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MOLECULAR BASIS OF IRON-INDUCED OXIDATIVE STRESS IN THE HONEYBEE BRAIN: A POTENTIAL MODEL SYSTEM OF OLFACTORY DYSFUNCTION IN NEUROLOGICAL DISEASES

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21.1 INTRODUCTION

It is well known that all living organisms are exposed to environmental stress. Many organisms are exposed to harsh environmental conditions such as extreme temperatures (freezing and heating), anoxia, desiccation, cross-tolerance, and oxidative stress, which may impair the operation of vital neuronal circuits and put animals in danger before these conditions directly cause cell and tissue death. Oxidative stress is defined as a condition that is produced by a disturbance in the cellular prooxidant-to-antioxidant ratio [1]. Reactive oxygen species (ROS) is a collective term that includes both oxygen radicals (such as superoxide, hydroxyl, peroxyl, and hydroperoxyl radicals) and nonradical oxidizing agents such as hydrogen peroxide (H_2O_2), hypochlorous acid, and ozone that can be converted into radicals. ROS are mainly produced by the mitochondrial electron transport chain and oxidation of polyunsaturated fatty acid (PUFA) [2].

In eukaryotic systems, reduction of molecular oxygen by one electron yields superoxide radical that has limited reactivity with some proteins but is not reactive with lipids or DNA (Fig. 21.1). Under the influence of superoxide dismutase (SOD), H_2O_2 is formed by the addition of 1 e^- and 2 H^+. H_2O_2 is not a free radical because it does not have an unpaired electron, but it is an effective nonradical oxidizing agent for many biological molecules because reduction of H_2O_2 yields hydroxyl radicals ($OH^\bullet$) [2]. Both superoxide ($O_2^{\bullet -}$) and hydroxyl ($OH^\bullet$) radicals are continuously generated during oxidative metabolism in biological systems. $O_2^{\bullet -}$ reacts with some proteins; however, $OH^\bullet$ radicals react with carbohydrates, proteins, lipids, and DNA (Fig. 21.1).

The level of oxidative stress is determined by the balance between the rate of induction of oxidative damage (depending on how fast ROS are generated) and the rate of efficient repair and/or removal processes (endogenous defense system including levels of repair enzymes and antioxidants) for such damage (Fig. 21.2). In response to low levels of ROS, the nuclear factor-erythroid-2-related factor 2 (Nrf2) translocates to the nucleus and regulates expression of genes involved in cell survival. At higher ROS concentrations, reaction between ROS and proteins or unsaturated lipids in the plasma membrane leads to a chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation, resulting in reduction of membrane fluidity and decreased activity of membrane-bound enzymes, ion channels, and receptors [3]. Furthermore, high ROS level prevents translocation of Nrf2 to the nucleus but stimulates nuclear factor-κB (NF-κB), a

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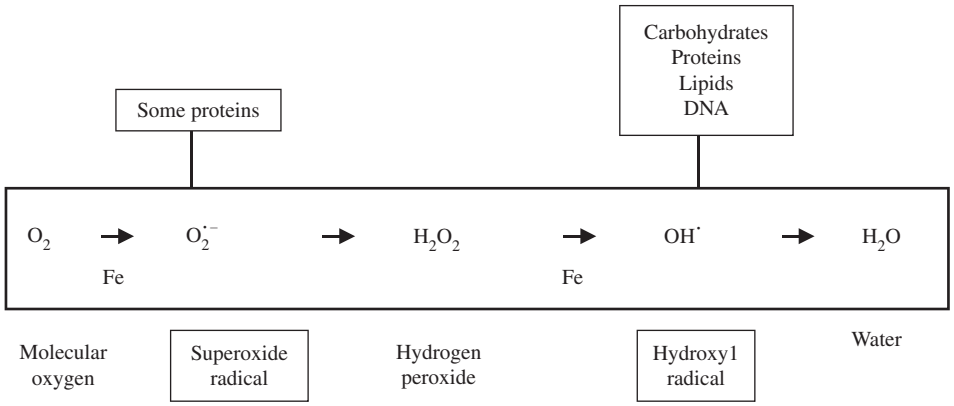

Fig. 21.1 Iron-induced reactive oxygen species (ROS) formation.

