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REVIEW

Dendritic spine remodeling and plasticity under general anesthesia

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Abstract
Ever since its first use in surgery, general anesthesia has been regarded as a medical miracle enabling countless life-saving diagnostic and therapeutic interventions without pain sensation and traumatic memories. Despite several decades of research, there is a lack of understanding of how general anesthetics induce a reversible coma-like state. Emerging evidence suggests that even brief exposure to general anesthesia may have a lasting impact on mature and especially developing brains. Commonly used anesthetics have been shown to destabilize dendritic spines and induce an enhanced plasticity state, with effects on cognition, motor functions, mood, and social behavior. Herein, we review the effects of the most widely used general anesthetics on dendritic spine dynamics and discuss functional and molecular correlates with action mechanisms. We consider the impact of neurodevelopment, anatomical location of neurons, and their neurochemical profile on neuroplasticity induction, and review the putative signaling pathways. It emerges that in addition to possible adverse effects, the stimulation of synaptic remodeling with the formation of new connections by general anesthetics may present tremendous opportunities for translational research and neurorehabilitation.

Keywords Neuroplasticity · General anesthesia · Dendritic spine dynamics · Cofilin · Actin cytoskeleton · Depression

Introduction

General anesthesia is one of the most widely used procedures in diagnostics and clinical care. It results from interruption of physiological communication between nerve cells in the brain induced by a single or multiple neuroactive agents, leading to a transient coma-like state with loss of protective reflexes. The procedure is carried out to enable a wide range of medical interventions that would be otherwise intolerably painful and even life-threatening. In the history of medicine, attempts of inducing a state of anesthesia can be traced back to ancient Assyria, Babylon, Egypt, and China, with the use of, e.g., opium extracted from poppy (*Papaver somniferum*) documented in numerous antique medical sources (Booth 1998; Robinson and Toledo 2012). The discovery of analgesic properties of diethyl ether in XV–XVI centuries by Ramon Llull and Paracelsus has opened a revolutionary new page in annals of anesthesiology, with the subsequent growing use of synthetic compounds and their mixture rendering general anesthesia more effective and better-controllable (Carter 1999; Gravenstein 1965). The modern tradition of the use of general anesthetics in diagnostics and surgery dates back to 1842 when Crawford Long administered diethyl ether to a patient and performed the first painless operation (Long 1991). Since the arsenal of general anesthetics has evolved and expanded, with delivery routes and controllability of anesthesia continuously optimized and improved. With the arrival of antipsychotic drugs, haloperidol and droperidol have been added to the practice of general anesthesia (Janssen et al. 1963). In modern days, general anesthetics are applied very commonly, and the procedure is assumed to be largely safe, or causing minor and reversible adverse effects, allowing full post-anesthesia recovery.

In recent years, however, concerns have been raised over the safety and reversibility of the procedures with lasting effects reported in the central nervous system (CNS) after the anesthetic agent is removed. Multiple reports suggest that perturbations of normal functions of neurons caused by general anesthesia may extend well beyond the treatment

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period (Wu et al. 2019). Experimental research in rodents, for example, has shown that the exposure to a variety of commonly used anesthetics at a young age produces social and behavioral impairments that may last into adulthood (Hofacer et al. 2013; Jevtovic-Todorovic et al. 2003; Satomoto et al. 2009; Wu et al. 2019). The majority of cognitive and behavioral impairments induced by general anesthesia have been assigned to their potential toxicity with pro-apoptotic effects, as well as suppression of neurogenesis, which has been shown in animals following their exposure to narcosis during the early postnatal period (Jevtovic-Todorovic et al. 2003; Stratmann et al. 2009). Likewise, in children under 3 years of age, general anesthesia has been reported to increase the risk of learning and memory deficit throughout puberty and into adulthood (Flick et al. 2011; Sun 2010; Wilder et al. 2009). An increasing number of reports have also shown detrimental effects of widely used general anesthetics in neuronal cultures, with outcomes varying, depending on the developmental stage of neurons (Head et al. 2009; Jiang et al. 2018). These findings are in agreement with numerous studies in rodents and primates, suggesting that the use of dissociative anesthetics during critical periods of development may induce lasting deficits of specific brain mechanisms and functions (Paule et al. 2011; Scallet et al. 2004). Hence, once considered safe, general anesthesia emerges to hold risks of long-term adverse impact, which calls for in-depth research and careful safety considerations.

Although the cause–effect relationship of disruptive neurobiological, cognitive, and behavioral consequences of general anesthesia remains to be determined, they have been so far viewed mainly in connection with impaired neurogenesis and synaptic toxicity, with both reported in vitro and animal studies after exposure to general anesthesia during the early postnatal development (Wei et al. 2008; Yu et al. 2013; Zhang et al. 2010). Structural alterations seem to be especially pronounced in neurons undergoing synaptogenesis and pruning, implying immature neurons and developing networks as the prime target of adverse effects. Recent advances of intra-vital imaging methods with the use of genetically encoded reporter proteins (GERPs) (Crowe and Ellis-Davies 2014; Ovsepian et al. 2017; Neuner et al. 2014) enabled monitoring of the effects of general anesthesia on synaptic plasticity and molecular processes in dendritic spines of an intact brain. As a prime anatomical locus of the majority of excitatory synapses, dendritic spines play a key role in neuronal communication and encoding memory traces. Importantly, these small protrusions of dendrites reveal a wide range of morphologies, with their changes related to a variety of specific neuronal processes and functions (Leuner and Shors 2004; Yuste 2010). Although the prevalent view is that developing neurons are especially sensitive to the lasting impact of general anesthetics, growing data shows that destabilizing effects of general anesthetics on spines and synaptic connections can occur in the mature brain as well, i.e., might be partially independent of the developmental processes in neurons (Crosby et al. 2010; Jiang et al. 2018; Platholi et al. 2014).

Considering recent progress in visualizing dendritic spines with conflicting findings, and the relevance of general anesthesia to clinical and translational neuroscience and patient safety, there is a pressing need for a critical assessment of emerging reports in the field, with implications for higher brain mechanisms and mental health. Such analysis is especially timely, given a growing number of COVID-19 patients receiving general anesthesia while maintained on artificial ventilation. Although the numbers are preliminary, according to recent estimates, in China ~ 3.2% of all COVID-19 patients received treatments involving intubation and general anesthesia over several days (Meng et al. 2020). Moreover, it was reported recently that the medically induced coma lasting > 6 h can also cause considerable restructuring of the cortical architecture and circuits in both young and adult mice (Wenzel et al. 2021). The detailed and critical assessment of the impact of general anesthesia on spine plasticity is, therefore, much pertinent, which as shown throughout this study, and extends the current knowledge beyond mere satisfaction of scientific curiosity, but provides clues for translational neuroscience, neurorehabilitation, and intensive care medicine (Table 1).

