Dynamic Neural Control of Insect Muscle Metabolism Related to Motor Behavior

Skeletal muscle innervation differs between vertebrates and insects. Insect muscle fibers exhibit graded electrical potentials and are innervated by excitatory, inhibitory, and also neuromodulatory motoneurons. The latter form a unique class of unpaired neurons with bilaterally symmetrical axons that release octopamine to alter the efficacy of synaptic transmission and regulate muscle energy metabolism by activating glycolysis. Octopaminergic neurons that innervate muscles with a high energy demand, for example, flight muscles that move the wings of a locust up and down, are active during rest but are inhibited during flight and its preparatory phase, a jump. Therefore, it is argued that these neurons are involved in providing locusts with the necessary fuel at takeoff, but then may aid the switch to lipid oxidation during flight. In general, the octopaminergic system may switch the whole organism from a tonic to a dynamic state.

General Aspects of Skeletal Muscle Innervation of Vertebrates and Invertebrates

Most animals, including humans, create movements by muscle contractions. Muscle contractions can be exerted over a wide range of speeds, forces, and durations. Depending on their function, vertebrate skeletal muscles are composed of distinct combinations of biochemically different fiber types such as slow and moderately fast twitch fibers with high oxidative capacity (red, type I, and type IIa fibers) or fast-twitch fibers with low oxidative capacity (fast-glycolytic, white, and type IIb and IIx fibers). In adult skeletal muscle, generally each muscle fiber is innervated by one motoneuron only (FIGURE 1A). By contrast, one motoneuron may innervate a large number of muscle fibers. The functional unit of one motoneuron and all of its target muscle fibers is named a motor unit. Each motor unit comprises muscle fibers of one type only. Most skeletal muscles are composed of many, often multiple hundreds of motor units. Variations in force and contraction speeds of each individual muscle are achieved by recruiting a variable number from its total pool of motoneurons, which all release acetylcholine (ACh) as transmitter and act via nicotinic ACh-receptors. Therefore, at the level of a single muscle fiber, the resulting electrical and mechanical response to a single action potential is set (except for synaptic changes or muscle fatigue).

The motoneurons are not uniform but exhibit different electrical and molecular properties and can be grouped into small and large motoneurons that cause slow or fast contractions. There is a tendency for small motor units to be composed of slow-twitch muscle fibers and those of larger motor units of fast-twitch muscle fibers. For recruitment of motor units, the size principle had been formulated stating that slower motor units will be recruited before the faster units (39, 40). As far as de-recruitment is concerned, the faster units will be de-recruited before the slower units. This ensures that the slowest, more fatigue-resistant units are recruited first and that the very fast-fatiguing motor units are reserved for infrequent high energy-demanding tasks such as jumping. Recently, however, the recruitment according to the size principle was refined into that of the task principle in which groups of motoneurons of a given muscle can be independently activated to fulfill special contraction requirements for specific motor tasks. These pools of motoneurons were called “task groups,” and thus during locomotion different recruitment strategies may be selected (42, 55, 71). However, the focus of this review is on invertebrate motor innervation and not vertebrate cholinergic motoneuron recruitment (for review, see Ref. 41). Here, it is sufficient to note that, in vertebrates, cholinergic motoneuron types with their different properties, biochemical target fiber types, and different recruitment strategies determine the contraction speeds, duration, and forces of skeletal muscle.