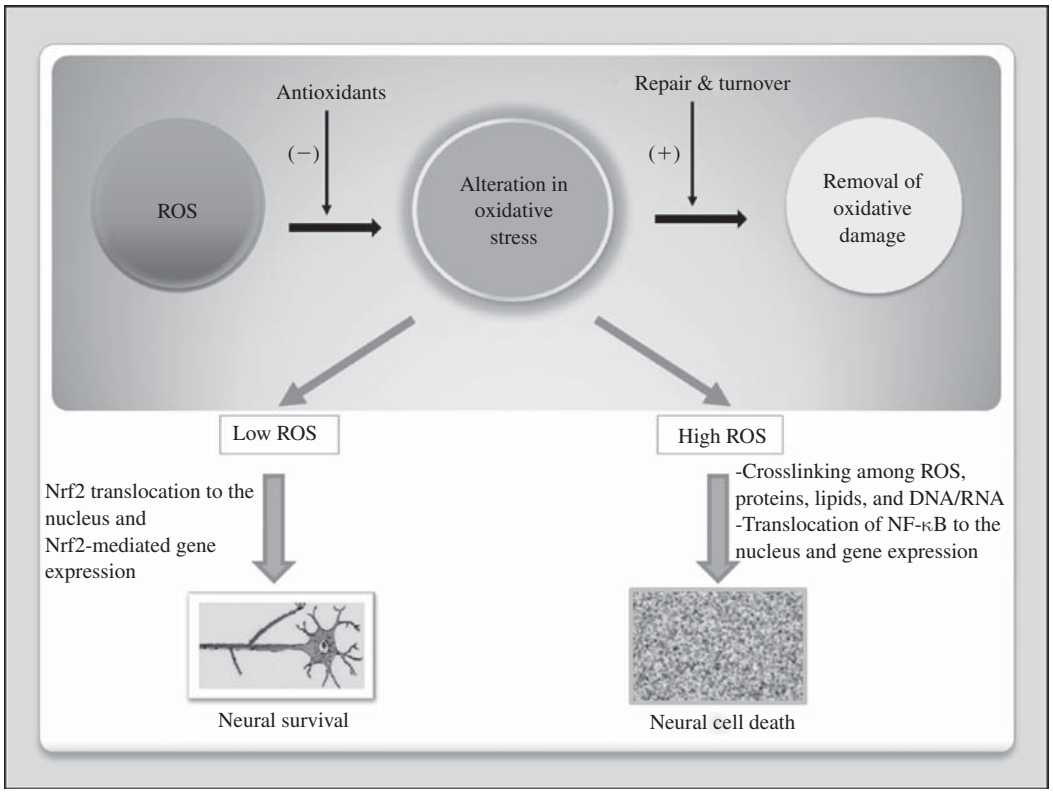

Fig. 21.2 Connection between reactive oxygen species (ROS)-mediated oxidative stress and abnormal functions. Increase in oxidant level, high ROS, decrease in antioxidants, and failure in repair and turnover result in functional abnormalities. Repair and turnover of oxidative damage, the so-called cellular defense mechanism, include DNA excision, resynthesis, and rejoining of DNA strands; repair of oxidized methionine residues in proteins; and normal membrane turnover releasing damaged lipids. Low ROS allow the translocation of nuclear factor-erythroid-2-related factor 2 (Nrf2) to the nucleus to regulate the expression of surviving genes for neural survival, whereas high ROS prevents translocation of Nrf2 to the nucleus. The cross-linking of high ROS occurs with proteins, lipids, DNA/RNA, which promotes neurodegeneration. *(See color insert.)*

transcription factor that, after translocating into the nucleus, induces expression of many genes involved in inflammation and oxidative stress and therefore promoting cell death (Fig. 21.2) [3]. The imbalance between ROS production and cellular defense mechanism is also implicated in destruction of neural cells in a wide variety of pathological conditions such as abnormal functions, aging, and diseases.

Iron, a transition metal ion, plays a crucial role in oxygen transport. Iron is a basic requirement for electron transport and cellular respiration and serves as a key element in most of the cytochrome enzymes involved in the oxidative phosphorylation of the Krebs cycle. Its overloading is potentially deleterious because of its involvement with lipid peroxidation in biological systems. Iron can induce generation of ROS through reduction of H_2O_2 to $OH^\bullet$ via the iron-catalyzed Haber–Weiss/Fenton reactions [4, 5]:

Haber–Weiss reaction:

$$H_2O_2 + OH^\bullet \rightarrow H_2O + O_2^{\bullet -} + H^+ \quad (1)$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^- + OH^\bullet \quad (2)$$

The Haber–Weiss cycle consists of the above two reactions. The Haber–Weiss reaction can form the hydroxyl radical ($OH^\bullet$) in an interaction between superoxide radical ($O_2^{\bullet -}$) and H_2O_2 in the presence of ferrous iron (Fe^{2+}) or ferric iron (Fe^{3+}).

Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\bullet + OH^\bullet \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^\bullet OHH + H^+ \quad (2)$$

Fe^{2+} is oxidized by H_2O_2 to Fe^{3+} and produces the $OH^\bullet$ and a hydroxyl anion in the Fenton reaction. Fe^{3+} is reduced back to Fe^{2+}, a peroxide radical (${}^\bullet OHH$), and a proton. The $OH^\bullet$ is highly reactive. It can abstract a hydrogen atom from PUFA to initiate lipid peroxidation [6]. Increased Fe^{2+} directly initiates additional lipid peroxidation with accumulated lipid hydroperoxides, resulting in changes in membrane structure and functional damage [6]. The $OH^\bullet$ attacks and damages every category of macromolecules such as lipids, proteins, carbohydrates, and DNA [7].

Brain is highly susceptible to oxidative damage. High rate of oxygen consumption, high lipid content, and relative scarcity in antioxidant enzymes compared to other tissues may account for the vulnerability in brain [8]. Several studies have demonstrated altered iron metabolism participating in the generation of ROS, resulting in marked increase in protein oxidation [9, 10], lipid peroxidation [11, 12], and DNA/RNA oxidation [13, 14] in the Alzheimer disease (AD) brain. Because of the excess of ferrous iron, oxidative stress has been shown to play a significant role in neurotoxicity associated with a variety of neurodegenerative and neuropsychiatric diseases (Table 21.1), affecting olfactory neuroepithelial cells at an early stage [15–33]. Increased oxidative stress is a prominent feature of vulnerable neurons in AD. Oxidative damage is shown to be increased in olfactory epithelium in biopsy specimens of AD [15]. ROS-mediated oxidative damage is markedly increased in olfactory epithelium from biopsy specimens of AD [15]. PAN-811 (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, or Triapine), a novel neuroprotectant, not only effectively suppresses intracellular ROS accumulation but also reduces ROS-mediated oxidative damage in both AD-derived and age-matched olfactory neuroepithelial cells [16]. It has also been shown that ROS-mediated olfactory deficit may be a characteristic feature of several neurological disorders including AD [15–17, 23], idiopathic Parkinson disease (PD), parkinsonism-dementia complex (PDC) of Guam [19, 20, 25], Huntington disease (HD) [23, 24], Guamanian amyotrophic lateral sclerosis (G-ALS), several forms of dementia [25, 30, 31], multiple sclerosis (MS) [27], schizophrenia [28, 29], idiopathic REM sleep behavior disorder [31], tauopathies [32], and open-angle glaucoma [33], supporting the view that there is a link among ROS, olfactory dysfunction, and neurodegenerative and neuropsychiatric diseases (Table 21.1).