Classification of general anesthetics based on their action mechanisms

There is a long-standing consensus that the coma-like state induced by general anesthesia results from direct or indirect inactivation of excitatory currents and receptors or enhancement of inhibitory drive in the CNS (Brown et al. 2011; Franks 2008; Franks and Zecharia 2011; Pavel et al. 2020). Despite major advances in elucidating the action mechanisms of individual anesthetics, the relative contribution of different neuronal types with specific ion channels and receptors to induction and maintenance of the state of general anesthesia remain elusive. Amongst the best characterized structural correlates of both, acute and long-lasting effects of general anesthetics in neurons are changes in morphology and number of dendritic spines and their distribution. Remarkably, there is considerable data implying differential sensitivity of various types of dendritic spines to general anesthesia, which are classified based on their morphological and functional characteristics into thin, stubby, mushroom and cup spines, and filopodia, and are thought to play a distinct role in neuronal functions and plasticity mechanisms (Nimchinsky et al. 2002; Rochefort and Konnerth 2012). In present-day medical and veterinary practices, a state of general anesthesia is typically achieved via

Table 1 Impact of general anesthesia on dendritic spine development and plasticity

Anesthetics	Region of the brain	PND 0—7	PND 7—21	PND 21—35	PND > 35	References
Isoflurane	Hippocampus	↓ Long thin spines only Number of spines remained the same, ↓	–	↑ in the CA1 region	↓ Anesthesia was supplemented with laparotomy	Schaefer et al. (2019), Kang et al. (2017), Qiu et al. (2020), Head et al. (2009), Yang et al. (2011), Briner et al. (2010), Landin et al. (2019)
	Cortex	–	↑	Filopodia pruning was reduced, ↑	–	
Sevoflurane	Hippocampus	Number of spines remained the same, ↓	↑	–	–	Briner et al. (2010), Qiu et al. (2016), Xiao et al. (2016), Jia et al. (2016), Liu et al. (2019), Zhou et al. (2019)
	Cortex	↑↓	↑	–	–	
Ketamine	Hippocampus	–	↑↓	↑, Number of spines remained the same	–	De Roo et al. (2009), Tan et al. (2009), Yang et al. (2011), Huang et al. (2016)
	Cortex	–	Number of spines remained the same	↑ fillopodia, ↓ motor learning-induced spine formation	↓ Motor learning-induced spine formation	
Opioids	Nucleus accumbens	–	↑ Thin spines ↓ Stubby spines	–	–	Geoffroy et al. (2019)
Propofol	Hippocampus	↓	↑	Number of spines remained the same	–	De Roo et al. (2009), Briner et al. (2011), Puskarjov et al. (2017), Huang et al. (2016), Zhang et al. (2017)
	Cortex	↓	↑↓	↑	Number of spines remained the same	

Dendritic spine density increase (↑); Dendritic spine density decrease (↓), *PND* Postnatal day

parallel and sequential use of several medications, to reach the desired characteristics of narcosis (Brown et al. 2018). Figure 1 schematizes four major groups of general anesthetics with their effects on neurophysiological parameters and functions of central neurons.

Dendritic spine remodeling by halogenated anesthetics

Although halogenated anesthetics comprise several group members used in the clinic and veterinary medicine, current discussion of their effects on dendritic spines is limited to isoflurane and sevoflurane (and very briefly desflurane), which are the most widely used and characterized members of the group.

Isoflurane

Isoflurane is a halogenated ether, and one of the most widely used inhalation anesthetic. It induces a generalized and reversible depression of CNS (Franks 2008; Franks and Zecharia 2011; Papich 2016). In research and clinic practice, isoflurane is applied for induction and maintenance of general anesthesia, although more recently, other substances are often used for initiation of the anesthesia, to avoid the initial airway irritation response caused by isoflurane (TerRiet et al. 2000). Electrophysiological studies showed that isoflurane inhibits glycine, $GABA_A$, *N*-Methyl-*D*-Aspartate as well as α7-nACh receptors (Jones et al. 1992; Zhang et al. 1997), and large-conductance BK_{Ca}^{2+} channels (Pancrazio et al. 1993). Isoflurane also potentiates serotonin response via $5\text{-}HT_3$ receptors, activates $KCNK_2$ (TREK-1) background K^+ channels (Liu et al. 2004; Lopes et al. 1998; Zhou et al.

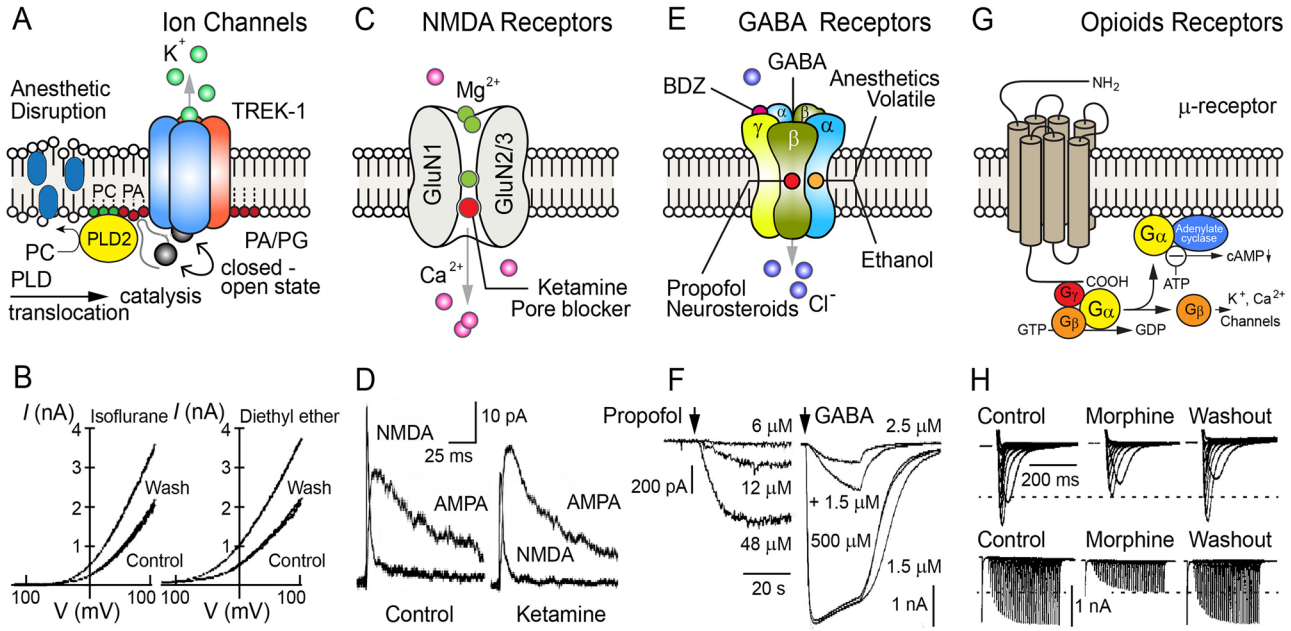

Fig. 1 Schematic illustration of four major primary molecular targets of general anesthetics with neurophysiological effects. **A**, **B** Isoflurane induced activation of TREK-1 channels resulting in the reduction of neuronal excitability. **A** TREK-1 activation by inhaled anesthetics results from disruption of monosialotetrahexosylganglioside-1 (GM1) rafts (blue ovals, left) in the surface membrane leading to aggregation of phospholipase D2 (PLD2) with TREK-1 and its substrate phosphatidylcholine (PC, green circle) in the affected area. After PLD2 hydrolyzes PC to phosphatidic acid (PA, red sphere), the anionic membrane lipids bind to the gating helix (grey circle and thread), which uncovers the TREK-1 channel, activating IK^+ and lowering membrane excitability. **B** Voltage–current relation of TREK-1 mediated IK^+ with effects of isoflurane and diethyl ether, respectively. Recordings were made in whole-cell mode using a 1-s ramp from a holding potential of − 80 mV (Pavel et al. 2020). **C**, **D** Schematic of NMDAR block with ketamine (red circle) (**C**) and induced changes of NMDA/AMPA ratio in fast-spiking interneurons of the medial prefrontal cortex of adult mice (example traces) (**D**) (Jeevakumar and Kroener 2016). **E**, **F** Schematic of $GABA_A$ receptor with binding sites of major agonists and enhancers: BDZ—benzodiazepine (**E**). Modulation of $GABA_A$ response in hypothalamic neurons by propofol, with examples of propofol-mediated currents in acutely isolated tubero-mamillary neurons (left) and potentiation of GABA induced currents by a different dose of propofol (Sergeeva et al. 2005) (**F**). **G**, **H** A schematic of opioid μ-receptor structure with downstream signaling and production of cAMP and Gβ, which modulate voltage-gated membrane currents via direct effects on ion channels or via indirect mechanisms, mediated through regulation of gene expression (**G**). Effects of morphine on INa^+ in isolated cardiac cells of rats (top) and on the recovery of INa^+ from inactivation (bottom). Membrane currents evoked by depolarizing pulses applied at 10 mV increments from − 60 to + 50 mV (top), and 20 ms test pulses used for measuring the recovered $INa+$ current after the first conditioning pulse, followed by washout from holding potential of − 80 mV (Hung et al. 1998)

