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Molecular physiology of manganese in insects

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Manganese is an essential element for maintaining life. Overexposure to the metal, however, can be toxic to organisms. Given the significant function of manganese in insects, agriculture, and human disease, as well as in the healthy ecology of the planet, the biological activities of manganese in insects needs consideration. Because of the role of manganese as a cofactor for essential enzymes present in different organelles, both over and underexposure to manganese has a multifaceted effect on organisms. At the physiological level, the effects of insect exposure to the metal on enzymatic activities and consequent alteration of insect behaviors are best explained through the metal's role in modulating the dopaminergic system. Despite numerous examples that alterations in manganese homeostasis have profound effects on insects, the cellular mechanisms that ensure homeostasis of this essential metal remain presently unknown, calling for further research in this area.

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Background

This literature review covers the physiological role of manganese in insects, at the cellular and tissue level, as well as the metal's impact on the whole animal, which can result in behavioral changes and affect ecological interactions. Insects have often been used as a model to study the effects of manganese overexposure, but only few studies have determined physiological consequences of manganese deficiency.

Manganese (Mn) can exist in a variety of oxidation states as Mn^{2+}, Mn^{4+}, and Mn^{7+} [1••]. Most experimental studies have examined the effects of Mn as $MnSO_4$ or $MnCl_2$ which are readily dissociated in water to Mn^{2+}. The presence of $MnSO_4$ in agriculture sprays and soil enrichment has increased the exposure of many species to this metal. Mn is essential for photosynthesis in plants (as part of the metal cluster supporting water oxidation in photosystem II, [2]) and promotes plant growth and health. A protein analysis in Arabidopsis revealed that 398 enzymes are predicted to contain Mn in a metal binding site, 20% of which have been verified experimentally to use Mn as a cofactor [3], explaining its use for agricultural crops. Application of $MnSO_4$ affects not only the insect populations directly feeding on plants, but also the soil runoff into water sources, including downstream and river-marine deltas. Relatively high levels of $MnSO_4$ are used to increase agricultural production, and these levels amplify throughout the ecosystem (e.g. blueberries, [4]). The effluents of mining and coal excavation also introduces Mn to the environment, serving as another source for exposure to insects. The environment and food web can amplify Mn concentration indirectly in insects in various ways as following: through exposure of pollen, thus affecting honey produced by bees; water sources, often containing developing mosquito larvae or other water borne insects; the bacteria and fungi in the soil that are fed on by termites; as well as other animal food sources used by carnivorous insects.

Dependence of biochemical reactions on Mn^{2+} ions

As in plants, Mn is essential to insects and other animals since it serves as a cofactor for numerous important cellular enzymes and organelle function (Table 1). This includes three mitochondrial enzymes: superoxide dismutase (SOD2; also referred to as manganese superoxide dismutase or MnSOD), arginase, and glutamine synthase [5••]; it also is a cofactor for glycosyltransferases in the Golgi [5••]. The DNA sequences encoding for MnSODs are conserved between animal species [6], suggesting that Mn serves as a cofactor for these enzymes throughout the animal kingdom [7]. MnSOD function has been studied in several insects, including the desert beetle *Micordera punctipennis* [8•], the Asiatic rice borer *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae [9••], the fruit fly *Drosophila melanogaster* [10•], and the insect cell line Sf-9 [11]. Since it is known that MnSOD is important to reduce oxidative stress, a lack of Mn decreases the ability of insects to withstand bacterial and viral infections [11]. Another important enzyme which requires Mn is the NAD-linked "malic" enzyme in skeletal muscle, studied in the tse-tse fly *Glossina morsitans* [12]. It is also important to note that temperature and pesticides may alter the expression of Mn-associated enzymes. Methyl paraoxonase activity was shown to increase in the tufted apple bud moth *Platyota idaeusalis* after exposure to Mn and in