TABLE 21.1 Olfactory dysfunction frequently observed in patients suffering from brain diseases

Disease	Reference
Alzheimer disease	14–16
Alzheimer disease, Idiopathic Parkinson disease, parkinsonism-dementia complex of Guam	14–16, 18–21, 24, 29, 30
Huntington disease	22, 23
Amyotrophic lateral sclerosis	24, 25
Multiple sclerosis	26
Neuropsychiatric disorders	17
Schizophrenia	27, 28
Idiopathic REM sleep behavior disorder	30
Tauopathies	31
Open-angle glaucoma	32

Very little information is available on ROS-mediated oxidative stress in the insect brain. Although honeybees are a well-known model system for behavioral studies for olfactory learning and memory because they can be conditioned to respond with feeding movements of the mouthparts (proboscis) to a variety of floral odors, which is called proboscis extension reflex (PER) conditioning [34–37], only a few studies have been attempted to demonstrate a correlation between ROS-mediated oxidative stress in the brain and its effect on the learning behavior in individual restrained honeybees [38–40].

The purpose of the present overview is to discuss our iron-induced oxidative stress model system in honeybee *Apis mellifera* brain, created by injecting ferrous ammonium citrate (FAC) in the antennal lobes, which results in impairment of olfactory learning and memory [40] suggesting that oxidative stress plays a major role in

olfactory dysfunction. Olfactory deficits have been reported in many neurological disorders (Table 21.1). A similar mechanism has been proposed for olfactory abnormalities in patients of AD and PD. Because of the similarities in cellular and molecular processes that govern neuronal plasticity in humans and honeybees [38], the author proposes that the honeybee can be used as a potential and relatively simple model system for understanding human olfactory dysfunction in neurological diseases.

21.2 A COMPARISION BETWEEN GENERAL PHYSIOLOGY OF OLFACTORY PROCESSING IN HONEYBEES AND HUMANS

The honeybee *Apis mellifera* olfactory system is well adapted to detect and discriminate a diverse array of odors [41]. The perceptual qualities of odors vary depending on several factors such as carbon chain length in the drug, shape, functional group, as well as concentration [42–49]. The honeybee olfactory system possesses olfactory sensory neurons inside the cuticle-covered sensillae along the antennae, which are equivalent to olfactory epithelia within the nasal cavity in vertebrates [38, 50]. The olfactory receptors are located on the olfactory sensory neurons in antennae. The recognition and discrimination of odor molecules starts at the antenna via binding of odor with olfactory receptor. From the antenna, information is carried directly to the antennal lobe via axons of olfactory sensory neurons. The antennal lobe is the structural and functional analog of the olfactory bulb in vertebrates that is subdivided into identified glomeruli. It processes incoming signals from broadly tuned olfactory sensory neurons [51, 52]. The axons of olfactory sensory neurons converge on two types of antennal lobe neurons: (1) local interneurons communicate within the antennal lobe, and (2) outgoing projection neurons mediate the signal information from the antennal lobe to the protocerebrum [53]. The synaptic contact between olfactory sensory neurons, local interneurons, and outgoing projection neurons takes place within the glomeruli arranged in a single layer around the antennal lobe [54, 55]. The glomerular layer in the antennal lobe probably contains the summarized representation of the receptor types activated by a given odorant. Thus olfactory information received from the antennae is processed in the antennal lobe and relayed to the mushroom body and the lateral protocerebrum via projection neurons, leaving the antennal lobe in three different antennocerebral tracts. The small mediolateral antenno-cerebral tract contains pluriglomerular cells, whereas lateral and median antenno-cerebral tracts contain axons of uniglomerular projection neurons [55, 56]. A synaptic response to focal electrical stimulation recorded in the mushroom body of the honeybee brain suggests involvement of the mushroom body in memory consolidation [57]. The synaptic plasticity exhibited in the mushroom body of the honeybee brain is similar to the synaptic plasticity occurring in the mammalian hippocampus [58]. Neuroplasticity in the honeybee brain, existing in the α lobe of the mushroom body, contributes to the associative long-term potentiation linked with olfactory learning and memory [59, 60]. This is in agreement with findings in *Drosophila* that all forms of olfactory learning (such as aversive, appetitive, and extinction) confined to the output sites of the mushroom body [61]. Moreover, GABAergic inhibitory interneurons in the antennal lobe of the honeybee brain modulate the overall activity and compue olfactory information by forming an odor-specific topographic map, relaying information from the antennal lobe to other brain regions via projection neurons [62–64].

Similar to the antennal lobe in honeybees, each glomerulus of the olfactory bulb in humans contains the axons of several thousands of olfactory sensory neurons, each expressing the same odorant receptor and the dendrites of mitral and tufted cells, which are the main input and output neurons of the olfactory bulb [65]. These neurons are activated by olfactory sensory neurons, but odorant information is further processed by lateral and feedback inhibitory pathways via activity of inhibitory interneurons, periglomerular cells, and granule cells [64–66]. During air inhalation, many volatile molecules reach the nasal cavity and interact with the odorant receptors located on the cilia of olfactory sensory neurons in the olfactory epithelium, eliciting an electrical signal that is transmitted to the second-order neurons in the olfactory bulb, which is then projected to pyramidal neurons in the olfactory cortex and to other brain regions such as thalamus and neocortex of the brain [38, 65]. Thus it is suggested that the peripheral olfactory systems involved in sensory transduction and early synaptic processing in the antenna and the antennal lobe of honeybees show a vast array of similarities to the olfactory epithelium and the olfactory bulb of humans [38, 68, 69].