2012), and Shaker-related K_V1 channels (Correa 1998; Li et al. 2018b) (Fig. 1A, B). Its effects on ion channels are partly mediated via an increase of membrane fluidity with activation of plasma membrane calcium ATPase protein (PMCA), which in turn facilitates binding to the D subunit of ATP synthase and NADH dehydrogenase, resulting in a reduction in membrane excitability (Borle 2014; Ou et al. 2020).

With such a broad range of effects, it is not surprising that isoflurane causes a variety of structural changes in neurons, although mechanisms underlying these alterations remain unclear. As a volatile anesthetic, isoflurane inhibits actin-based motility and causes depletion of F-actin in dendritic spines, which leads to destabilization of the molecular skeleton with the collapse of spines (Herold et al. 2013; Kaech et al. 1999; Platholi et al. 2014). The effects of isoflurane on dendritic spines were investigated in primary neuronal culture, where its application caused reversible inhibition of spine formation and decreased actin motility (Kaech et al. 1999). Head et al. (2009) have shown that exposure of neuronal culture to isoflurane inhibits the release of synaptic tissue-type plasminogen activator (tPA), promotes the pro-BDNF, and reduces the mature BDNF (mBDNF) signaling. In PND5-7 mice pups in vivo, 2 h isoflurane exposure was shown to cause a reduction in the total number of excitatory synapses. This effect was attenuated by pretreatment of animals with a membrane-permeable inhibitor of low-affinity neurotrophic receptor p75NTR signaling TAT-Pep5 (Head et al. 2009). Of note, another report showed that in PND7 and PND18 mice, the deficit of glutamatergic long-term potentiation (LTP) with impairments of memory and dendritic spine loss caused by a 4 h isoflurane exposure could be attenuated by inhibition of mTOR pathway, or by NO-donor treatment (Kang et al. 2017; Schaefer et al. 2019). As noted, the exact cellular processes underlying isoflurane-induced anesthesia and their contribution to structural alterations in dendritic spines remain currently unclear. Emerging evidence suggests that via changes in dendritic spine density and dynamics, isoflurane can act on a range of neuronal processes and functions, altering synaptic activity and connectivity. Importantly, the effects of isoflurane on dendritic spines may vary considerably, depending on the duration and dose of exposure, age, and time that has passed since the treatment (Briner et al. 2010; Crosby et al. 2010). In PND7 mice, for instance, single isoflurane anesthesia lasting 4 h reduced the number of thin spines in the hippocampus for 2 weeks, with a total number of spines remained unchanged (Schaefer et al. 2019). In vivo evidence has suggested that isoflurane can also cause dose-dependent apoptosis (Jevtovic-Todorovic et al. 2003). These observations were supported by the results of the report by Head and coworkers showing that 4 h exposure of 5-day-old neuronal cultures to isoflurane induces extensive death of neurons. Such effects appear to be age-dependent, as similar treatment of 14 or 21-day-old cultures did not cause cell death. In agreement with these findings, in vivo studies showed a reduction of the density of hippocampal synapses after 4 h exposure of PND5–7 mice to isoflurane (Head et al. 2009).

The impact of isoflurane on spine development has been also studied longitudinally in vivo by Yang et al. (2011) using two-photon microscopy. In layer V pyramidal neurons of the somatosensory cortex of PND30 mice, while isoflurane did not affect filopodia formation, it caused a significant reduction in filopodia elimination. Importantly, dendritic spine remodeling induced by isoflurane was also described during the late postnatal life of mice, with 40 min of general anesthesia in conjunction with laparotomy lowering dendritic spine density in the CA1 region in 16-month-old mice, measured 8 days after the procedure. These effects were antagonized by NMDARs blocker memantine or calpain inhibitor MDL-28170, with both preventing the loss of dendritic spines (Qiu et al. 2020). Remarkably, similar measurements in 18-month-old rats exposed to 2 h isoflurane showed no changes in synaptic density, despite cognitive impairments found in behavioral tests. Unlike reports in mice, this study carried out in rats performed spine counting 29 days after isoflurane exposure (Lin et al. 2012). Contrary to these findings, 1 h isoflurane anesthesia of PND16 rats increased the spine density of both apical and basal dendrites in layer V pyramidal cells of the medial prefrontal cortex, which became more pronounced after longer exposure to isoflurane (Briner et al. 2010). A recent report of the effects of 40 min exposure of pubertal rats to isoflurane also showed higher spine density in the same brain region. What is more, this study showed an increase in spine density also in pyramidal neurons of the hippocampal CA1 but not the CA3 area (Landin et al. 2019).

Overall, while results of experimental tests agree that isoflurane can cause age- and time-dependent changes in dendritic spines, they are far from simple and need careful analysis and interpretation. Region-specific differences of isoflurane effects, for instance, might be because, like in humans, different brain regions of experimental models reach maturity at different ages. Indeed, in 1–2-month-old rats, most of the dendritic spines in the somatosensory and visual cortex stabilize, while those in frontal and associative areas remain dynamic, and reach stability in 4–5 months of age (Crosby et al. 2010). Differential effects might be also partly due to the activation of different molecular mechanisms by various doses. Whether neuroplasticity changes caused by isoflurane contribute to the induction of general anesthesia or its adverse neurobehavioral effects remains to be shown.

Sevoflurane

Sevoflurane is another halogenated ether widely used for induction and maintenance of general anesthesia. As an inhalational anesthetic, it shares many features with isoflurane, with the principal difference being lower solubility, which renders the anesthesia onset and recovery faster (Ibrahim et al. 2001; Papich 2016). Like isoflurane, sevoflurane affects several voltage- and ligand-gated channels, with K^+ and Na^+ channels and α7 nAChR, recognized as primary targets (Barber et al. 2012; Stock et al. 2018). Positive modulation of Shaw $K_V3.2$ conductance by sevoflurane emerges to result from (1) shifting conductance–voltage relation towards more negative potential, while potentiation of Shaker-related K_V1 channels results from the prolonged open state of $K_V1.2$, $K_V1.3$, and $K_V1.5$ subunits containing channels (Lioudyno et al. 2013; Stock et al. 2018). The inhibitory effects of sevoflurane on Na^+ channels, on the other hand, are due to prolongation of the activation time constant (Barber et al. 2012), while effects on α7AChR and NMDA receptors result from non-competitive blockade and downregulation of their expression (Brosnan and Thiesen 2012; Tang et al. 2018).