Table 1

The action of Mn on various enzymes and metabolic processes

Insect	Enzyme/compound	Action of Mn	Citation
Aphids (*Myzus persicae*)			
	Catalase	Increased activity	[14]
	Glucose-6-phosphate dehydrogenase	Increased activity	
	Superoxide dismutase	Increased activity	
	Peroxidase malondialdehyde (biomarker of oxidative stress)	Increased activity	
		Decreased activity	
	Digestive enzymes:		
	α-amylase	Inhibited activity	
	Trypsin	Inhibited activity	
	Chymotrypsin	Inhibited activity	
	Elastase	Inhibited activity	
Bumble-bee (*Bombus spp.)*	Fructose 1,6-diphosphatase	Inhibited activity in body wall muscle	[15]
Drosophila melanogaster	Beta4-galactosyltransferase	Increased activity	[16]
	Manganese-dependent Metallopeptidase	Increased activity	[17]
	GTP cyclohydrolase	Inhibited activity	[18]
	CMP-sialic acid synthetase Sugar donor enzyme for sialylation	Increased activity	[19]
House fly (*Musca domestica*)	Glycerophosphodiesterase	Increased activity	[20]
German cockroach & tobacco budworm	Methyl paraoxonase	No effects	[13]
Mosquito (*Aedes aegypti*)	Ribonuclease	Increase activity	[21]
Silkworm (*Bombyx mori*)	Farnesyl pyrophosphate Synthetases I and II	Increased activity	[22]
	Catalase for cinnabarinic acid	Increased activity	[23]
	Cyclic GMP phosphodiesterase	Increased activity	[24]
	Adenylate cyclase Pupal fat body	Increased activity	[25]
Termite (*Neotermes koshunensis*)	β-glucosidase	Increased activity	[26]
Tufted apple bud moth (*Platynota idaeusalis*)			
	Methyl paraoxonase	Increased activity	[13]

building resistance to the pesticide azinphosmethyl [13]. Using current approaches to analyze total mRNA expression (RNA-Seq) for whole animals of small size or even single cells within defined tissues with single cell RNA-Seq, one could assess the effects on gene expression with depletion or over exposure of Mn in order to have a more complete understanding in the protein connectome by such transcriptomic evidence.

Effects of Mn at the cellular level

Mn must be transported into the cell. The *Malvolio* (*Mvl)* gene encodes a proton-coupled metal ion transporter in the SLC11 family which transports Fe^{2+} and Mn^{2+} into cells [27]. Mutations in this gene in *Drosophila melanogaster* affects taste sensation [28•]. Taste in *Drosophila* is dependent on the dopaminergic system within the CNS [29•], so it is of interest to know how Mn may affect the dopaminergic neurons. Additionally, altered neuronal *Mvl* expression in bees affects social behavior [30••]. It is worth mentioning that a high-affinity Mn transport system has been hypothesized in *Drosophila* [5••], although the specific transporter involved has not been identified.

Ceramide phosphoethanolamine, which is an integral part of *Drosophila* cell membranes, is likely inactivated in conditions with low manganese [5••]. Additionally, since MnSOD helps reduce the amount of free radicals, low levels of Mn is thought to cause damage to cells due to increased oxidative stress; however, excessively high levels of Mn are known to also increase oxidative stress [31]. These examples help illustrate that Mn deficiency as well as excess Mn levels are damaging to various cellular activities of insects. However, in twenty-three species belonging to two different fly families (*Tephritidae* and *Drosophilidae*), no differences in Mn accumulation were observed between the families, suggesting that strong homeostatic mechanisms for metal control are present in insects [32]. The nature of such regulation for Mn at the cellular or systemic level remains unknown.

Effects of Mn on behavior

In social insects, environmental exposure to Mn has been associated with alterations in social tasks, as worker honeybees had higher concentrations of manganese in their body as compared to bees with other tasks for the colony [30••]. Importantly, Mn supplementation in the beehive altered the distribution of bees from defenders of the hive to foragers [30••]. Mn concentration within honey is used as an indicator of Mn in the environment [33,34]. Mn-treated flies also had a significant increase in acetylcholinesterase (AChE) activity [35] and a simultaneous decrease in dopamine levels and tyrosine hydroxylase activity [35]. In mammals, Mn was shown to accumulate in a region of the brain where dopamine syntheses is high [36,37]. Synchrotron X-ray fluorescence microscopy techniques in insects could be used to examine the differential location of Mn within the body and within specific tissues [38–40].