Most invertebrates, including all arthropods such as insects and crustaceans, possess striated
muscles with biochemical fiber types not qualitatively different to those of vertebrates (43, 44). By contrast, a fundamental difference to vertebrate striated muscle is that insect skeletal muscle fibers generate graded electrical potentials, which in turn cause graded contraction forces. In arthropods, each muscle is innervated by a pool of excitatory motoneurons. In contrast to vertebrate motoneurons, these are not cholinergic but glutamatergic. In arthropods, the number of excitatory glutamatergic motoneurons per muscle can vary between 1 and 10. Depending on their intrinsic properties and the muscular contractions that they initiate, they can be classified as fast motoneurons, where a single action potential causes a powerful twitch, and as slow motoneurons, which generate gradual contractions with the amplitude dependent on the frequency of action potentials. Between the fast and slow motoneurons are those of intermediate properties. In *Drosophila*, the excitatory, glutamatergic larval motoneurons can be of two different types: the Ib-type innervating one muscle and possessing large boutons (Ib-terminals) or the Is-type innervating a large number of muscles and possessing small boutons (Is-terminals). Both types may cause slightly different contractions. However,
there are several additional fundamental differences in the innervation scheme of invertebrate and vertebrate muscle (FIGURE 1B). First, arthropod muscle fiber can be innervated by more than one type of motoneuron and, thus, belongs to several motor units (22). In fact, in some insect muscles, each fiber may be innervated by fast, slow, and intermediate excitatory motoneurons (4, 73). Second, unknown to vertebrate muscle, inhibitory neurons release γ-aminobutyric acid (GABA) at the neuromuscular junction (NMJ) and cause muscle relaxation and inhibition of tonic fibers, which functions to allow a muscle to be used in dynamic tasks, for example walking (FIGURE 1B; Refs. 3, 6, 93). Although GABA is not found at the vertebrate neuromuscular junction, it should be noted that *Xenopus* embryonic striated muscle expresses receptors for GABA, glutamate, acetylcholine, and glycine receptors (11). As maturation progresses, AChR expression prevails, but expression of other neurotransmitter receptors can be maintained in the case of activity-dependent homeostatic changes in the transmitter complement of spinal neurons (11, 12). Third, all insect skeletal muscles are additionally supplied by octopaminergic/tyraminergic (OA/TA) neurons, a class of neurons specific to the insect central nervous system (CNS) (FIGURE 1B). Most of the results mentioned in this article were gained on locusts, either the desert locust *Schistocerca gregaria* or the migratory locust *Locusta migratoria*, but similar functions for OA/TA containing neurons have also been found to be true for other insects such as moths, cockroaches, and fruit flies, and some of the findings may apply to different invertebrate phyla. This casts light on how important comparative studies are for understanding how nervous systems were built and shaped during evolution.

Within the nervous systems of locusts (FIGURE 2A), paired clusters of OA/TA neurons exist predominantly in the brain (49, 78). Most OA/TA neurons of the ventral nerve cord ganglia (FIGURE 2B), however, have unique properties in that they have median cell bodies either on the dorsal or ventral surface of the nerve cord, and they are unpaired as they originate from the large unpaired median neuroblast and, thus, possess bilaterally symmetrical axons (FIGURE 2C). These features gave them their name: dorsal or ventral unpaired median (DUM or VUM) neurons (Ref. 45; for review, see Refs. 18, 80). One such population of unpaired OA/TA neurons comprises efferent neurons found in each of the segmental ganglia. They innervate peripheral tissues such as most likely all skeletal and visceral muscles, and some glands or even sense organs. All members of this population generate overshooting action potentials in the soma and have additional axonal spike initiation sites (36, 38). A second population of unpaired OA/TA neurons is exclusively confined to the CNS where their types range from large projection neurons descending through the whole ventral nerve cord or ascending to the brain to more local neurons of the brain or ganglia (14, 19). Thus, all major neuropils of brain and ganglia are densely supplied by OA/TA fibers. Octopamine is synthesized from tyramine by tyramine β-hydroxylase, and tyramine in turn is made from tyrosine by tyrosine decarboxylase. Since tyramine can also act as an independent transmitter and OA-receptors, TA-receptors, and mixed OA/TA-receptors have been identified, the whole range of actions is far from understood (31, 86). Occasionally, some special muscles may receive additional peptidergic innervation such as from neurons releasing allatostatin (51, 52), or some motoneurons may...
release peptides such as proctolin as co-transmitters (62, 63, 10), the latter of which is also used for modulating contractions.

The Effects of Octopamine on Insect Skeletal Muscle Contraction

What are the consequences of octopamine release from motoneurons on arthropod skeletal muscles? First, release of octopamine alone does not result in an electrical potential change or a contraction of the muscle fiber. However, octopamine modulates multiple aspects of neuromuscular operation via G-protein-coupled receptors. Presynaptically, it acts to increase glutamate release from the motor axon terminal, and this may account for an ~5% increase in twitch amplitude. More significantly, an increase in the relaxation rate of skeletal muscle via affecting chloride and potassium conductances is observed (Ref. 89; FIGURE 3). The latter effect allows that, in an alternating locomotory movement, each muscle will relax quickly enough to ensure fast contractions during subsequent glutamatergic excitation and to reduce muscle stiffness to ensure maximal joint movement during the contraction of antagonistic muscles. In contrast to skeletal muscle, in visceral muscle, octopamine reduces contraction amplitude, and may even cause myogenic contractions to cease (30, 48, 54, 64, 66).