Similar to isoflurane, the broad range of effects caused by sevoflurane is expected to influence a variety of functional and molecular processes, with transient and long-term impacts on structural plasticity. In PND 6, 7, and 8 rats, three consecutive 2 h sevoflurane exposure caused a lasting reduction of dendritic spine density in CA1 pyramidal neurons, as revealed at PND 50 (Jia et al. 2016). Sevoflurane also reduced levels of BDNF, synapsin-1, MAP-2, PSD-95, c-fos, pCREB/CREB, acetylated histones H3 and H4. Another group of rats, also treated with sevoflurane but receiving histone deacetylase inhibitor sodium butyrate from PND 6 to PND 50 did not show spine density alterations (Jia et al. 2016). Based on these findings, it was concluded that the effects of sevoflurane on dendritic spines and related behavioral abnormalities might depend on lasting histone acetylation. A recent report tested long-term developmental effects of repeated sevoflurane exposure in utero, on gestational days 13, 14, and 15. Consistent with observations made by Jia et al. in these tests, sevoflurane caused a decrease in dendritic spine density in the CA1 region, with a reduction in BDNF expression and histone acetylation. Of note, a single sevoflurane exposure on gestation day 14 was insufficient to produce morphological changes, while neuroplasticity effects induced by the repeated treatment could be reversed by higher physical activity (Wu et al. 2018). Multiple exposures to sevoflurane were shown to disrupt the tPA/PAI-1 signaling, causing inhibition of the cleavage of pro-BDNF to BDNF production in rats. The latter resulted in downregulation of the TrkB signaling and suppression of hippocampal synaptic plasticity in pubertal rats (Dong et al. 2020). Effects of sevoflurane-induced anesthesia (6 h) were also studied on PND 7 mice. It was found that sevoflurane caused a decrease in spine density in the hippocampus and increased PTP1B expression, with signs of apoptotic changes and synaptic degeneration also evident. In the same study, pharmacological inhibition of PTP1B attenuated disruptive effects caused by sevoflurane (Liu et al. 2019). Xiao et al. compared short- and long-term effects of sevoflurane, demonstrating that 1 h and 6 h anesthesia of PND 7 rats lowered the density of apical dendritic spines in the CA1 pyramidal cells at PND 21. Of note, changes in spine density induced by this anesthetic correlated with the expression of presynaptic target-SNARE syntaxin. The increase in the expression of another presynaptic target-SNARE SNAP-25 was, however, evident only in the group with 6 h sevoflurane exposure (Xiao et al. 2016). It is worth noting that other presynaptic markers, such as synaptophysin and α-synuclein remained overtly unchanged. Importantly, in vivo experiments with 30 min sevoflurane anesthesia did not show any signs of neurotoxicity or apoptosis in the hippocampus of PND 7 and PND 15 rats, whereas sevoflurane exposure for 4 h caused a marked enhancement in the number of active caspase-3-positive cells in the CA1 region in PND7 rats (Qiu et al. 2016). Interestingly, the sevoflurane-induced dendritic spine remodeling seems to involve changes in astrocytes. Zhou et al. showed for instance that 4 h treatment of PND 7 mice with 2.5% sevoflurane followed by morphometry at PND 21 revealed considerable changes in astrocyte morphology, which correlated with synaptic overgrowth and spine remodeling. Of note, these effects were specific to mushroom-type spines in basal dendrites of cortical pyramidal neurons but were absent in the hippocampal CA1 region (Zhou et al. 2019). Analysis of sevoflurane effects on Ca^{2+} homeostasis in astrocytes showed significant changes, leading to downregulation of ezrin expression, which is an actin-binding membrane protein implicated in morphogenesis of neurons (Fig. 2A–C). Sevoflurane-induced remodeling of dendritic spines was functionally associated with changes of spontaneous synaptic activity and NMDA/AMPA current ratio in cortical pyramidal neurons (Fig. 2C). Overall, disruptive effects of sevoflurane, similar to those of isoflurane, seem to involve actin remodeling, causing de-polymerization of its fibrillary form (F-actin) with the collapse of dendritic spines and detrimental functional consequences. Activation of RhoA/Rho-kinase signaling seems to be also contributing towards the shortening of dendritic protrusions caused by sevoflurane (Zimering et al. 2016).

In juvenile rats (PND 16 rats), on the other hand, 30 min sevoflurane anesthesia increased spine density on apical and basal dendrites in the layer V pyramidal cells of the medial prefrontal cortex over 6 h, with longer anesthesia causing a further increase in spine density on apical dendrites only (Briner et al. 2010). Notably, the increase in spine density by

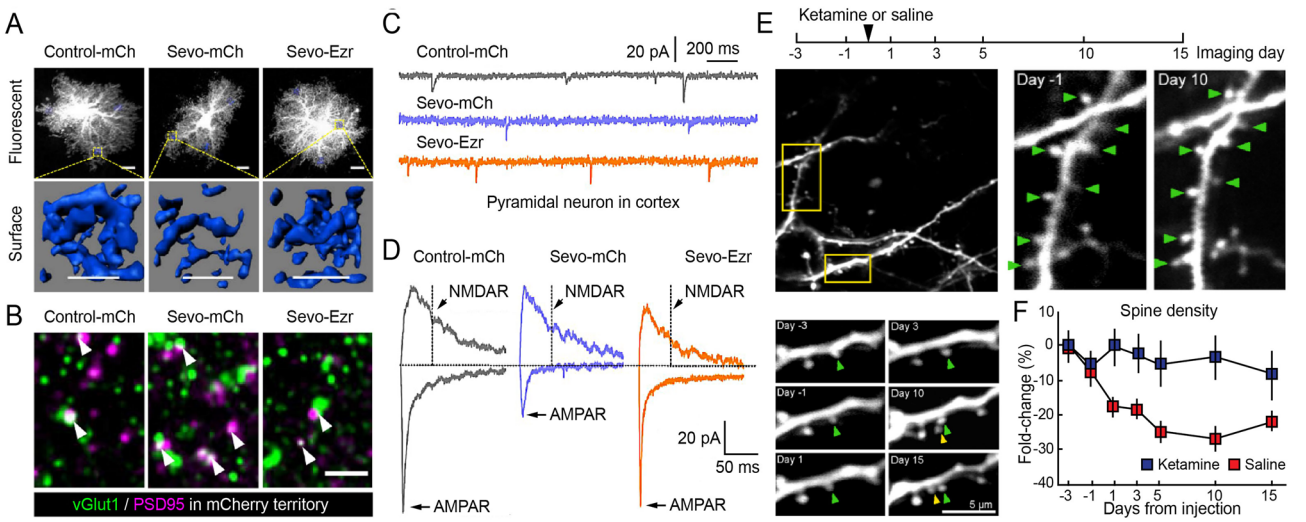

Fig. 2 Structural and functional alterations induced by general anesthetics in astrocytes and neurons. **A**, **B** Representative confocal images of cortical astrocytes and 3D reconstructed distal fine processes demonstrating changes induced by sevoflurane and rescue by ezrin overexpression (top to bottom). Scale bars, 10 µm. **B** Confocal images of vGluT1 (green) and PSD95 (magenta) within mCherry-positive territories with expressional changes in response to sevoflurane and rescue by ezrin. Scale bar, 2 µm. **C**, **D** Representative mEPSCs traces of L3–5 cortical pyramidal neurons and eEPSCs traces recorded in mouse brain slices from three groups at P22–P27 showing changes of mEPSC frequency and NMDA/AMPA current ratio. Adapted with permission (Zhou et al. 2019). **E** Systemic ketamine administration leads to higher dendritic spine density for at least 2 weeks relative to controls. Top: timeline of the experiment. Ketamine was administered at a dose of 10 mg/kg through intraperitoneal injection. An example imaging field of view acquired in Thy1-GFP-Mouse (top left). Yellow boxes indicate the dendritic branches shown enlarged at specified dates after treatment (right and bottom). Green arrowheads, stable spine (top right). Yellow arrowhead points to the new spine. **F** A summary graph of changes in dendritic spine density across days expressed as a fold change from the value measured on the first imaging session. The mouse was injected with either ketamine (blue square) or saline (black circle) (red arrowhead). Values are reported as the means. Adapted with permission (Phoumthipphavong et al. 2016)

