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Introduction

As a consequence of modern medicine and healthier living, our population is aging beyond our current understanding of geriatric physiology. One inevitable fact society must face is the growing number of cases of age-related cognitive decline and neurodegenerative diseases. The costs of these conditions in terms of monetary value and in terms of human quality of life are unquantifiable. We have identified numerous genetic and lifestyle risk factors for development of Alzheimer’s disease (AD), yet penetration across populations is sporadic [1,2]. Thus, the actual mechanistic triggers or tipping points to initial the pathological cascades leading to AD or other forms of cognitive decline have remained elusive.

One of the most prominent risk factors for the development of late onset Alzheimer’s disease (LOAD) is the presence of heterozygous or homozygous ApoE4 alleles. ApoE and cholesterol have a complex inter-relationship in CNS. Astrocytes are the major producers of cholesterol and neurons are the major consumers, using the cholesterol transported by ApoE particles to build their elegant membrane structures and maintain the synaptic superhighway of information. Therefore, dysfunction in transport, uptake or trafficking of cholesterol may represent major contributing factors to the development of AD [3-5]. Along those lines of reasoning, other factors which potentially disrupt cholesterol biogenesis, synthesis and mechanisms of action, could exacerbate already damaged cells, leading to clinical pathology.

Voluntary general anesthetics (vGA) interact directly with cholesterol in the plasma membrane, through an undefined mechanism, and alter signal transduction cascades [6]. These effects can be considered acute or chronic [7] depending on the precise protein or receptor affected. However, the actual chemical structure of individual anesthetics may dictate these interactions. Isoflurane (ISO) and sevoflurane (SEVO) have been shown to have opposing effects compared to desflurane (DES), due to differences in the degradative byproduct trifluoroacetic acid or the shorter action time [8,9].

Postoperative Cognitive Dysfunction (POCD) is a well described clinical syndrome found in elderly patients following surgery, with duration and symptomology widely varying and found to be associated with use of inhaled anesthetics. Any combination of AD risk factors and common surgical procedures may be considered a triggering event in the long pathological decline leading to full-blown AD disease. This review will discuss: a) the implications of vGA exposure in the development of cognitive impairment, b) theories supporting vGA as an initiating trigger of AD pathology and c) identify some potential therapeutic targets to attenuate the consequences.

Genetic risk factors and volatile anesthetic interactions

The Alzheimer’s Association (Alz.org) currently estimates around 5.3 million AD patients in the US alone and the number will escalate as the percentage of people >65 years increases in the population. Bioinformatics, combined with robust genomic
analyses, have identified new cohorts of risk factors associated with development of sporadic Alzheimer’s disease (SAD) or LOAD. What we currently lack is an understanding of how the daily human experience and the molecular mechanisms of risk factors genes can synergize, leading to AD clinical manifestations. One significant environmental factor which has the potential to alter physiology both transiently and chronically, is the use of volatile anesthetics during surgical procedures [10]. Anesthetics such as ISO are commonly administered to all age populations and have been linked to early childhood cognitive issues [11] and elderly POCD [8]. Older adults represent a large percentage of surgical patients, with cardiovascular surgery and joint replacements common procedures requiring extensive use of anesthetics.

Physiological interactions at a molecular level between variant risk gene proteins and volatile anesthetics may likely determine the initiation of dementia and classical AD pathologies in any number of individuals. Familial Alzheimer’s disease (FAD) has a clear genetic component. Mutations and duplications of human amyloid precursor protein (APP) and the presenilins 1 and 2 (PSEN1; PSEN2) have been identified in large family cohorts of patients [12], but, these cases represent only a minor fraction of current clinical presentation. The etiology of the remaining cases is still an open debate. Newly identified AD risk variants, typically single nucleotide polymorphisms – SNPs – form interesting cluster patterns, ranging from cholesterol biology, intracellular trafficking to neuro inflammation [1,2,5,13–16]. Table 1 lists current information on genes with risk identified SNPs, cell type most affected and the cellular function if defined.

Precisely how these variant proteins can alter normal CNS homeostasis and result in AD related cognitive decline or how individuals with these genetic alleles avoid AD pathology are key questions. Until we have a significant pool of fully sequenced humans and a lifetime to study their particular nurturing and environmental interactions, the latter will remain unanswered and unobtainable. However, we have begun to unravel connections between AD risk factors and anesthetic exposure [10]. POCD has been clinically recognized and has clearly defined operational criteria [17]. How, when and where the transition or switch from simple POCD to the long pathway of synapse loss, neuronal damage and neuroinflammation occurs has been confounded by the use of transgenic AD mouse models, non-human CNS cell cultures and a lack of definitive long term human clinical studies.

