBON-γ5β′2a/β′2mp/β′2mp_bilateral than MBON-γ2α′1.	Wake-promoting	Sitaraman et al., 2015b
		Cross-brain-area neural pathways			
	Sleep		Relaxation oscillators balance sleep homeostasis between dFB neurons, EB-R2 neurons, and central complex interneuron spiral cells.	Sleep-promoting	Donlea et al., 2018
			T1 DANs (arousal-promoting), R59E08 neurons (neurons labeled by R59E08-Gal4 driver, which label sleep-promoting PB interneurons), and R52B10 neurons (neurons labeled by R52B10-Gal4 driver, which label arousal-promoting P-FN neurons) project from PB to ventral FB and NO to form a sleep-wakefulness regulatory circuit (PB-FB pathway).	Wake-promoting	Tomita et al., 2021

Continued

		Mechanism	Effect	Reference
		FB cholinergic interneurons promote arousal, receive excitatory input from the PB-FB pathway, and are associated with dFB neurons, and therefore may be a circuit regulating sleep.	Wake-promoting	Kato et al., 2022
Whole brain	GA	Anesthetics reduce various neural responses in the brain.	Induction	Cohen et al., 2016, 2018
		Whole-brain calcium imaging of *Drosophila* under isoflurane anesthesia.	Induction	Troup et al., 2023
	Sleep	Whole-brain calcium imaging during sleep and wakefulness.		Tainton-Heap et al., 2024
		Whole-brain electrophysiology of sleep and wakefulness.		Van De Poll & Van Swinderen, 2024

GA, general anesthesia; PLD2, phospholipase D2; PA, phosphatidic acid; NADPH, nicotinamide adenine dinucleotide phosphate; SNAP-25, synaptosome-associated protein 25; BRP, bruchpilot; DAT, dopamine transporter; K2P, two-pore-domain potassium; DA, dopamine; dFB, dorsal fan-shaped body; ORK1, outwardly rectifying K^+ channel-1; GABA, γ-aminobutyric acid; RDL, resistance to dieldrin; DopR, D1-like receptor; PAM, protocerebral anterior medial; DANs, dopamine neurons; MB, mushroom body; KCs, Kenyon cells; MBONs, mushroom body output neurons; NMDA, N-methyl-D-aspartic acid; NMDAR, N-methyl-D-aspartic acid receptor; 5HT, 5-hydroxytryptamine; PDF, pigment dispersing factor; FB, fan-shaped body; PPL1, posterior lateral protocerebrum 1; VNC, ventral nerve cord; EB, ellipsoid body; IP3R, inositol-triphosphate receptor; EPG, ellipsoid body-protocerebral bridge-gall; PB, protocerebral bridge; NO, noduli.