The modulatory effects of octopamine play major roles during skeletal muscle contractions as

**FIGURE 3. The action of octopamine on different insect muscles**

A and B: single twitches of a locust abdominal muscle by electrical stimulation of its nerve (arrows). Summation of muscle tension occurs in the absence of octopamine (A). In the presence of octopamine (B), each twitch tension returns to zero (from Ref. 19). C and D: a flight steering muscle is stimulated by 20 Hz and then by a 40-Hz stimulus (see arrows) and develops a catch effect after stimulation (C), which is prevented in the presence of octopamine (D) (from Ref. 81). E: a schematic drawing of the stimulus regime for selectively stimulating the OA/TA containing DUM neurons innervating a flight “power” muscle (M 119) via suction electrodes (stimulation electrode 1 or 2), recording the activity of the antidromically stimulated DUM neuron from nerve 4 (N4) by a recording electrode and then testing the muscle for the concentration of F2,6P2 (left M119, stimulated muscle; right M119, control muscle) (from Ref. 59). F: the concentration of F2,6P2 in 20 individual animals; control muscles (red bars) to stimulated muscles (blue bars). The mean values are shown in graph at right. *Highly significant difference.
observed during crawling or slow walking. Accordingly, OA/TA containing neurons innervating walking or crawling muscles are connected to the same premotor interneurons as excitatory glutamatergic motoneurons. This results in a well timed cycle-by-cycle activation of OA/TA neurons during crawling (47) or walking (7, 37, 59). By contrast, the very fast-contracting insect flight muscles are innervated only by fast motoneurons and aminegic neurons and exhibit a single twitch per fast motoneuron action potential. In insects with synchronous direct flight muscles, wing-beat frequencies range between 10 and 30 Hz, and no muscle tone is built up. Therefore, during flight, increases in muscle relaxation speed and contraction amplitude as mediated by octopamine seem functionally obsolete. Nevertheless, all insect flight muscles receive abundant innervation by OA/TA releasing DUM/VUM neurons (15, 29). What might be the functional role of flight muscle aminergic innervation?

**Direct Control of Muscle Metabolism by Octopaminergic Motoneurons**

Bath application of octopamine to isolated insect skeletal muscles causes intramuscular increases in fructose-2,6-bisphosphate (F2,6P2), a key regulator of glycolysis (8, 90). In the presence adenosine-monophosphate (AMP), F2,6P2 activates the enzyme phospho-fructo-kinase (PFK), which in turn stimulates glycolysis as a means for muscle ATP synthesis. However, insect flight is one of the most energy-demanding motor tasks, and, therefore, flight cannot be sustained with carbohydrate as a fuel for more than 5–10 min (90). In fact, many insects fly for much longer durations, as swarming locusts have been reported to cross the Atlantic ocean (56). In fact lipid metabolism is utilized during prolonged insect flight (53, 91), just like in marathon runners after mile 21. Accordingly, locust flight power muscles exhibit a number of ultrastructural specializations for prolonged oxidative metabolism (9). In addition, octopamine plays a major role as a hormone in lipid mobilization from fat body (see below). The skeletal flight muscle innervation by OA/TA containing motoneurons provides a mechanism to allow direct control of muscle metabolism by the CNS.

The favorable anatomical feature of individually identifiable OA/TA containing neurons in the midline of the locust CNS (DUM neurons) with bilaterally symmetrical efferent axons allowed for directly testing the effects their on flight muscle metabolism. Antidromic stimulation of individual identified OA/TA containing neurons could be conducted such that octopamine was released onto the target muscle on one side of the body, while the same type of target muscle on the other side did not see octopamine release from OA/TA containing motoneurons. Both muscles, however, shared the same behavioral history of the animal, so that baseline metabolism was identical. Upon a “mild” OA/TA containing motoneuron stimulation of 1 Hz for 20 min, the muscle that had received octopamine release showed a sixfold increase in F2,6P2 over the internal control muscle (FIGURE 3F). Additional pharmacological experiments revealed that protein kinase A (PKA) activated via the octopamine receptor is necessary but not sufficient. Therefore, Gs-protein-coupled OA receptors as well as another pathway are activated in parallel by octopamine release from OA/TA containing motoneurons to increase F2,6P2 and thus stimulate PFK and glycolysis (58). These experiments provided clear evidence for the control of muscle metabolism by OA/TA containing neurons residing in the CNS.