Known volatile anesthetic effects

Volatile anesthetics interact directly with the lipid bilayer, modifying the local lipid and protein environment. Three theories have been proposed for the interactions of lipids and volatile anaesthetics: 1) direct binding to membrane proteins modifying their conformation and signaling activity; 2) binding to and weakening cholesterol and phospholipid associations which alters plasma membrane localized ion channel activity; and 3) binding specifically to lipid raft domains, modifying their lateral organization, interfering with many required protein–protein interaction in signal transduction pathways [18].

As the chemical structures of this class of anesthetics are diverse, the interactions with lipids and proteins are likely to differ and have differential effects. ISO has a weakening effect on cholesterol–phospholipid interaction in lipid raft domains, suggesting that ISO acts as a “replacement” or substitute for cholesterol [19,20]. Propofol (PFF), on the other hand, can interact with raft or non–raft domains and when PPF induced metabolic changes, after exposure, were compared to SEVO post–exposure, distinct differences in fatty acid or glucose metabolism, after exposure, were compared to SEVO post-exposure, distinct differences in fatty acid or glucose oxidation were seen. SEVO increases fatty acid transport and PPF increases pyruvate dehydrogenase activity without affecting GLUT4 [20].

Within the CNS, these volatile anesthetics can interact differentially within the membrane environment based on phospholipid composition and differentially with local areas of membrane specialization, such as the pre or postsynaptic zones or lipid rafts. vGA effects may also be temporally different throughout lifespans. We are now realizing the profound phenotypic differences between young glia and old glia in terms of priming, cytoskeletal integrity and mitochondrial function. The same can be said for neuronal phenotypes; young cells are highly plastic, while older neurons have a myriad of dysfunctional cell operations, such as autophagy [21].

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**Table 1: GWAS Identified AD risk factors.**

| Gene            | Cell Type       | Cellular Functions               |
|-----------------|-----------------|----------------------------------|
| HLA-DRB5-DRB1   | microglia       | innate immune system             |
| CLU             | microglia       | innate immune system; cholesterol regulation |
| CR1             | microglia       | innate immune system             |
| TREM2           | microglia       | innate immune system             |
| INPP5D          | microglia       | innate immune system             |
| MEF2C           | microglia, neurons | innate immune system; synaptic regulation |
| MS4A/MS4A6E     | microglia       | undefined                        |
| EPHA1           | microglia, neurons | innate immune system; synaptic regulation |
| ABCA7           | microglia       | cholesterol regulation           |
| TREM2           | microglia       | innate immune system             |
| CD33            | microglia       | innate immune system             |
| Neuronal dysfunction | Cellular Functions |
| PTK2B           | neurons         | cellular signaling               |
| NME8 locus      | neurons         | cytoskeletal regulation          |
| ZCWPW1 locus    | neurons         | axonal growth                    |
| CELF1 locus     | neurons, astrocytes | cytoskeletal regulation          |
| FERM2           | neurons         | cytoskeletal regulation          |
| CASP4           | neurons         | actin dynamics                   |
| SORL1           | neurons, astrocytes | cellular trafficking              |
| APOE4           | neurons, astrocytes | cholesterol regulation          |
| PICALM           | neurons, astrocytes | cellular trafficking           |
| PLD3            | neurons         | cellular signaling               |

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How volatile general anesthetics (vGA) can induce AD pathology

The hallmark pathology of AD has long been considered extracellular deposition of toxic Aβ – amyloid plaques – and/or the intracellular accumulation of oligomerized tau protein, known as neurofibrillary tangles (NFT), but this concept is currently challenged [22]. Still, the controversy rages on and recent data clearly suggests that these pathologies are probably symptomatic not causative [23]. vGAs have the capacity to increase Aβ processing [10,24,25]. Importantly, 2% ISO treatment for six hours drives β-secretase activity over α-secretase activity, increasing the Aβ42-toxic cleavage product, leading to aggregation over short time points [24,26]. When these cell culture studies are repeated in transgenic AD mouse models however, inconsistent data is found across compounds. In their 2011 review, Papoun et al, conclude that although it appears little differences in APP processing occurs in normal mice following vGA exposures, transgenic mice with known increased Aβ plaque formation are at increased risk for increased aberrant APP processing following exposures [27].