21.3 OLFACTORY NEURONAL NETWORK IN HONEYBEES

In honeybees, the olfactory system is comprised of three major components: (1) antennae, (2) antennal lobes, and (3) mushroom bodies [38]. Honeybees use antennae to detect odors by making use of olfactory receptors that are located on olfactory sensory neurons in the antennae. Olfactory sensory neurons project their axons into the antennal lobes. The pathway involving olfactory sensory neurons is called the conditioned stimulus (CS) pathway, where CS represents an odor. Honeybees have

taste receptors located on olfactory sensory neurons in the antennae that project their axons into the antennal lobes. This pathway is called the unconditioned stimulus (US) pathway, where US represents nectar or sucrose. The primary sensory afferents (CS and US pathways) converge into the glomeruli of the antennal lobe.

In the honeybee *Apis mellifera*, an identified ventral unpaired medial cell (VUMmx1; an octopaminergic neuron) produces an associative link between US and CS pathways in the antennal lobe. The VUM interneuron releases octopamine into most if not all glomeruli of the antennal lobe innervating antennal lobe, mushroom body, and lateral protocerebrum. Electrical stimulation of VUM neuron substitutes for the unconditioned stimulus (sucrose) in an associative olfactory learning paradigm, supporting its role in olfactory learning and memory [70]. Local injection of octopamine into defined areas of brain increases honeybee's learning ability as well as recall, suggesting that octopamine is involved in regulation of learning and memory [71]. Actually, exactly how VUM interneuron couples the CS and US pathways is not clear except that this neuron has a reinforcing property. The convergence of two types of stimuli suggests that the antennal lobe is a site where part of olfactory memory consolidates [72]. The release of octopamine from VUM neuron or acetylcholine from olfactory sensory neuron in the synaptic cleft may induce GABA release from GABAergic local interneuron in the antennal lobe [38]. GABA-mediated inhibitory response is considered to be important for shaping and tuning the output from the antennal lobe [73].

21.4 ROS, OLFACTORY DYSFUNCTION, AND AGING

Increased oxidative stress is a prominent and early feature of aging and neurodegenerative diseases; therefore, oxidative damage has been considered an important factor in the progression of pathological and nonpathological age-related functional declines including olfaction. Again, this harmful condition occurs when there is either an excess of free radicals or a decrease in antioxidant levels, resulting in variety of changes in cells and tissues. Free radicals cause oxidative damage by attacking PUFA in neural cell membranes and generate metabolic waste products that interfere with cell-cell communication, damaging cellular proteins, lipids, and DNA, lowering energy levels and vitally impeding biochemical processes. The source of the devastating actions of ROS is mainly the oxygen molecule's unpaired electron, which makes it unstable and electrically charged [74]. Although sufficient ROS accumulation makes an organ system unable to function, endogenous defense mechanisms can bring ROS levels back down to a normal level consistent with organ function. Increased levels of oxidative stress markers have been observed in cultured human olfactory neurons in AD patients compared to control subjects [75]. Oxidative stress in the aging murine olfactory bulb has shown significant changes in steady-state levels in olfactory bulbs of 20- versus 1.5-month-old mice, demonstrating greater carbonylation and nitration of specific proteins with aging [76]. Nitric oxide (NO) and derived nitrogen species in CNS may interact with catecholamines, thus modifying not only NO regulatory actions but also producing oxidants and free radicals that are likely to trigger toxic effects. Peroxynitrite, a potent oxidizing agent formed by the interaction between superoxide anion and nitric oxide, produces nitration of tyrosine groups present in the proteins and inactivates several enzymes [77]. Peroxynitrite appears to be involved in the pathophysiology of many neurodegenerative diseases. Increased oxidative stress in olfactory epithelium of AD patients causes modification in olfactory receptor proteins, resulting in olfactory impairment [78]. Moreover, abnormal processing of the proteins in AD supports contribution of RNA oxidation [14]. In humans, olfactory function deteriorates progressively with increasing age, even in the absence of overt medical problems [79]. Similarly, a marked deficit has been observed in olfactory learning and recall in healthy caged aged honeybee workers (30 days old) compared to young workers (4 days old), indicating that olfactory learning and memory processing in the honeybee brain appear to be vulnerable to aging [38]. Collective evidence supports the notion that ROS-mediated oxidative stress may have a strong impact on olfactory dysfunction with aging.

21.5 IRON INDUCES OXIDATIVE STRESS IN THE HONEYBEE BRAIN

Iron, an important ion used for biological electron transfer in aerobic systems, also exhibits a chemical dark side with side reactions that form ROS, resulting in oxidative stress. ROS-mediated lipid peroxidation has been suggested to be an important factor in posttraumatic neuronal degeneration [80]. Addition of FAC allows evaluation of a quick onset of oxidative stress that is free from other conditions, such as neuroinflammation, which are produced by chronic levels of iron injury during neurodegeneration [80, 81]. By applying in vivo injections of FAC into the antennal lobes of the honeybee brain to produce $OH^{\bullet}$ radical, and monitoring the subject's responses to odorants by proboscis extension reflex conditioning assay, we have examined whether oxidative stress can be induced into the antennal lobes of the honeybee brain and whether or not ROS-associated alterations in brain can modulate olfactory

response of honeybees [40]. We have used different concentrations of FAC in each antennal lobe to evaluate its dose-dependent response and showed that the level of acquisition response in FAC-treated groups (0.5 mM to 5 mM range) significantly decreases with increasing dose (Fig. 21.3A), suggesting that iron interferes with olfactory learning in a dose-dependent manner. Testing of subjects in each group with conditioned odor (C), molecularly similar odor (S), and molecularly dissimilar odor (D) for retention and generalization responses show that iron interferes with retention in a dose-dependent manner but impairs generalization gradient at all FAC concentrations tested (Fig. 21.3B).