How are these neurons activated or inhibited during behavior to adjust muscle metabolism to the specific needs as occurring during rest and flight? The full potential of the insect preparation comes to light when identified neurons (that can be recognized as individuals in different animals) are recorded during motor behavior in intact, semi-intact, or restrained animals. Recordings of locust OA/TA containing motoneurons (DUM neurons) during various motor behaviors in semi-intact preparations revealed that all DUM neurons innervating leg muscles are strongly activated during walking or jumping (FIGURE 4A; Refs. 23, 28, 37, 59). In fact, these modulatory motoneurons receive direct input by the same central pattern generating circuits that drive the activity of excitatory glutamatergic motoneurons (7, 28). By contrast, all DUM neurons innervating flight power muscles are strongly inhibited during flight (FIGURE 4B; Ref. 28) and also during jumping, which often precedes flying (FIGURE 4B; Ref. 23).

Inhibition of those TA/OA containing motoneurons that innervate flight power muscles during flight reconciles with the metabolic effects of neuronal octopamine release onto muscle (FIGURE 5). OA/TA containing motoneurons innervating flight muscle are active at low spike frequencies when the animal is resting, thus stimulating glycolysis (FIGURE 5). Therefore, locust flight power muscles stay poised for rapid energy utilization, as is needed during escape motor behavior, which usually comprises a jump followed by a short distance of flight (58, 68). OA induced intramuscular elevations of F2,6P2 require the presence of AMP to induce high glycolytic rates in muscle. Therefore, OA/TA release onto resting muscle ensures accumulation F2,6P2 to prime the muscle for high glycolytic rates upon demand. This provides the necessary surplus of energy for the animal’s take-off.
FIGURE 4. The activity of individually identified OA/TA containing DUM neurons during motor activity

A and B: DUM neuron activity recorded during kicking of a locust that corresponds to the motor pattern during a jump. A: the activity of a DUM neuron, which innervates the extensor tibiae muscle and other leg muscles of the locust (trace 1: intracellular recording via a glass microelectrode from the soma; traces 2 and 3 show electromyogram recordings from the left and right flexor muscles, respectively; trace 4 shows a record of the movement of the tibia of the jumping leg (arrow down flexion, arrow up extension). The kick occurs at the big upward arrow. B: intracellular recordings from two DUM neurons innervating wing power muscles during a similar kick show that these neurons, in contrast to the one shown in A, are inhibited. Again, the kick occurs at the upward arrow (from Ref. 23). C and D: the activity of DUM neurons innervating leg muscles (C) and wing muscles (D) during a short flight sequence. Trace 1 shows intracellular recordings from the neuron soma; trace 2 shows extracellular recording from the motor nerves revealing motor activity during these bouts of flight (from Ref. 28). E: a schematic drawing of events shows how a jump leads to flight activity. The jump motor program consists of co-contracting extensor and flexor tibiae muscles that generate force isometrically that is stored involving a number of specialized features of the joint. Once a flexor muscle is inhibited, the force is released, and, as the rapidly extending tibia pushes toward the ground, the animal is lifted off the ground and then starts takeoff by opening the wings and flying (for other reference, see Ref. 23).
**FIGURE 5.** The octopaminergic control of fuel availability and catabolism in locust flight power muscle during rest, takeoff, and flight