Physiological effects of Mn

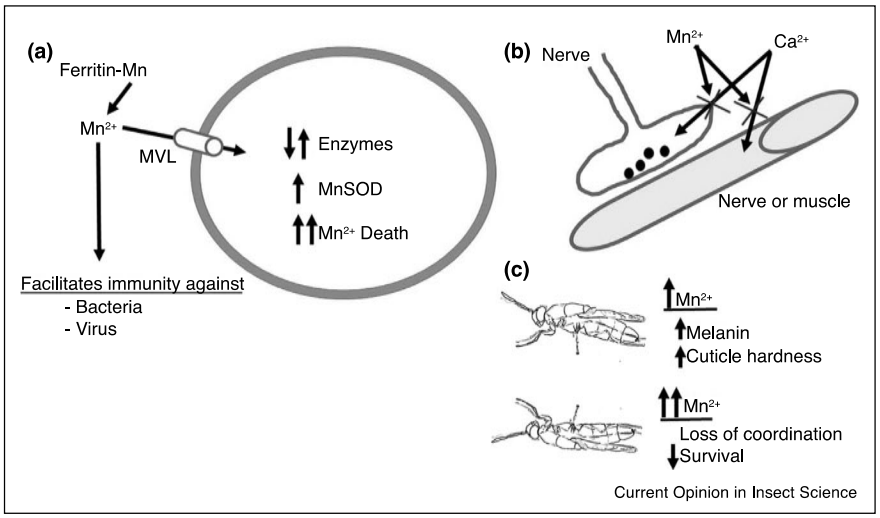
Few studies have directly investigated the effects of Mn on physiological functions of tissues and systems in insects. Considering that Mn^{2+} can block Ca^{2+} flux in skeletal muscle in cockroaches [41], it is not surprising that muscle excitation and contraction are also affected in skeletal, smooth, and cardiac muscle function in other insects. This is demonstrated by studies indicating that the hindgut of the cockroach stops contracting after exposure to 2 mM Mn^{2+} [42] and that the heartbeat of *Drosophila* larvae drastically reduces when exposed to 2.5 mM and is eliminated at 15 mM $MnCl_2$ or $MnSO_4$ [43••]. The effects of manganese on locomotion and skeletal muscle function are a combination of effects, likely involving Mn^{2+} blocking voltage-gated Ca^{2+} channels in the presynaptic nerve terminals [43••]. Thus, muscles will show reduced contraction. The body wall muscle in larval *Drosophila* have graded the excitatory junction potentials, which is related to graded contractions, and demonstrate a dose-dependent depression with Mn [43••]. Additionally, acute exposure of 2.5 $MnSO_4$ mM blocks nerve evoked synaptic transmission [43••]. Depending on the ion channel subtypes and density of channels in a neuron, a Mn block of Ca^{2+} influx may even result in a depolarized state if a constitutively active calcium-activated potassium channel ($K_{(Ca)}$) is compromised. If this occurs, then a membrane potential may not repolarize as quickly and result in overexcitation of a neuron and synaptic responses if there is still enough Ca^{2+} influx for synaptic transmission to take place [44]. This may explain the hyperexcitability of crustacean sensory neurons when a nerve is bathed in low Ca^{2+} [45]. However, the action potential amplitude of cockroach motor neurons recorded with an intracellular electrode was compromised by 40 mM Mn^{2+}, which slightly enhanced the amplitude and prolonged the width of the action potential [46]. This supports the notion that Mn^{2+} can reduce a $K_{(Ca)}$ current by blocking Ca^{2+} channels. The body wall muscle in most insects has Ca^{2+} channels on the plasmalemma of the muscle to allow Ca^{2+} influx for muscle contraction when depolarized. The direct actions of Mn on muscle, independent of neural innervation, can have consequences due to voltage-gated Ca^{2+} channels on the plasma membrane, essential to electrical depolarization [43••,47]. However, the depolarization of the larval body wall muscle fibers in a beetle (*Xylotrupes dichotomus*) were maintained in the presence of 40 mM Mn^{2+} [48], suggesting the Ca^{2+} channels present were not blocked by Mn, but instead manganese was able to permeate through the channels. The role of Ca^{2+} association in promoting various transport processes are also likely affected by Mn exposure. However, vitellogenin uptake in a cockroach was shown not to be inhibited by Mn [49].

Toxicity effects of Mn

Considering Mn has a role in maintaining an effective immune response, low Mn levels can reduce the life span by causing insects to be more vulnerable to bacterial, viral and parasitic infections. If cellular processes are disturbed in various tissues, it is reasonable to expect that there would also be behavioral consequences from altered Mn levels. Since primary sensory neurons, synaptic transmission at neuromuscular junction and cardiac function are all dampened in response to high Mn levels, it is not surprising that life span may be compromised. Various studies have investigated the mortality rate after Mn exposure in *Drosophila*. The fruit fly is a model organism commonly utilized for pathological conditions related to mammals [50–54]. One study exposed adult *Drosophila* to 10 mM $MnCl_2$ and examined locomotor behaviors as well as survival and showed about 20% mortality after 5 days and nearly 100% mortality after 20 days [55]. The increase in mortality correlated with a rise in reactive oxygen species and a decrease in locomotor behaviors [55]. In another study, adult *Drosophila* exposed to 30 mM/Kg in their diet for seven days resulted in a 20% mortality [56]. The effects of 5 mM $MnCl_2$ or $MnSO_4$ in food eaten by adult *Drosophila* were similar to controls, but 15 mM and 30 mM significantly increased mortality. Eight days of feeding 30 mM $MnSO_4$ resulted in 100% mortality; feeding 30 mM $MnCl_2$ for the same duration was less severe as some animals survived [43••]. $MnSO_4$ or $MnCl_2$ at 30 and 100 mM are toxic to 1st instar *Drosophila* larvae; at 15 mM of either compound, there was still significant mortality (15 mM $MnSO_4$ resulted in 50% of the larvae pupating and eclosing as adults, [43••]). A behavioral analysis of larvae consuming Mn 30 mM $MnSO_4$ or $MnCl_2$ for 24 hours resulted in significant decreases in body wall and mouth hook contractions; 15 mM Mn had a milder but still significant effect [43••].