sevoflurane was primarily due to the formation of small head mushroom spines. Importantly, the spine promoting effects of halogenated anesthetics seem to be substance-specific, as, unlike sevoflurane and isoflurane, the exposure of age-matched rats to desflurane up to 1 h resulted in no change in density of spines on either apical or basal dendrites. Analysis of apoptotic effects of desflurane and comparison with isoflurane and sevoflurane, on the other hand, suggests its significantly higher neurotoxicity (Kodama et al. 2011). The plasticity-enhancing effects of sevoflurane were confirmed by another report showing that even a short (30 min) exposure of PND 15 rats could increase the dendritic spine density in layer II/III of medial prefrontal and somatosensory cortices, and the CA1 region (Qiu et al. 2016). The same group showed that sevoflurane treatment at PND 7 caused a decline of spine densities in above mentioned neocortical regions, with no effects in pyramidal neurons of the CA1 area. Testing the effects of brief sevoflurane exposure on spine density over 3-month period revealed no difference from age-matched saline-treated controls, implying that sevoflurane-induced changes fully reverse over a long time (Qiu et al. 2016).

In summary, sevoflurane anesthesia emerges to have a potent bidirectional effect on dendritic spines, which depend on the developmental stage of experimental animals, exposure time, and brain regions. While alterations in spine density induced during early development can last for several weeks, these changes generally are reversible. Dendritic spine remodeling by sevoflurane seems to involve not only direct effects on neurons but also astrocytes. The mechanisms underlying spine remodeling under sevoflurane warrant further investigation, with emerging data supporting downregulation of ezrin and F-actin depolymerization as potential causatives of the loss of dendritic spines. The full impact of these changes on brain connectome, cognition, and behavior remains to be shown.

Ketamine: dissociative anesthetic and neuroplasticity stimulant

Ketamine is one of the best characterized dissociative anesthetic widely used in the clinic and veterinary medicine. There is increasing evidence for ketamine-induced rapid synaptic remodeling and changes in dendritic spine morphology with related functional alterations. Modifications of spine density and synaptic dynamics by ketamine are thought to contribute to cognitive impairments and

psychedelic symptoms associated with its intake, with precise mechanisms underlying these effects remaining a matter of debate and future studies (Ali et al. 2020; Moda-Sava et al. 2019). Unlike inhalational anesthetics, such as sevoflurane and isoflurane, ketamine is administered in a liquid form via intravenous, intraperitoneal, subcutaneous, or intramuscular injection. While ketamine is widely known for its mild analgesic effects, it is used in medicine primarily as a general anesthetic. Due to its growing abuse as a recreational drug with hallucinogenic and dissociative effects, with strong addictive potential, ketamine has been placed on the list of a controlled substances and is registered as a schedule III drug (Sassano-Higgins et al. 2016). At low concentrations, ketamine works predominantly as a non-competitive antagonist of NMDA glutamate receptors (Ogden and Traynelis 2011; Sleigh et al. 2014) (Fig. 1C, D). Lodge and Anis (1984) have pioneered in demonstrating the inhibitory effects of ketamine on the polysynaptic excitatory transmission in spinal cord circuits of the cat. The more recent work by this group showed that ketamine inhibits both, NMDAR-dependent short- and long-term synaptic plasticity (STP and LTP) in the hippocampus, effects that might be linked to changes in dendritic spine dynamics and contribute to its dissociative effects with impairments in the short term memory (Ingram et al. 2018). At a higher dose, ketamine can also modulate opioid, monoamine, purinergic, cholinergic, and adenosine mechanisms in the brain (Papich 2016; Persson 2010). Such a wide variety of effects mediated via multiple receptors is likely to contribute to a considerable variability of the ketamine-induced changes in dendritic spines morphology and plasticity in neuronal cultures, and in vivo in animal models as discussed below. The role of ketamine-induced dendritic spine remodeling in inducing the state of general anesthesia remains unclear and requires further research.

Both, acute and lasting effects of single or repeated ketamine exposure on dendritic spines has been shown by numerous reports. Dendritic spines of developing neurons emerge to be especially sensitive to ketamine, with a single time exposure of 5 DIV rat neuronal cultures causing a decrease in both, spine number and length (Jiang et al. 2018). Under these settings, inhibition of RhoA/ROCK signaling by Y27632 was reported to attenuate the effects of ketamine on spines, suggesting that ketamine-induced changes in dendritic spine morphology and synaptic plasticity involve Rho/ROCK mechanisms (Jiang et al. 2018). In PND 7 male rats, on the other hand, a single infusion of a sub-anesthetic dose of ketamine (10 mg/kg) stimulates the formation of short and thick mushroom-type spines, resulting in an overall higher spine density as compared to saline-injected controls (De Roo et al. 2009). These effects were accompanied by an increase in the diameter of dendritic spines, and have been suggested to be associated with enhanced expression of CRMP2 protein in rat cortical neurons (Zhang et al. 2020b). A report by De Roo et al. showed that 5 h ketamine anesthesia also increases the density of dendritic protrusions in the somatosensory cortex in PND 15 and PND 20 mice, with, however, no effects observed in the more advanced age group (PND 30 mice). Similarly, the total number of protrusions increased in the hippocampal CA1 pyramidal neurons but only in the PND 15 mice. Of note, the remaining spines showed reduced head width in PND 15 ketamine-treated mice in both, somatosensory cortex and hippocampal CA1 pyramidal neurons (De Roo et al. 2009). Another report presented data suggesting that repeated ketamine exposure of neonatal mice causes short-lived changes in dendritic spine dynamics and their extensive loss, with effects becoming negligible in more advanced age groups (Tan et al. 2009). In this study, animals were treated with ketamine from PND 8 to PND 12, with a notable decrease in spine density and increase in spine length observed in the CA1 region at PND 13, respectably; similar experiments in PND30 mice revealed no changes in spine characteristics (Tan et al. 2009; Yang et al. 2011; Al-Hasani and Bruchas 2011; Cao et al. 2010; Wei et al. 2008).