A: list of different behavioral states with associated catabolism before a flight, during takeoff, and during prolonged flight. B: hemolymph (yellow) concentrations of octopamine, AKH, and free lipids during the different behavioral states listed in A. Black arrows indicate effects of hemolymph levels of one substance onto those of another one. C: levels of metabolic products, fuel, and metabolic intermediates in flight power muscle (blue) during the three behavioral states depicted in A. Black arrows indicate interactions between these molecules. Phosphofructokinase (PFK) is activated during takeoff in the presence of high levels of F2,6B2 and AMP to boost glycolysis. AMP is high because of high ATP turnover during takeoff (see red arrow). F2,6B2 is high in flight muscle before a flight due to activity of octopamine releasing DUM neurons to flight muscles at rest, before takeoff (D, red). DUM neurons to flight muscles are inhibited during takeoff and prolonged flight (D), so that octopamine levels in flight muscle decrease as soon as the animal becomes airborne. Therefore, F2,6B2 levels in flight muscle decrease (black arrow), consequently PFK activity decreases (black arrow), and ATP production is ensured by a switch to lipid oxidation (see green arrow), as free lipids become available in the hemolymph while the animal is airborne (see B).
or “start energy.” As soon as the animal engages into a jump or into flight, all TA/OA containing motoneurons to flight power muscles are inhibited (23, 29), and the OA content is quickly diminished in these wing “power” muscles (FIGURE 5; Ref. 90). Consequently, the glycolysis promoting effect of octopamine release from motoneurons ceases shortly after takeoff, which relies on fast ATP availability (FIGURE 5). During prolonged flight, trehalose as a fuel for flight power muscles would be empty after a short time so that these switch to lipid oxidation (90). Apparently, the octopaminergic system is switched off in flight “power” muscles to allow for the switch from glycolysis to lipid oxidation. In contrast, leg and flight steering muscles cannot gain energy through lipid oxidation and thus have to stimulate glycolysis and rely on mechanisms of carbohydrate oxidation. Accordingly, OA/TA containing motoneurons to these muscles are activated during all motor tasks regardless of jumping, flying, or walking (81).

The Role of Amines as Hormones in Mobilizing Fuels

To cover the rapid demand of ATP during takeoff as well as the prolonged demand of high energy during sustained flight, the respective fuels have to be available to the flight power muscle. In this respect, OA acts as a one of the hormones involved in lipid and carbohydrate (trehalose) mobilization from insect fat body and thus has hyperglycaemic action (25, 94, 95). Note that insects have an open circulatory system, and thus fuel supply cannot be regulated by diverting blood flow to skeletal muscle. Therefore, fuels are made available globally in the hemolymph. In fact, OA is the first hormone to be released into the hemolymph during motor activity (FIGURE 5; Refs. 1, 65) or in states of hunger (92), and significant elevations in blood OA are measured after a few minutes of motor activity, which, in turn, will provide enough trehalose. Although the source of OA release as a neurotransmitter remains speculative, there are clusters of cells in the brain that project to neurohormonal glands such as the corpora cardiaca or corpora allata (13). In addition, there are special OA/TA containing motoneurons in abdominal segments that exclusively project to the heart and potentially could release there (33, 79). And finally, some members of the segmental OA/TA containing motoneurons show neurohaemal release sites along their axons in peripheral nerves (15, 17). The latter are good candidates for mediating elevations in hemolymph OA during flight (FIGURE 5; Ref. 67) because they continuously fire during flight (28). However, the demand for lipids during prolonged flight is crucially dependent on the release of adipokinetin hormones I to III (AKHI to III) from the Corpora cardiaca, an endocrine gland associated with the brain (reviewed in Ref. 85). Although OA containing neurons of the brain may be involved in AKH production and release, recent evidence points to important roles of a cocktail of peptide transmitters in this process (88). However, OA containing neurons may be informed about AKH levels because they express AKH receptors (92).

Orchestration of Muscle Metabolism, Fuel Availability, and Broader Behavioral Context

The Central Role for Octopamine is in Correspondence to Its Peripheral Role

Fully compatible with its role in motor behavior, OA exerts multiple actions within the CNS, ranging from direct effects on motor CPGs to affecting foraging, feeding, motivation, aggression, and learning. In many insect species, OA induces flight motor patterns by direct actions on the CPGs (27, 77, 87), but the endogenous sources for central OA release onto CPG networks remain to be identified. On the cellular level, OA enhances synaptic transmission in neurons, and depending on the ion channel bouquet may induce plateau potentials in flight interneurons (69, 70). In addition, octopaminergic neurons located in the brain orchestrate overall behavioral states biasing the animal for high energy-demanding motor activity, such as motivational states favoring locomotion (46), aggression (5, 82), and foraging (35, 74). Correspondingly, in the presence of OA, the habituation of reflexes takes longer, and sensitivity of sensory receptor cells is increased (16, 32, 57) in the case of antennal olfactory neurons even in a circadian manner (34). Thus there is an intricate and, it seems, finely tuned relationship between the central and peripheral effects of OA in all motor behaviors that links neural and metabolic pathways together. Correspondingly, a recent study on the Drosophila larval neuromuscular system (50) demonstrates that starvation causes increased formation of octopaminergic type II terminals and thus globally enhances neuromuscular transmission. The general effect of this activity-dependent plasticity of octopaminergic neuron axon terminals is to enhance locomotion during starvation.