Reduced glutathione (GSH), an intracellular antioxidant, is known to prevent the oxidation of protein sulfhydryl groups and to help in maintaining cellular thiols in reduced state (-SH) [82]. To evaluate the reversal of FAC-mediated inhibitory effect on olfactory learning and memory, we injected GSH in each antennal lobe before injecting FAC, with an assumption that presence of excess thiol compound in the brain may efficiently protect honeybees against toxic effects of ROS on olfactory learning and memory. We observed that the FAC-mediated inhibitory effect on acquisition response is fully reversed in presence of GSH (Fig. 21.4A). Pretreatment of antennal lobes with GSH before injection of FAC also results in complete reversal of iron-mediated inhibition of retention response as well as showing a characteristic generalization gradient (Fig. 21.4B). This suggests that increased concentration of antioxidant forms an important component of the defense by reversing the oxidative stress created by free radicals [40].

Several novel brain-permeant and multifunctional ion chelators (such as HLA20, M30, VK-28, and M32) have been tested for their ability to inhibit iron-dependent lipid peroxidation in rat brain homogenates [83]. The brain-permeant iron chelator VK-28 has been shown to prevent *N*-methyl-4-phenyl-1,2,3,6-tertahydropyridine (MPTP)-induced neurotoxicity in mice [84] and 6-hydroxydopamine (6-OHDA) lesion in rats [85], without showing

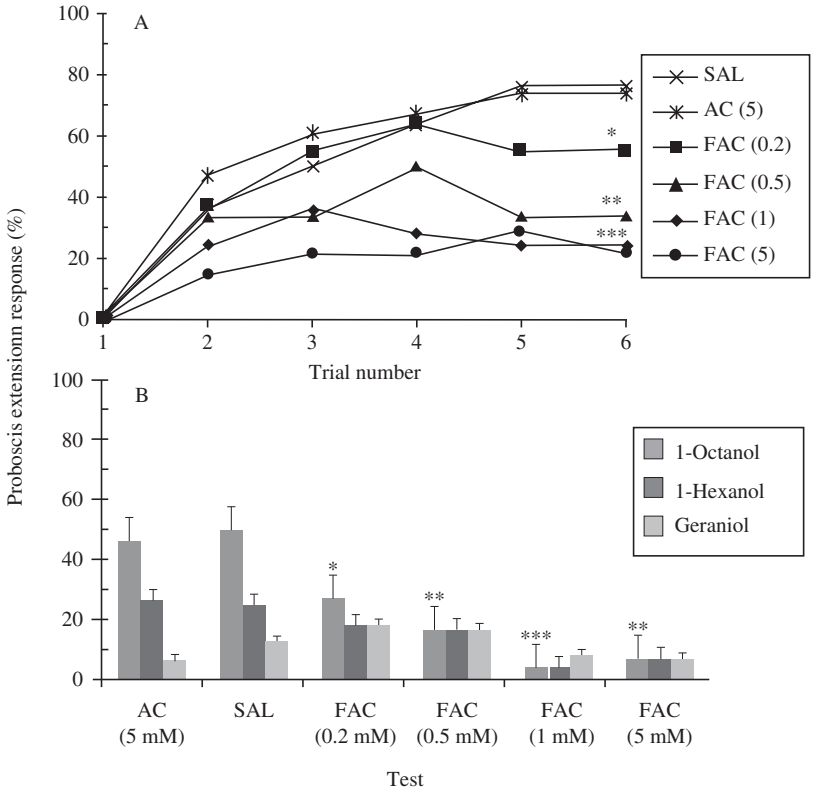

Fig. 21.3 Dose-response effect of ferrous ammonium citrate (FAC) on olfactory learning and memory. (A) Acquisition of the conditioned odor C: In this experiment, honeybees received 4 nl of saline (SAL), ammonium citrate (AC), or FAC at 0.2 mM, 0.5 mM, 1 mM, and 5 mM in each antennal lobe. Twenty-four hours later, subjects in each group were conditioned with 1-octanol (odor C). (B) Test with odors C, S, D: Ninety minutes after conditioning, subjects in each group were tested with C, molecularly similar (S), and molecularly dissimilar (D) odors in randomized order. *ns*, Not significantly different from controls (SAL, 5 mM AC). Asterisks indicate significant differences of respective points from control group: $*P = 0.05$, $**P = 0.005, 0.001$, $***P = 0.0001$). This figure is modified from reference [40]. *(See color insert.)*