Effects of ketamine anesthesia were also investigated on neurons in the somatosensory and medial frontal cortices of mice, using longitudinal two-photon microscopy via cranial window implants (Yang et al. 2011; Phoumthipphavong et al. 2016). In Yang et al. study, different groups of mice were subjected to 1 h or 4 h anesthesia with a mixture of a varying dose of ketamine and xylazine. In line with results of earlier reports in juvenile mice, it was found that ketamine dose-dependently increases the density of filopodia in both 1 h and 4 h anesthetized groups, however, causing no change in the total number of dendritic spines. Importantly, the filopodia formation rates of non-anesthetized and anesthetized mice were comparable at 12 h after ketamine exposure, inferring the transient character of neuroplasticity changes. Of note, administration of a non-competitive NMDA receptor antagonist MK801 caused similar alterations in spine dynamics as ketamine, implying that transient increase in filopodia results from ketamine-induced blockage of NMDA receptors (Yang et al. 2011). A similar study of the effects of multiple ketamine exposures after PND14 in the primary motor cortex using transcranial two-photon microscopy in PND 30 mice did not reveal changes in the parameters of dendritic spines (Huang and Yang 2015). In the same vein, a follow-up report by Huang et al. found no effect of ketamine on baseline spine dynamic in the motor cortex in PND 30 mice after a single or repeated treatment at a younger age (PND 7–11). Notably, this study found that PND 30 and PND 60 mice triple injected by ketamine–xylazine at PND 7–11 exhibited a decrease in motor learning-induced spine formation, an effect that could be mitigated by CX546—an allosteric modulator of AMPA receptors, which was infused

with the mixture of ketamine–xylazine (Huang et al. 2016). These results differ from ketamine-induced changes in dendritic spines in medial frontal cortical neurons, where a single-time infusion of ketamine caused an increase in spine density over 2-week period, which resulted from the new spine formation, with spine elimination rate remaining unchanged (Fig. 2E). Importantly, a fraction of newly formed spines became stable, consistent with their developmental maturation and engagement in functional networks (Phoumthipphavong et al. 2016).

Finally, the relationship between ketamine anesthesia in utero and postnatal dendritic spine development with plasticity was also investigated. In gravid rats, exposure to 3 h ketamine on a gestational day 14 followed by the analysis of dendritic spines in PND 30 offspring revealed a lower density of dendritic spines in hippocampal pyramidal neurons (Li et al. 2017). In a follow-up report, Li et al. analyzed spine density and dynamics in rat fetuses exposed to ketamine over 3 h at gestation day 19, which caused an increase in specific markers for neuronal autophagy, oxidative stress, and apoptosis in the hippocampal region. Of note, it was found that midazolam, an anticonvulsive drug, and $GABA_A$ enhancer, administered 20 min before ketamine, mitigated the neurotoxic effects (Li et al. 2018a). A very recent study analyzed the effects of 3 h ketamine anesthesia at embryonic day 14 on activation of the Wnt/β-catenin pathway, known to be involved in dendritic spine plasticity (Ochs et al. 2015). The authors found decreased expression of mRNA of Wnt3a and Wnt7a as well as Cyclin D1, c-myc in PND 30 offspring (Zhang et al. 2020a). These changes were associated with a decline in p-GSK-3β levels in ketamine treated group.

Collectively, these findings highlight the potential role of the Wnt/β-catenin pathway in ketamine-induced neuroplasticity and in governing the developmental dynamics of dendritic spines, which may vary, depending on the dose of ketamine and the age of experimental animals.

Propofol: neuroplasticity induction by $GABA_A$ enhancers

Propofol is one of the most widely used general anesthetics in outpatients, intensive care, and surgery (Trapani et al. 2000; Zhao et al. 2018). Overwhelming data suggests that general anesthesia induced by propofol results from potentiation of GABAergic synaptic drive, mediated largely via $GABA_A$ receptors, and to a lesser extent via $GABA_B$ receptors (Ito et al. 1999; Schwieler et al. 2003; Yip et al. 2013). $GABA_A$ receptor is coupled to a chloride channel. Upon binding GABA, it becomes activated, causing rapid membrane hyperpolarization and hypo-excitability. $GABA_B$ receptor is, on the other hand, a member of G-protein linked receptor superfamily, enriched at presynaptic terminals, mediating inhibition of synaptic transmission via activation of outward IK^+ and suppression of inward ICa^{2+}. Enhancement of $GABA_A$-mediated Cl^- current by propofol has been suggested to involve dual mechanisms: (1) allosteric potentiation of GABA effects on receptors-channel complex, independently of receptor subunits, and (2) direct activation of $GABA_A$ receptor, acting on β_3 subunit (Jones et al. 1995; Yip et al. 2013) (Fig. 1E, F). It was shown that a single point mutation in the β_3 subunit is sufficient to abolish the effects of propofol on $GABA_A$ currents (Eaton et al. 2015; Quinlan et al. 1998). Upon binding to the $GABA_A$ receptor, propofol decreases the dissociation rate of GABA from the receptor-channel complex, thereby, increasing the duration of GABA-activated opening of the chloride channel. In addition to a high selectivity for GABA receptors, propofol was shown to acts also on other ionotropic receptors, including glycine, nicotine, and glutamate receptors (Fodale and Santamaria 2004; Orser et al. 1995; Trapani et al. 2000), as well as G-protein coupled metabotropic M1 muscarinic receptor, but with much higher IC_{50} (Murasaki et al. 2003).

Similar to other general anesthetics discussed so far, the exposure of neurons to propofol destabilizes dendritic spines and induces synaptic remodeling, which depends on the dose and duration of the treatment, and developmental stage of neurons. Briner et al. used single or repeated doses of propofol to generate brief or lasting (6 h long) anesthesia in young rats. It was found that while 6 h anesthesia affects neither the overall length of dendrites nor their branching, even brief exposure to propofol was sufficient to induce rapid and lasting changes in spine density in the medial prefrontal cortex. Indeed, both, short term and extended propofol anesthesia lowered the number of dendritic spines in layer V pyramidal cells at PND 5 and PND 10 (Briner et al. 2011). On the other hand, similar treatments of rats at PND 15, 20, or 30, caused an increase in dendritic spine density. Quantitative electron microscopy revealed that propofol-induced increase in spine density was accompanied by the rise in the number of synaptic connections. It is important to note that the effects of propofol depend on the morphology of dendritic spines, affecting selectively those with a head diameter of 0.3–0.4 µm at PND 10, but not those with larger head size. In contrast, the increase in spine density in PND 15 groups was primarily caused by a rise in the number of spines with smaller heads, i.e., thin spines. It is worth noting that up to 6 h propofol anesthesia did not cause changes in dendritic spine morphology when applied at PND 60 and PND 90, whereas the reduction in dendritic spines induced by PND 5 anesthesia persisted up to PND 90, suggesting that its effects on dendritic spine plasticity are limited to the critical period of neuronal development (Briner et al. 2011). Of note, prolonged exposure to propofol also caused an increase in

dendritic protrusions in the somatosensory cortex of PND 15 and PND 20 mice. In the hippocampal CA1 region, on the other hand, the number of dendritic spines was only increased in PND 15 mice, with no change observed in PND 20 mice. These findings are in agreement with the results of earlier reports indicating that the effects of propofol on dendritic spine plasticity are age-dependent (De Roo et al. 2009). Intriguingly, the same report showed that similar remodeling of dendritic spines in the somatosensory cortex and hippocampus can be induced by either anesthetics attenuating glutamatergic or enhancing GABAergic synaptic drive, implying that the induction of neuroplasticity with spine remodeling can be independent of specific mechanisms, and might present a non-specific compensatory response of neurons to an overall change in neuronal activity (De Roo et al. 2009).

A more recent report by Puskarjov et al. (2017) showed a reduction in spine density in layer II/III of the somatosensory cortex after propofol was administered at PND 10. This study tested the hypothesis that in developing neurons the neuroplasticity-inducing effects of propofol depend on transient upregulation of potassium–chloride (K–Cl) co-transporter (KCC2). Using in utero manipulation of KCC2 expression, the authors showed that propofol-induced decrease in dendritic spine density can be mitigated by upregulation of KCC2, signifying the key role of changes in KCC2 mediated chloride transport in age-dependence of the effects of propofol on dendritic spine plasticity (Puskarjov et al. 2017). Finally, Toni et al. investigated if developmental stage-dependent effects of propofol can be replicated in adult-born neurons in the mouse brain (Krzisch et al. 2013). Using eGFP labeling, authors investigated the impact of propofol on the density of dendritic spines and integration of adult-born neurons into existing circuits of the hippocampal dentate gyrus. It was found that propofol causes neither changes in dendritic spine density nor the formation of synaptic connections with granule neurons.