This mechanistic correspondence between aminergic effects on motor control and feeding seems strongly conserved through invertebrates. In Caenorhabditis elegans, starvation directly activates octopaminergic neurons, which in turn activate neck motoneurons to generate locomotion. When food is sensed, dopaminergic neurons are activated that make inhibitory connections to the octopaminergic and the neck motoneurons, and thus locomotion ceases to allow the animal to ingest food (83,
Therefore, dopamine and octopamine link starvation (hunger) to locomotion. Similarly, high hemolymph OA titers are positively correlated with high levels of aggression in crickets (82) and fruit flies (5), and mutant fruit flies that lack the gene for the OA-synthesizing enzyme are less aggressive than wild-type fruit flies (96). In addition, OA also regulates interactions of males during courtship behavior (26), which is closely related to aggression.

Despite the multiple roles of OA in the CNS and for modulating sensory sensitivities, muscle contraction properties, and metabolism, insects with pharmacologically depleted OA stores can fly (21), although detailed studies on flight performance, behavioral thresholds, and fuel availability are not yet available. However, for pharmacological depletions of OA stores, it remains unclear whether all OA is removed, whether receptors may be overexpressed in response to lowered amine levels, and whether more than one amine transmitter is affected. Like the mouse or zebra fish for vertebrates, the fruit fly Drosophila melanogaster provides a genetically accessible organism for insects. Drosophila is known to use carbohydrate oxidation during flight, and its asynchronous flight mechanism (no 1:1 correlation between the neuronal pattern and wing beats) differs from that of the large insects, which use a neuronal pattern synchronous during flight, and its asynchronous flight mechanism (no 1:1 correlation between the neuronal pattern and wing beats) differs from that of the large insects, which use a neuronal pattern synchronous with the wing beats. Like in all insects, its whole muscular system is supplied by similar types of OA/TA containing motoneurons (24, 75, 76). In tβh-mutant Drosophila (60, 61) that cannot convert TA into OA, flight performance is greatly impaired. Although these animals can fly, flight bouts last significantly shorter, and takeoff likelihood is significantly reduced compared with wild-type Drosophila (20). Although these behavioral genetic data further underscore the importance of OA/TA neurons for motor behavior and in particular for an enduring performance, it remains unclear whether flight performance defects are caused by central or peripheral effects of OA, or by both. In addition, tβh-mutant Drosophila have up to eightfold increased TA level (61), and TA also has marked effects on multiple motor behaviors in flies (20, 72). C. elegans (2), and bees (35). Specific genetic manipulations, such as temporally restricted and tissue-specific knockdown of distinct classes of amine receptors will be necessary to decipher the multiple roles for OA on insect motor behavior.

**Concluding Remarks**

In contrast to vertebrate skeletal muscle, arthropod skeletal muscle receives innervation by OA/TA containing motoneurons in addition to excitatory motoneurons. OA/TA containing motoneurons are not only modulators of neuromuscular transmission but are also modulators of catabolic pathways, linking motor and metabolic demands. Although speculative at present, OA containing neurons of the brain may not only be involved in the initiation of locomotion but also in controlling metabolic systems that are required for energy-demanding activity levels of central neural circuitry during behavior. Such links to catabolic pathways might be common for other transmitters as well, although this, to our knowledge, is one of the best understood systems. With increasing evidence that both vertebrate and invertebrate animals share a common ancestor that was already equipped with a nervous system, comparative studies should provide insight into common design principles as well specific adaptations to particular life styles. Although OA/TA containing motoneurons are unique to arthropods, some key functions of amine release resemble ones known from the sympathetic nervous system in vertebrates and, in addition, from pathways of motor control. Last but not least, it is noteworthy that, despite the enormous evolutionary distance between insects and vertebrates including humans, the behavioral “key” contexts of the action of biogenic amines appear to be similar.

No conflicts of interest, financial or otherwise, are declared by the author(s).

**References**