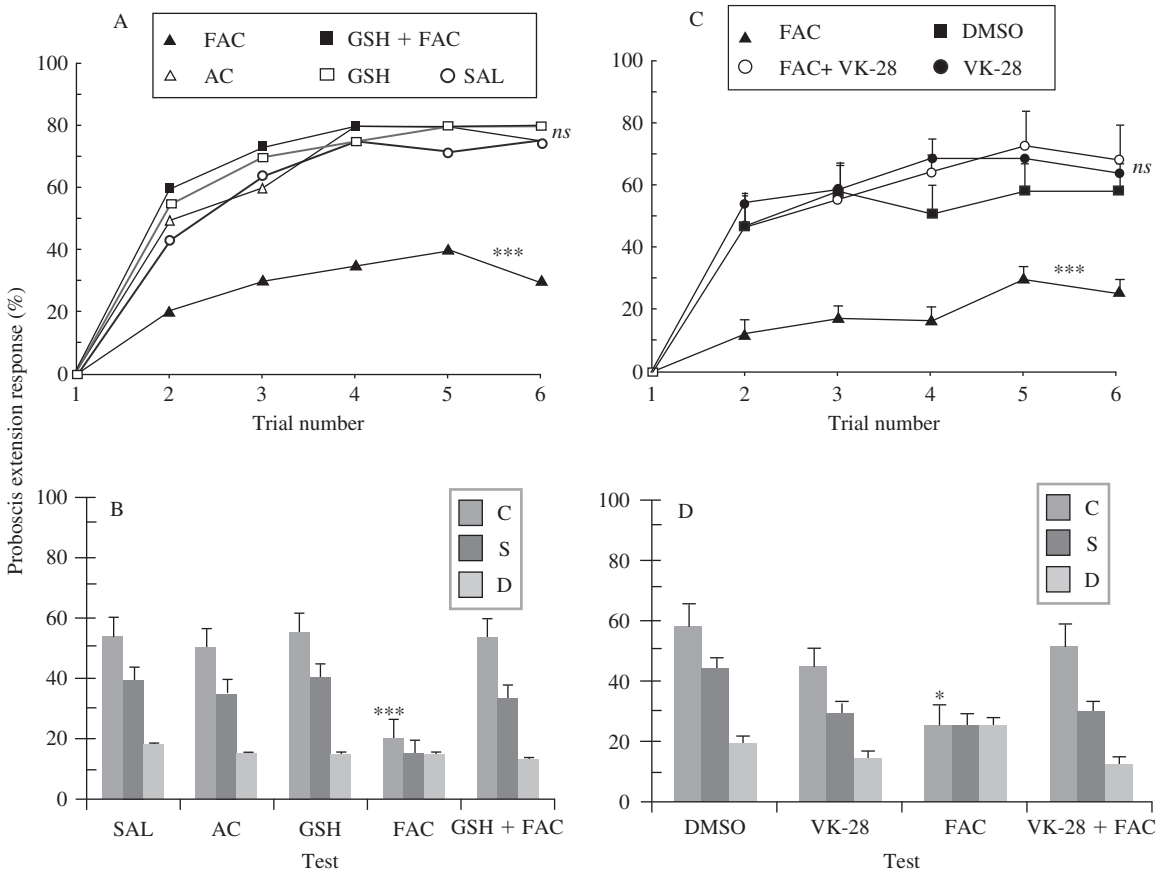

Fig. 21.4 Glutathione and VK-28 can reverse ferrous ammonium citrate (FAC)-mediated oxidative stress. (A) Acquisition of the conditioned odor C: In this experiment, subjects received 4 nl of either saline (SAL) or 2.5 mM reduced glutathione (GSH) in each antennal lobe. Two hours later, 4 nl of 500 μmol/l ammonium citrate (AC) or 500 μmol/l FAC were injected in each antennal lobe. Twenty-four hours later, subjects were conditioned with 1-octanol. (B) Test with odors C, S, D: Ninety minutes after conditioning, subjects in each group were tested with different odors (1-octanol, C; 1-hexanol, S; and geraniol, D) in randomized order. (C) Acquisition of the conditioned odor C: In this experiment, subjects received 4 nl of either dimethyl sulfoxide (DMSO) or VK-28 or DMSO + VK-28 in each antennal lobe 30 min before injection of FAC. Twenty-four hours later, subjects were conditioned with 1-octanol. (D) Test with odors C, S, D: Ninety minutes after conditioning, subjects in each group were tested with C, S, and D odors in randomized order. Asterisks indicate significant differences of respective points from control group (*$P = 0.05$, ***$P = 0.001$). *ns*, Not significant. This figure is modified from reference [40]. *(See color insert.)*

any appreciable inhibitory effect on monoamine oxidase (MAO) [86]. VK-28 inhibits both basal and Fe/ascorbate-induced mitochondrial membrane lipid peroxidation [85]. To confirm that FAC-mediated inhibitory effect on learning and memory is due to the addition of a high concentration of iron, we injected VK-28 into the antennal lobes of the honeybee brain before injecting FAC. We observed that the combination of FAC and VK-28 treatment restores learning response to 100% (Fig. 21.4C), implying that the FAC-mediated inhibitory effect on learning is due to increased ferrous iron. Subjects in the FAC-treated group show 40–50% reduction in retention response compared to controls and do not show a characteristic generalization gradient (Fig. 21.4D). However, FAC + VK-28-treated group overcomes these inhibitory responses due to iron-chelating effect (Fig. 21.4D). Collectively, FAC-induced oxidative stress in the antennal lobes of the honeybee brain markedly inhibits olfactory learning and memory, supporting a major role of iron in olfactory dysfunction [40].

The inhibitory patterns of olfactory learning have also been observed by disruption of octopamine receptor function by either receptor antagonism or gene silencing [72]. However, addition of GSH into the antennal lobes prior to mianserin/dsRNA treatment does not reverse octopamine receptor disruption-mediated inhibitory responses of olfactory learning and memory [40], suggesting that octopamine receptor disruption and iron-mediated oxidative stress confer two independent mechanisms that impair olfactory learning and memory in honeybees.