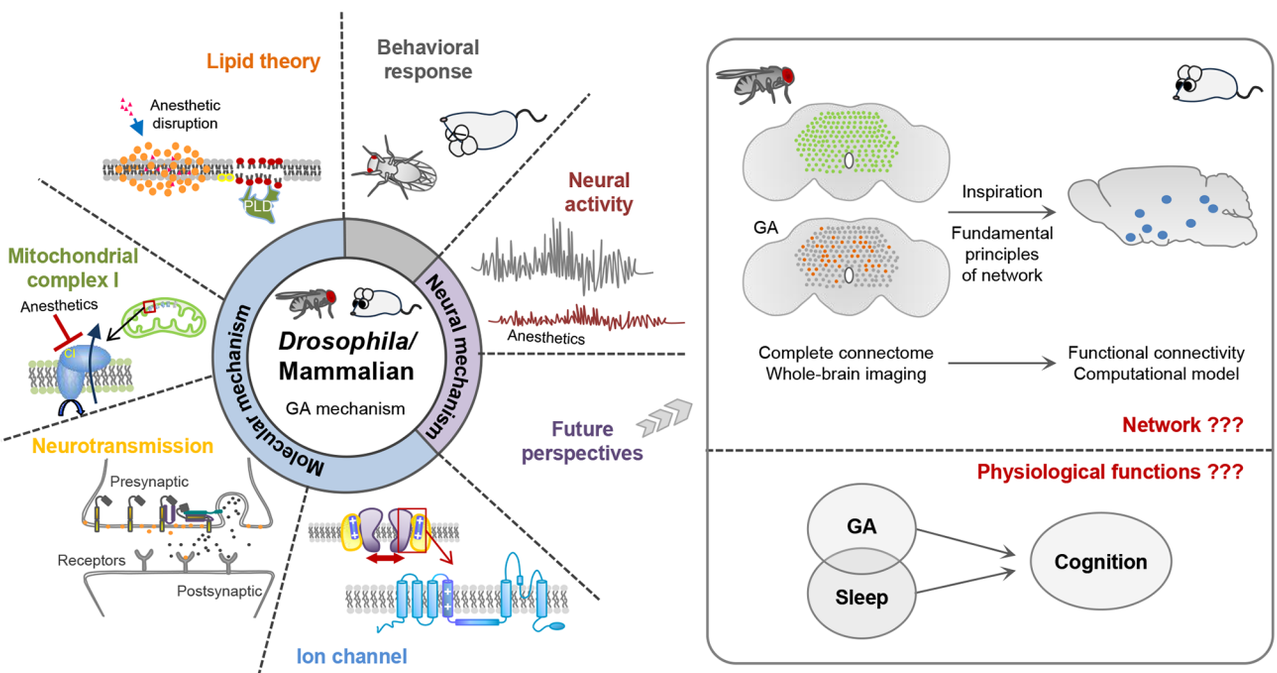

Figure 4 Graphical summary of GA-related molecular and neural mechanisms between *Drosophila* and mammals and future research directions

et al., 2024), offering comprehensive local and global circuit diagrams that serve as an invaluable framework for resolving GA-induced network-level effects.

The ability to genetically manipulate defined neurons and circuits in *Drosophila* allows precise functional analysis at the single-cell level. When combined with the complete brain connectome, this system provides a powerful framework for investigating how information is processed and how anesthetics alter network activity. For example, the central complex—particularly the fan-shaped body (FB) and ellipsoid body (EB)—is characterized by dense internal and reciprocal connectivity (Lin et al., 2024). Although some FB and EB neuronal subtypes appear unresponsive to GAs (Troup et al., 2023), further investigation on cell type specificity in response to GA and functional connections may clarify how anesthetics affect local circuitry. These insights could guide the development of computational models to simulate circuit-level responses under anesthesia.

These discoveries in *Drosophila* offer valuable perspectives for understanding mammalian, and potentially human, brain function. Advances in *Drosophila* whole-brain imaging have enabled the dissection of network spatiotemporal dynamics during the induction, maintenance, and recovery phases of GA. The integration of a well-defined connectome with brain-wide imaging techniques presents a powerful framework for delineating the neural transitions underlying GA states. This approach may also facilitate the development of a theoretical model of brain-wide network behavior, marking a significant advancement in the field.

GA and sleep exhibit several shared features (Table 3). Both states involve reduced responsiveness and overlapping patterns of suppressed activity, hinting at partially convergent mechanisms related to decreased awareness. Nevertheless, each state also displays distinct neurophysiological signatures, highlighting their mechanistic divergence. Whole-brain imaging under GA and natural sleep offers a comprehensive perspective on brain function in non-wakeful states, offering insights relevant to anesthetic development

and the pathophysiology of sleep-related disorders. However, studies on the interactions between GA and natural sleep remain in their infancy. Future efforts are required to exploit shared and divergent pathways, with the dual aim of improving anesthetic precision and mitigating adverse effects.

Although *Drosophila* serves as an ideal animal model for exploring conserved molecular and neural mechanisms and fundamental principles, the simplicity of its brain structures, basic physiological systems, and restricted behavioral repertoire pose significant challenges for the direct translation of research findings to mammals. For example, the absence of sophisticated circulatory and respiratory systems in *Drosophila* limits investigations into the systemic pharmacodynamics of anesthetics —critical components of mammalian anesthesia. Moreover, while fly studies have advanced understanding of learning and memory (Huang et al., 2024), their capacity to model higher-order cognitive outcomes such as postoperative cognitive dysfunction and delirium remain relatively limited, highlighting gaps in translating findings across species. Thus, integrating diverse methodologies across multiple species is essential for a comprehensive understanding of the mechanisms underlying GA. This multifaceted approach, rather than a singular reliance on any one system, is essential to fully unravel the neural mechanisms of GA.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conceptualization and framing of the work. All authors contributed to the drafting of the manuscript and made critical revisions to important content and diagrams. All authors read and approved the final version of the manuscript.

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