Tau protein is a small molecular weight microtubule-associated protein. When bound to polymerized microtubules in neuronal axons, tau acts as stabilizing struts to maintain the shape and flexibility of long axonal processes. When phosphorylated by GSK-3β, a major effector kinase in this pathway, tau falls off the microtubule and depolymerization can begin. Things go awry when this phosphorylation process become unregulated or the known phosphatase PP2A loses functionality – reviewed by [28]. Tau is continually phosphorylated on multiple serine and threonine residues – hyperphosphorylation – an initiating step in the pre-tangle stages of tau aggregation [29,30]. vGA can accelerate this process by increasing pS and pThr sites directly [31] or through inhibition of PP2A [27]. A principle side effect of vGA exposure during surgical procedures is significant drop in body temperature, hypothermia. The enzymatic activity of PP2A is directly tied to temperature, thus the lowered thermostat has been found to inhibit the dephosphorylation of tau by indirect means, abnormally increasing hyperphosphorylated tau [27,32,33]. SEVO induces increased p-tau/t-tau ratios via inhibition of mammalian target of rapamycin (mTOR); a pathway mediated by GSK-3β, indicating vGAs can directly influence intracellular kinase activities [34,35].

However, since both amyloid plaques and NFTs may take years to manifest and POCD is a more acute ramification of vGA exposure, other mechanisms are most likely in play. Zhang et al, have made a strong case that induction of neuroinflammation by vGA in older patients is the primary factor in the development of POCD [35]. Exposure to ISO or PPF in elderly rats increased IL-1β and increased expression of the microglial biomarker, Iba-1. Eight days post-surgery, the rats also displayed significantly longer target whole identification in a Barnes Maze learning and memory task. In addition, the rats showed reduced levels of freezing in fear conditioning behavioral tests. A second study directly compares adult and aged mouse cytokine expression and behavior three days post ISO exposure and finds elevated expression of canonical neuroinflammatory markers, such as IL-1β, TNF-α, IFN-γ, in the aged mice, along with impaired spatial learning memory [36]. Proposed models of AD pathology implicate neuroinflammation as a precipitating event in the development of disease [37,38], so these data represent a perplexing conundrum to clinicians. Do we risk exposing elderly patients to potentially harmful compounds and forgo life-saving care or do we recognize this as a significant clinical issue and begin to unravel the molecular causes to find a solution?

The search for an intervention when a mechanism is not yet fully elucidated: POCD to AD

Armed with the idea that neuroinflammation may be the underlying condition fueling POCD and ultimately AD, several groups have begun exploring novel anesthetic pre- or post-treatments to reduce the activation of microglia in the hippocampus and other brain regions, reviewed by [39]. Berberine is a natural isoquinoline alkaloid, with known anti-inflammatory properties, acting through suppression of the TLR-4/NF-κB signaling pathway. It can cross the BBB but has pleiotropic effects and very low bioavailability. A recent 2016 study has found that 10mg/kg intraperitoneal (IP) injections of berberine following 1.5% ISO exposure in a mouse model during laproscopic surgery, was enough to reduce expression of canonical inflammatory markers and prevent POCD in an accepted clinical animal model of POCD [40]. The antibiotic minocycline has become a CNS anti-inflammatory drug of choice with the ability to cross the BBB and to moderate microglial phenotypes. When mice were pre-treated with 45 mg/kg IP minocycline 12 hours prior to 1.5 – 2% ISO exposure, POCD associated behavioural changes and markers of neuroinflammation were reduced. More importantly, the neuroprotective cytokines, IL-4 and IL-10 were concomitantly upregulated [41].

Two other compounds are also being evaluated, which have indirect anti-inflammatory properties. The α2-adrenergic receptor agonist, dexmedetomidine, has CNS protective effects. When administered 30 minutes prior to vGA exposure at 15 – 25μg/kg IP, activation of the α2-adrenergic receptor significantly reduced the expression of neuroinflammatory markers [42]. However, another study from 2014 demonstrated non-hypothermic dependent hyperphosphorylation of tau when dexmedetomidine was used as a standalone GA [43], making this compound less attractive as an interventional therapy. By an intriguing and novel mechanism, deferoxamine shows protection against POCD in aged rats, by reducing the activation of microglia following vGA exposure. Microglial activation is preceded by a burst in oxidative phosphorylation, mediated by iron containing mitochondrial proteins. Pan, et al, theorized chelation of iron for six days prior to surgery could attenuate POCD symptoms, by preventing the metabolic burst required for full microglial activation [44]. They found that they were able to stave off microglia activation by oxidative stress mechanisms, using deferoxamine, and were also able to prevent the associated neurotoxicity and cognitive deficits.