21.6 MOLECULAR BASIS OF IRON-INDUCED OXIDATIVE STRESS

The brain is susceptible to iron-induced oxidative stress in both vertebrates and invertebrates. Oxidative stress in the olfactory system is a major factor associated with age- or disease-related olfactory impairment. Patients of AD at the early stage of the disease exhibit perceptual deficits in odor identification, although the molecular mechanisms are not completely understood. Protein oxidative modification in aging murine olfactory bulb has shown the presence of the specific carbonylated proteins in astrocytes and mitral/tufted neurons but nitrated proteins in the vasculature, as molecular substrates of age-related olfactory dysfunction [87]. Studies on redox-competent copper and iron indicate that redox activity in AD resides exclusively within the cytosol of vulnerable neurons, and chelation with deferoxamine or diethylenetriaminepentaacetic acid (DTPA) removes this activity [88].

The role of mitochondria as a potential source of redox-active metals and oxygen radical production is assuming more prominence. Increased mitochondrial DNA and cytochrome *c* oxidase activity but reduction in the number of mitochondria in AD brain indicates accelerated mitochondria turnover [15]. Neurons, and also the surrounding epithelial cells, in biopsy specimens have been observed with increased lipid peroxidation and heme oxygenase-1, whereas no increase in nucleic acid or protein oxidation was observed in vulnerable neurons in AD [15], suggesting that the abnormality in array of oxidative stress markers may occur depending on different cell types in AD.

A hypothetical scheme is shown in Fig. 21.5 to present molecular mechanism(s) involved with olfactory dysfunction in the honeybee brain. In the iron-induced oxidative stress model, phospholipase A_2 (PLA_2) peroxidizes PUFA that are present in phospholipids into peroxidized phospholipids, which are better substrates for phospholipase A_2 than native phospholipids in the honeybee brain (Fig. 21.5). This process results in the formation of free peroxidized arachidonic acid (ARA). The oxidation of ARA ultimately results in the generation of 4-hydroxynonenal, which impairs mitochondrial electron transport and produces ROS. Increased ROS production causes oxidative stress that damages proteins, DNA/RNA, and lipids, causing olfactory dysfunction (Fig. 21.5). Reduced glutathione protects brain against oxidative stress by modulating the redox state of specific thiol residues of target proteins, whereas VK-28 chelates iron from the system and therefore slows down the formation of ROS. Oxidation of catecholamines may form quinones, which can form ROS, leading to olfactory dysfunction (Fig. 21.5). Furthermore, octopamine receptor function is disrupted by mianserin (octopamine receptor antagonist), which competes with octopamine in binding to octopamine receptors and therefore blocks receptor function, resulting in olfactory dysfunction (Fig. 21.5). Octopamine receptor function disruption by octopamine receptor dsRNA (AmOA1-dsRNA) silences octopamine receptor gene expression, which inhibits its protein synthesis, resulting in olfactory dysfunction (Fig. 21.5). The receptor functional disruption mechanisms (mianserin/AmOA1-dsRNA) seem to be independent of ROS formation. The receptor functional disruption is also not a random process but appears to be associated with increased oxidation of specific proteins [89]. Similarly, disruption of noradrenergic receptor or production of dopamine quinones in the olfactory bulb of vertebrates (including humans) may contribute to increased levels of oxidative stress, resulting in olfactory dysfunction.

21.7 HONEYBEE MODEL SYSTEM FOR OLFACTORY DYSFUNCTION

The olfactory system is phylogenetically highly conserved among invertebrates and vertebrates. Honeybees have the ability to learn about the association of odor with reinforcement. There are well-established behavioral paradigms, such as proboscis extension reflex, for monitoring olfactory learning in honeybees. They have a short life span, which makes them suitable for study of aging. They are straightforward to propagate and easy to manipulate in the lab. They have short gestation periods that produce large numbers of offspring, and their genome sequence is available. Their brains can be easily dissected out to focus on the relevant neurons in a specific region such as antennal lobe from the honeybee brain because of its larger brain size compared to other insect models such as *Drosophila*. Finally, the antennal lobe of the honeybee brain is a functional analog to the mammalian olfactory bulb.

Oxidative damage has been demonstrated to be increased in the olfactory system (including neurons and the surrounding epithelial cells) in the brains of AD patients [15]. Neuropathological studies in the brains of PD patients also suggest neuronal damage in the olfactory system (including olfactory bulb and the anterior olfactory nucleus) [90]. These findings support our findings in the honeybee brain, where oxidative stress induced in the antennal lobe of the brain results in olfactory dysfunction [40]. All of the criteria mentioned above for a better model system for olfactory research, neuropathological studies performed in AD and PD brains [15,90], and our findings associating oxidative stress and olfactory dysfunction in the honeybee brain [38, 40] support the honeybee brain as a potential model system for olfactory dysfunction during aging and neurodegenerative diseases.