When comparing freshwater midges, *C. javanus* was equally or more sensitive to environmental metals than

Figure 1



Overview of the effects of manganese on cells, tissue, and the whole animal. **(a)** Labile Mn^{2+} may be transported and potentially regulated in hemolymph by ferritin [64]. The proton-coupled metal ion transporter expressed by the *Mvl* gene has been suggested to transport Mn^{2+} into cells. Increases in Mn^{2+} can be beneficial in helping to maintain an increased immune response to bacteria and viruses. Mn^{2+} concentration within cells can result in some enzymes increasing in expression, while others decrease. In particular, MnSOD increases in expression. However, high levels of intracellular Mn^{2+} can trigger apoptosis and cell death. **(b)** Mn^{2+} can block voltage-gated Ca^{2+} channels on presynaptic nerve terminals as well as on muscles (i.e. body wall and cardiac). **(c)** Dietary Mn^{2+} promotes melanin production to protect from solar radiation and increases cuticle hardness. High levels of dietary Mn^{2+} lead to reduced locomotory function and decreased survival.

Chironomus javanus [57•]. The nymphs of a dragonfly (*Tramea cophysa*) were less sensitive than two tropical ostracod (Crustacea) species (*Chlamydotheca* sp. and *Strandesia trispinosa*) to various metal with Mn being the least toxic of the four metals (i.e. Cd > Hg > Cu > Mn) studied [58•].

Mn neurotoxicity

Mn as well as iron (Fe) and copper (Cu) are associated with Parkinson's disease [59••]. Likewise, Mn, along with Zinc (Zn), Cu, aluminum (Al) influence the severity of Friedreich's ataxia [60]. *Drosophila* may serve as a useful model to study the effects of metals on movement disorders, since Mn exposure damages dopaminergic neurons in the central nervous system of *Drosophila melanogaster* [59••]. Alterations in the dopaminergic neural circuits affect locomotion in *Drosophila melanogaster* [59••,61]. It is not yet known if Mn is directly affecting the dopamine receptors or exerting its effects through a specific cellular process. Since larval heartrate is also influenced by dopamine it is possible that Mn might alter heart rate by blocking dopamine receptors [62] as well as other cellular processes. The further exploration of the effects in various insect models needs to continue in order to facilitate understanding the effects of Mn poisoning in other animals [63].

A general overview in the impact of Mn at a cellular level to the whole animal is schematically illustrated in Figure 1.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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57. • Shuhaimi-Othman M, Yakub N, Umirah NS, Abas A: **Toxicity of eight metals to Malaysian freshwater midge larvae *Chironomus javanus* (Diptera, Chironomidae).** *Toxicol Ind Health* 2011, **27**:879-886

Larvae of freshwater midge Chironomus javanus were exposed over four days at controlled levels. Mortality was assessed and median lethal concentrations (LC(50)) were calculated. LC(50)s for 96 hours was conducted with a ranking was Cd & Cu & Fe & Pb & Al & Mn & Zn & Ni in toxicity. Comparison of LC(50) values for metals for this species with those for other freshwater midges reveals that *C. javanus* is equally or more sensitive to metals than most other tested dipteran.

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The aim of this study was to evaluate the acute toxicity of four metals (Cd, Cu, Mn, and Hg) in a tropical dragonfly nymph of Tramea cophysa and two tropical ostracod species (*Chlamydotheca* sp. and *Strandesia trispinosa*). The order of metal sensitivity was different for the three test species: T. cophysa: Cu & CdHg & Mn, Chlamydotheca sp.: Cd & Cu & Hg & Mn, and S. trispinosa: Cd & Hg & Cu & Mn. However, manganese was the least toxic metal tested for all three species, which is hypothesized to be due to a possible metal transfer to the cuticle of the moulting test species.

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These authors showed that acute (15 mM for up to five days) or chronic (0.5 mM for up to 15 days) exposure to Fe, Mn and Cu reduced life span and locomotor activity in adult fruit flies (*Drosophila melanogaster*). The metals accumulated in the fly's head and were capable to destroy dopamine-ergic neurons. This work provides for the first time metal-induced Parkinson-like symptoms in *D. melanogaster*.

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