Taken together, these findings suggest that during development, propofol can induce significant changes in dendritic spine density and synapse formation in various cortical regions, thereby affecting neuronal connectivity throughout the critical phases of synaptogenesis. The developmental effects of propofol in the juvenile brain seem to be limited to selected groups of dendritic spines and depend on the upregulation of the KCC2 transporter, with other mechanisms also potentially contributing. Remodeling of existing spines by propofol during development and its incompetence to alter dendritic spine plasticity of adult-born neurons in mature brain imply differential mechanisms of neuroplasticity in adult-born neurons from those formed during prenatal development.

Opioids in general anesthesia and spine remodeling

One of the key priorities of anesthesiology is reaching the state of general anesthesia with keeping major homeostatic and physiological parameters relatively unchanged. Difficulties with attaining effective and readily manageable anesthesia using a single agent endorsed the application of a combination of drugs (Brown et al. 2018; Klimscha and Zimpfer 1998). In current medical and veterinary practice, while general anesthesia is typically induced by one substance, adjunct treatments are often applied to achieve better sedation. Due to potent anti-nociceptive effects and versatile use in postoperative pain management, opioids by far are the most commonly used supplements (Egan 2019). Rapid onset of addiction with high incidents of post-surgery abuse, however, not only urge to move away from the opioid use but call for research of their effects on neuronal biology and synaptic plasticity mechanisms. In general, the effects of opioids are mediated via five groups of receptors: delta (δ), kappa (κ), mu (μ), nociceptor, and zeta (ξ) (Snyder and Pasternak 2003; van Rijn et al. 2010; Waldhoer et al. 2004). These are G-protein coupled receptors (GPCR), which can modulate neuronal functions via two independent mechanisms: (1) stimulation of cAMP response element-binding protein (CREB) phosphorylation (Al-Hasani and Bruchas 2011; Cao et al. 2010; Wei et al. 2008), leading to activation of genes controlling neuronal plasticity, and (2) dissociation of the Gα and Gβγ subunits, with Gβγ acting on voltage-gated calcium channels (VGCC) and KIR3, inward rectifying K^+ channels, causing inhibition of Ca^{2+} currents and attenuation of synaptic transmission (Endo and Yawo 2000; Sesena et al. 2014), or membrane hyperpolarization with hypoexcitability (Matsui and Williams 2010; McFadzean 1988) (Fig. 1G, H).

There is considerable evidence suggesting that some of these effects can play a key role in addiction. Specifically, induced by morphine and heroin synaptic plasticity in medium spiny neurons of striatum and nucleus accumbens (NAc) has been proposed as a key in developing opioid dependence (Graziane et al. 2016; Hearing et al. 2016; Koo et al. 2014). Structural remodeling of dendrites induced by opioids, similar to halogenated ethers and dissociative NMDA receptor antagonists, has been reported to involve changes in actin dynamics of dendritic spines (Martin et al. 2019; Robinson et al. 2002; Spiga et al. 2005), an alteration observed also in several neuropsychiatric diseases. It is well known that physiological dynamics of actin are critical for stabilizing excitatory synapses and dendritic spines, which can be disrupted by opioids, leading to functional impairments (Martin et al. 2019). Using

targeted gene-transfer methods, Martin et al. showed that in NAc, overexpression of Drebin, which bundles actin filaments to maintain synaptic integrity, is sufficient to increase dendritic spine density and decrease drug-seeking behavior in experimental animals. This process is specific to medium spiny neurons, and depends on histone modifier HDAC2, as verified by inhibition of histone deacetylases (Martin et al. 2019). Analysis of the effects of acute and chronic (hours, days) exposure of primary cortical neurons to morphine, DAMGO, etorphine, and methadone in vitro, and in μ-receptor knock-out mouse models, has been carried out by Liao et al. (2005, 2007), to elucidate the mechanistic link between internalization of μ-opioid receptors and changes in dendritic spine morphology. By activating μ-receptor at glutamatergic synapses, morphine leads to the collapse of dendritic spines and decreases AMPA receptor expression. The fact that these effects were antagonized by a non-selective, competitive opioid receptor antagonist naloxone, and were absent in mice lacking μ-receptor supports the key role of the latter in morphine-induced plasticity of dendritic spines (Liao et al. 2005). Authors speculate that induced by the same mechanism internalization of μ-opioid receptors can regulate dendritic spine homeostasis under physiological conditions, with extended morphine-treatment resulting in spine loss. Of note, activated by DAMGO and etorphine internalization of μ-opioid receptors has had opposite effects, promoting the development of new spines (Liao et al. 2007). In the same study, methadone at low dose induced modest internalization of μ-opioid receptors, causing morphine-like remodeling of dendritic spines, while at higher concentrations, it induced robust internalization and caused a further increase in spine density. It was proposed that differences in the rate of μ-opioid receptor internalization could contribute to a range of effects of endogenous opioids on dendritic spines (Liao et al. 2007). Importantly, these findings imply that by regulating the constitutive activity and turnover of μ-receptor, endogenous opioids might contribute to the maintenance of normal structure and functions of dendritic spines. Whether structural changes in dendritic spines induced by opioids are development-dependent remains to be determined.

Geoffroy et al. (2019) investigated the effects of chronic low-dose morphine treatment (14 days) on medium spiny neurons of the core and shell of rat NAc. Analysis of spine morphology showed a selective increase in the density of thin spines and a decrease in stubby spines in the shell of morphine-treated rats. Because morphine is known to increase corticosterone activity, which could, in turn, mask its effects on dendritic spines, authors repeated these experiments in adrenalectomized rats. Removal of the effects of corticosteroids caused a significant increase of mushroom spines in the shell and stubby spines in the core after morphine treatment. The increase of thin spines in adrenalectomized rats was lower when compared to morphine-treated intact rats, with no change detected in stubby spines within the shell of NAc (Geoffroy et al. 2019). These findings agree with the results of another report investigating the impact of morphine in the post-traumatic stress disorder (PTSD) model (RaiseAbdullahi et al. 2019). Young–adult rats exposed to a single episode of stress have received a morphine injection at four different timepoints (0, 6, 12, 24 h). At 25d post-stress, morphine-treated groups showed increased dendritic spines, with the highest effects observed in the group injected with morphine 24 h after inducing a stress response. Of note, the stimulating effects of morphine on dendritic spine formation were antagonized by naloxone (RaiseAbdullahi et al. 2019). Another study in mice also using chronic (6-day) morphine treatment followed by abstinence for 2 months showed an increase in spine density in the frontal cortex and NAc, which persisted over 2 months. Changes in dendritic spine density in this report were accompanied by the upregulation of Shank 1 post-synaptic proteins (Pal and Das 2013). In stark conflict with NAc and frontal cortex, in the primary visual cortex of rats, chronic morphine treatment for 10 days caused a reduction in spine density and shortening the dendritic length of the layer III pyramidal cells (Li et al. 2007).

Taken together, the accumulating evidence suggests significant changes in morphology and density of dendritic spines induced by opioids, which similar to other general anesthetics, depends on anatomical location, dose, and duration of the treatment, with effects mediated via impairments of F-actin dynamics. Morphological changes induced by opioids are accompanied by alterations of key postsynaptic markers, inferring changes in glutamatergic synaptic transmission. Importantly, the emerging evidence shows also that endogenous opioids might regulate constitutive dynamics of dendritic spines and synaptic homeostasis in general, and, therefore, might contribute to the development of addiction response.