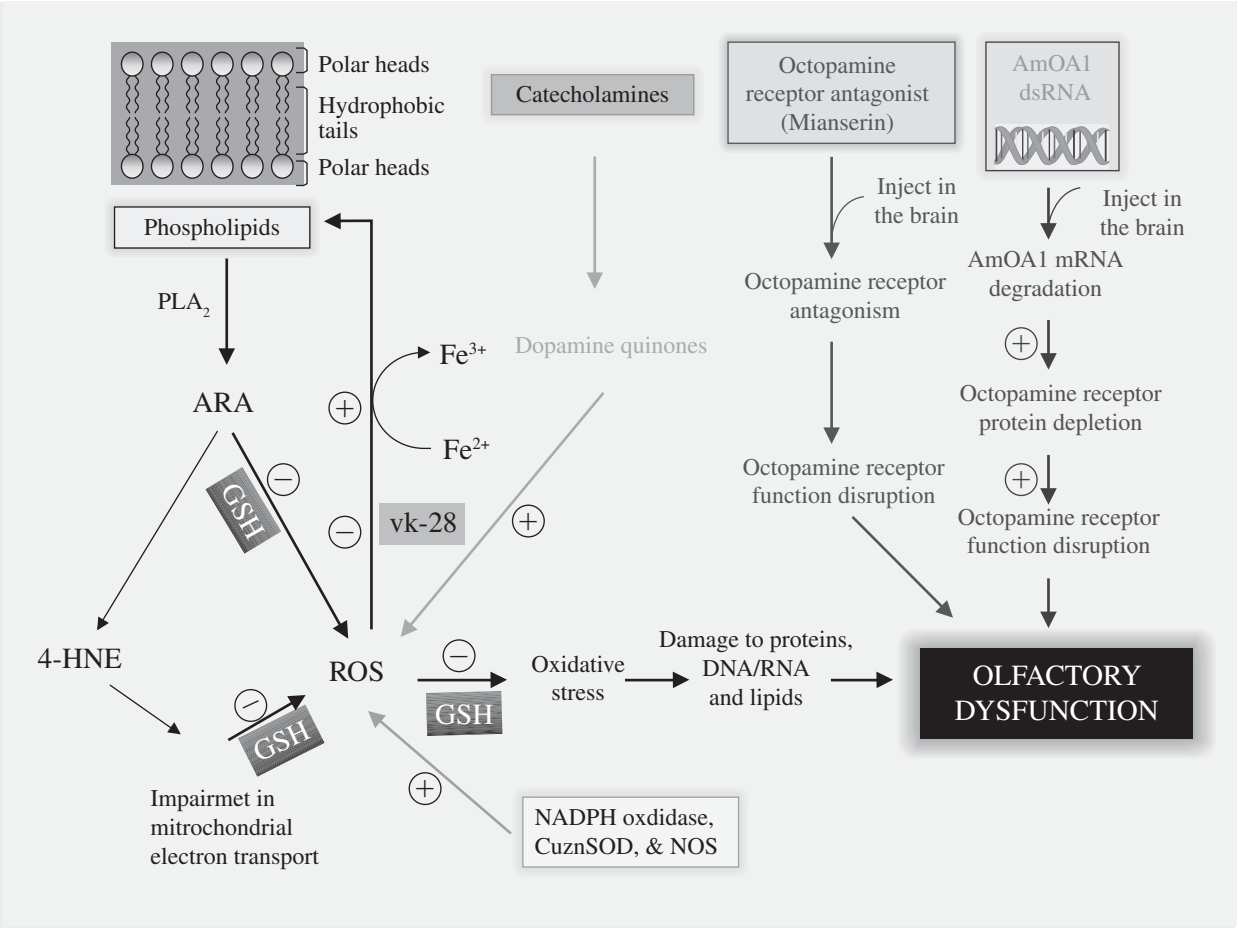

Fig. 21.5 Hypothetical molecular mechanism underlying reactive oxygen species (ROS)-mediated olfactory dysfunction in the honeybee brain. Iron-induced oxidative stress: Iron peroxidizes polyunsaturated fatty acids into peroxidized phospholipids, which are considered better substrates for phospholipase A_2 (PLA_2) than native phospholipids. PLA_2 catalyzes this reaction, forming arachidonic acid (ARA). The nonenzymatic oxidation of ARA results in the generation of 4-hydroxynonenal (4-HNE) that impairs mitochondrial electron transport, producing ROS. Increased ROS produces oxidative stress that damages proteins, DNA/RNA, and lipids, impairing olfactory processes (encoding, consolidation, and/or retrieval processes). Both octopamine receptor antagonism by mianserin (MAS) and octopamine receptor protein depletion by octopamine receptor double-stranded RNA (AmOA1-dsRNA) result in functional disruption of octopamine receptor, which leads to olfactory dysfunction. Oxidation of catecholamines forms quinones, which results in formation of ROS, leading to olfactory dysfunction. GSH protects brain against oxidative stress by modulating the redox state of specific thiol residues of target proteins. VK-28 chelates excess iron from the system. Monoamine oxidase inhibitor (MAOI) inhibits monamine oxidase enzyme and therefore prevents oxidation of catecholamines. ROS can also be produced by activation of NADPH oxidase. *(See color insert.)*

21.8 CONCLUSION AND FUTURE PERSPECTIVE

Iron plays a role as a catalyst in free radical generation. Increase in ferrous iron catalyzes $OH^{\bullet}$ radical production in the antennal lobes of the honeybee brain, which causes impairment in learning and memory. The inhibitory effect of iron-induced oxidative stress on learning and memory can be reversed by antioxidant as well as iron chelator in the antennal lobes, suggesting that the honeybee brain is highly susceptible to iron-induced oxidative damage and can be used as a model of olfactory dysfunction.

Iron is not regarded as the underlying cause of olfactory dysfunction in neurodegenerative diseases, but it does play an important role in progression of neurodegenerative diseases, such as AD and PD, by participating in redox reactions that catalyze the formation of ROS that produces increased oxidative stress, leading to olfactory abnormalities. Thus newly developed antioxidants and brain-permeant iron chelators (such as VK-28, HLA20, M30, and M32) can be screened for their rescuing effect on olfactory dysfunction in honeybees during aging and iron-induced oxidative stress. The efficacy of these drugs may depend on their ability to penetrate the subcellular

compartments and cellular membranes where iron-dependent free radicals are generated.

A reliable biomarker for oxidative stress is isoprostane, which is generated from cell membrane-bound ARA by free radical attack [91]. Isoprostanes have been described in vertebrate systems [92]. However, this information is not known in invertebrate systems. Therefore, another important challenge for future studies with the honeybee model system will be to determine levels of isoprostanes in the antennal lobes of FAC-treated and untreated brains, which can be used as an index of lipid peroxidation.

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