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Two studies, published in early 2017, have intriguing data on other possible selective targets and pathways to mitigate POCD. The first study attenuates POCD associated neurodegeneration in aged mice with PPARγ activation using the agonist pioglitazone prior to ISO exposure. Antagonizing the receptor reverses the positive benefits of pre–treatment [45]. The already approved pioglitazone treatment for diabetes lowers blood sugar levels but has potential cardiac risks with long–term treatment. However, the suggestion that short term or acute administration prior to surgery can provide behavioural benefits makes this pathway an attractive target and well worth investigating in clinical trials. Since vGA exposure – SEVO in particular – increases p–tau/t–tau ratios and inhibits the autophagy pathway through mTOR [34,35], a compound that targets both p–tau generation and moderates autophagy pathways is another method worth investigating. The nutriceutical selenomethionine (Se–Met) has shown efficacy in the retention of cognitive function in mouse transgenic AD models [46]. This compound reduces p–tau by modulation of the key GSK–β3/PP2A pathway and concomitantly activates the AMPK–mTOR axis to enhance autophagic clearance. Animals were treated for 3-month, not short term, and the effects were modest but as proof of concept studies, they establish the basis to screen for other similar compounds with faster kinetics and pharmacodynamics.

Volatile anesthetics and the “Two–Hit” concept of AD

Although many studies have established the vGAs have the ability to mimic or induce AD phenotypes, the definitive connection between development of POCD and the long road to AD dementia is unclear. Current thinking about the trek down the cyclic path towards AD is muddied by the failures of promising anti–Aβ therapies (solanezumab – Eli Lilly, 2016). Newly emerging hypotheses revolve around the concept of multiple small causative risk factors combining together to create a favorable environment for loss of synapses and neuronal death to occur. The Two–Hit hypothesis was initially proposed in 2004 by Zhu, et al, [47] and refined by Zlokovic [48] in 2011. The relevance of this concept has been greatly strengthened with genome wide association studies (GWAS) variant identifications [1,2]. In theory, patients can have heterozygous variant risk genes across specific CNS cell types. In a homeostatic situation, these individual risk factors play a marginal role in brain dysfunction. However, if contributing second factors, like a concussion (TBI), aberrant glucose utilization, metabolic cholesterol dysfunction, or exposure to vGA, are added, the balance can shift to pathogenic pathways.

Whether exposure to vGA is an accelerating factor in people predisposed to AD by eliciting POCD through neuroinflammatory routes or by directly inducing initial Aβ or p–tau mechanisms needs more experimental data. One idea worth exploring is the use of CRISPR/Cas9 genome–edited human induced pluripotent (hiPSC) stem cells to directly model the effects of vGA on wild–type versus genetic variants of each CNS cell type [49–52]. (Figure 1) Use of relevant human cells instead of transgenic animal models provides a more accurate

**Conclusion**

Considering our reliance on volatile general anesthetics in modern medicine, it is surprising how little we actually understand about the immediate and long–term consequences of exposures. Clearly these complex compounds interact on a molecular level with cholesterol in cell membranes and with many genetic AD risk factor variants clustered in cholesterol metabolic pathways, much more precise information about is needed to understand the vGA effects on variant human neurons. The same can be reiterated about microglial function and phenotype. Newly discovered variants specific for these cells are clustered around phagocytosis and markers of activation. Whether vGA can destabilize already primed variant microglia, to elicit enhanced neuroinflammation, is a key question that needs further study.

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**Figure 1:** Schematic representation of genome-editing using CRISPR/Cas9 to model the effects of vGA on differentiated neurons and microglia. hiPSC cells are grown and target genes editing from WT to AD risk factor variants. Then the edited cells are differentiated into specific cell types. Once differentiation is confirmed, the cells are exposed to vGAs and readouts for AD pathology and neuroinflammation are assessed.
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