Summary and conclusions

Almost 200 years ago, the first successful use of general anesthesia for surgery has launched one of the greatest medical miracles, which enabled countless life-saving diagnostic and surgical interventions without pain and traumatic memories. Despite the ubiquitous use and intense research of action mechanisms, there is a lack of clarity as to how general anesthetics induce the reversible coma-like state. Extensive research has been carried out elucidating functional effects of general anesthetics at presynaptic terminals of central neurons, affecting synaptic vesicle dynamics and parameters of neurotransmitter release (Hemmings

et al. 2005; Perouansky and Hemmings 2003; Torturo et al. 2019; Baumgart et al. 2015). With progress in unraveling the mechanisms and effects of general anesthetics, the notion that the state of general anesthesia results from the potentiation of GABAergic or depression of glutamatergic activity appears as an oversimplification and is incomplete. Indeed, the more recent data pinpoints the involvement of far complex structural and molecular processes and mechanisms, and extended neural circuits in the induction of the state of transient coma, including changes in the activity of hypothalamic, midbrain, and brain stem modulator networks (Brown et al. 2010; Ching and Brown 2014). Although the recent demonstration of reversible activation of a selected group of neuroendocrine neurons in the supra-optic area by several general anesthetics (Jiang-Xie et al. 2019) offers a unifying framework for a common neural substrate for the effects of general anesthetics, it leaves unanswered a wide range of questions over their transient or lasting actions on structure and functions of cortical and hippocampal neurons, including changes in dynamics and plasticity of dendritic spines, with long-lasting neurocognitive and behavioral impact. Notwithstanding the multiplicity of primary targets and action mechanisms, general anesthetics seem to converge on a few shared processes which in one way or another destabilize dendritic spines and stimulate synaptic remodeling (Fig. 3). Whether these changes contribute towards the initiation and maintenance of the coma-like state or their neuro-behavioral after-effects, remains to be shown.

As discussed throughout this review, the most commonly used general anesthetics seem to be capable of stimulating rapid onset neuroplasticity, with, however, considerable variability of effects, dependent on the developmental stage of neurons, the dose of anesthetics, as well as specific anatomical regions in the brain. While there is considerable overlap in their effects as reviewed throughout this study, it should be stressed that they cannot be extended over all general anesthetic used to date in clinical and veterinary practice. Despite the agreement that destabilizing effects of anesthetics on dendritic spines are the highest in neurons undergoing synaptogenesis and pruning, recent data shows that general anesthetics can also destabilize established synaptic connections and spines in a mature brain, where the majority of spines have reached stability. In mice, for instance, by 1–2 months of age (puberty and early adulthood), approximately 50% of spines in the somatosensory cortex and over 70% in the visual cortex are stable, with 4–5 months of age, these numbers increasing to 70% and 90%, respectively (Trachtenberg et al. 2002; Zhou et al. 2012). The emerging data for stimulation of dendritic spine formation and synaptic remodeling by general anesthetics in the mature brain, thus, extend their effects beyond modulation of neurodevelopmental processes, onto many fundamental neurobiological mechanisms involved in synaptic stability and plasticity,

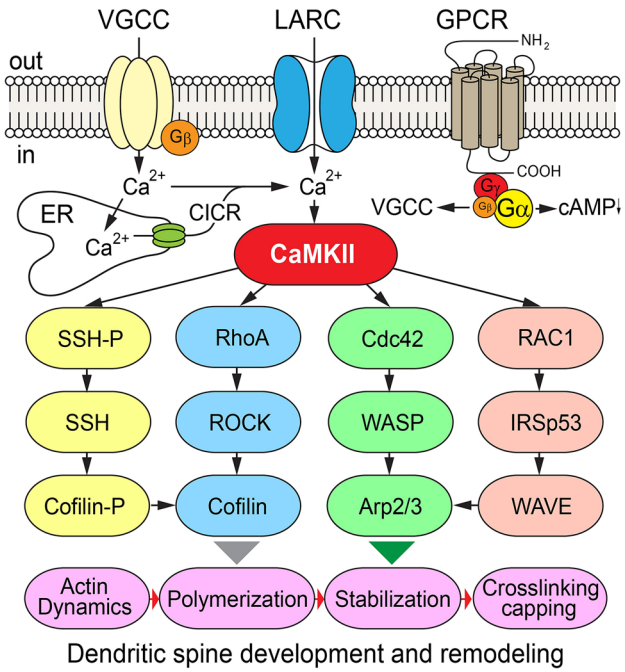

Fig. 3 Schematic representation of putative mechanisms and molecular pathways contributing to dendritic spine remodeling induced by general anesthetics. The majority of general anesthetics activate Ca^{2+} influx via stimulation of voltage-gated calcium channels (VGCC), ligand-activated receptor channels (LARC) or stimulation of G-protein coupled receptors (GPCR), with the downstream enhancement of VGCC. An increase of intracellular free Ca^{2+} can also cause Ca^{2+} induced Ca^{2+} release (CICR) from intracellular stores and primarily from the endoplasmic reticulum (ER). As a second messenger, Ca^{2+} binds and activates calmodulin, which acts as part of a Ca^{2+} signal transduction pathway by modifying its interactions with various target proteins, with the Ca^{2+}calmodulin-dependent kinase II (CaMKII) playing a key role in controlling molecular processes underlying the stability and dynamics of the cytoskeletal protein actin, governing the dynamics of dendritic spine remodeling and plasticity. This involves four major signaling pathways mediated via several members of the Rho family of small GTPase, PAK-phosphatases slingshot, Cdc42, WASP, IRSp53 proteins converging on two effectors—cofilin and Arp2/3 proteins. While the former, cofilin, when activated, causes severing of polymerizing actin with shrinkage of dendritic spines (grey arrowhead), Arp2/3 promotes the assembly of actin and branching (green arrowhead), with enlargement and stabilization of dendritic spines

with potentially lasting consequences for mental health and behavior.

It is important to note that in addition to implicit neurobiological and functional challenges, the destabilizing effects of general anesthetics on synapses and dendritic spines open tremendous opportunities for therapeutic intervention. However, these are likely to come with potential downsides as well, including neurotoxicity and degeneration of neurons and synapses, which are largely attributed to the activation of an array of apoptotic factors, increase in PTPB activity as well as excessive release of cytochrome c from

mitochondria, known also to cause impairments and loss of dendritic spines and synapses (Cheng et al. 2015; Erturk et al. 2014; Ovsepian et al. 2019; Liu et al. 2019). The fact that stable spines in the adult brain can be switched into a dynamic state by injury, neuropathic pain, as well as neurodegenerative and psychiatric conditions infers a possible compensatory role of enhanced spine plasticity. Likewise, antidepressant properties of dissociative anesthetics, such as ketamine, emerge to be related to remodeling of dendritic spines with restorative effects. Activation of dendritic spine remodeling by general anesthetics in a mature brain, therefore, upgrades the observations discussed throughout this review from the rank of discoveries satisfying general scientific curiosity to unveiling exciting and previously unrecognized translational opportunities. If successfully induced in selective groups of neurons and circuits, the elevated neuroplasticity could enable adjustments of synaptic functions and circuit dynamics, with potential benefits for clinical neurology and psychiatry.

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Declarations

Conflict of interest The authors have no conflict of interest to report.

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