

Report on the 13th symposium on invertebrate neurobiology held 26–30 August 2015 at the Balaton Limnological Institute, MTA Centre for ecological research of the Hungarian Academy of Sciences, Tihany, Hungary

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Abstract This report summarizes the lectures and posters presented at the International Society for Invertebrate Neurobiology's 13th symposium held 26–30 August 2015, at the Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary. The symposium provided an opportunity for scientists working on a range of topics in invertebrate neurobiology to meet and present their research and discuss ways to advance the discipline.

Keywords Invertebrate · Neurobiology · International · Symposium

Around 60 neurobiologists attended the 13th meeting of the International Society for Invertebrate Neurobiology (ISIN) which was held in Tihany, 26–30 August, 2015. The participants came from 11 countries, with the largest contingents from Russia, Hungary and Japan. The programme consisted of 6 plenary lectures, including the Ernst Florey and Janos Salánki memorial lectures. Both Professor Florey and Professor Salánki were instrumental in establishing ISIN in 1989, and Professor Salánki was a former Director of the Institute. In addition to the plenary lectures, there were 22 oral communications and 35 poster communications. As with previous ISIN symposia, the animals of choice for study were predominantly gastropods and insects, making up around two-thirds of the studies. Other animals used included annelids, nematodes, echinoderms, lamellibranchs, bryozoans, phoronids, cnidarians, brachiopods and isopods. Rats and eels became honorary

invertebrates in 3 studies. In describing the work presented, a reference has been included where possible so the reader can more easily access further information on the research.

Professor Karoly Elekes opened the symposium and welcomed everybody to Tihany (Fig. 1) and stated that it was almost 50 years since the first meeting organized by Professor Janos Salánki at the Institute in 1967. Professor Frederic Libersat (Ben-Gurion University of the Negev, Israel) delivered the introductory lecture, the Ernst Florey Memorial Lecture, on a topic which his laboratory has been researching for a number of years, the relationship between a parasitic wasp and its host. The jewel wasp, *Ampulex compressa*, has evolved the ability to partially paralyse its host, the cockroach, *Periplaneta americana*, and the latter then serves as a food source for the wasp's larva. Having located a cockroach, the female wasp grabs it by its pronotum and stings the cockroach in the thorax (Libersat and Gal 2014). This causes a temporary flaccid paralysis of the front legs. The first sting contains a high concentration of γ -aminobutyric acid (GABA) which acts to open neuronal chloride channels which inhibits synaptic transmission, resulting in temporary central paralysis. Next the wasp injects venom directly into the cockroach's brain, specifically into the sub-oesophageal ganglion and the central complex of the supra-oesophageal ganglion. The second dose of venom contains GABA, taurine and dopamine, as well as many proteinous components; dopamine was shown to initiate grooming behaviour which lasts for about 30 min and is followed by a state of hypokinesia, the latter lasting for up to 6 days. The wasp then drags the cockroach into a prepared burrow and lays an egg which it glues to the cockroach's body and finally buries the cockroach. The wasp egg hatches and the larva uses the cockroach as a food source, initially feeding on its hemolymph and later chewing its way into the body of its host

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and eating its internal organs. The larva then pupates and hatches after about a month. Under laboratory conditions, a stung cockroach in the absence of a wasp larva will recover after 5–6 days, indicating the effect of the venom is reversible. Libersat and his colleagues have investigated the interplay between dopamine, octopamine and opioids on cockroach behaviour and related this to the response following the injection of the wasp's venom. Dopamine injected into the hemolymph will induce grooming in a cockroach, while the presence of a dopamine antagonist reduces the grooming effect following venom injection. In contrast, injection of an octopamine antagonist fails to reduce the grooming time. The decrease in the drive for walking following venom injection can be countered by the injection of an octopamine agonist into the supra-oesophageal ganglion. When opioid antagonists were injected into a cockroach prior to being stung by a wasp, the startle threshold following electric foot shock was decreased, but the converse was true when an opioid agonist (loperamide) was injected in controls. Opioid agonists and antagonists had no effect on grooming (Gavra and Libersat 2010). The second talk by Ms Stav Emanuel from the same university continued the theme of the previous lecture but considered the relationship between natural quiescence of a cockroach and the lethargic state induced by the wasp's venom. In the lethargic state, cockroaches show no escape behaviour.

Electromyograms were recorded from cockroaches' third leg coxa segment to monitor slow neuron tonic activity which was reduced in the quiescent state and further depressed in animals that had been stung. The regular tonic firing of the slow motoneurons was absent in stung cockroaches. Recordings from the central complex of the supra-oesophageal ganglion showed that evoked activity from this area was greatly reduced in both quiescent and lethargic animals. Ms Emanuel concluded that neuronal modulation associated with quiescence and venom-induced lethargy may share common mechanisms. The first session ended with a talk by Dr. Dmitry Vorontsov (Institute of Developmental Biology, Russian Academy of Sciences, Moscow) on hearing in mosquitoes. In his studies, Dr. Vorontsov used three species, viz. *Anopheles messeae*, *Aedes excrucians* and *Culex pipiens* (Lapshin and Vorontsov 2013). The acoustic sensory organ in mosquitoes, Johnston's organ, is a complex mechanoreceptive structure, composed of 15,000 receptors in the male and about half this number in the female. These receptors are highly sensitive and used to detect females of the same species. Behavioural studies indicate that these receptors can discriminate frequencies and this has now been confirmed using electrophysiological studies. Individual receptor properties in females were investigated using a positive feedback stimulation paradigm. It can be concluded that



Fig. 1 **a** The meeting organizer Professor Karoly Elekes (*centre*) outside the beautifully situated meeting venue, Balaton Limnological Institute, Ecological Research Centre of the Hungarian Academy of Sciences, with two of the presenters Dr. Anna Crisford (*left*) and

Professor Lindy Holden-Dye (*right*). **b** Two invertebrate inhabitants enjoying the hillside in Tihany. **c** The monastery of the Benedictine Order at Pannonhalma, founded in 996A.D., which provided a fascinating venue for an afternoon excursion

individual auditory receptors are tuned to different frequencies. The auditory system in females may play a role in precopulatory identification, detection of host movements and avoidance of birds and dragonflies.

The second session consisted of three talks: one on bryozoans, one on ants and the third one on a gastropod mollusc. The first talk by Dr. Viktor Starunov (Zoological Institute of the Russian Academy Sciences and St Petersburg State University, St Petersburg, Russia) investigated the neuromorphology of three freshwater bryozoans, viz. *Cristatella mucedo*, *Plumatella repens* and *Fredericella sultana*. Using confocal laser scanning microscopy and antibodies staining against acetylated α -tubulin, serotonin and FMRFamide, their distribution was compared in the nervous systems of the three bryozoans (Shun'kina et al. 2013). The three species chosen differ in their colony and lophophore structure and their zooidal size. While *C. mucedo* and *P. repens* both have horseshoe-shaped lophophores, *F. sultana* has a circular lophophore, and all three species have a single cerebral ganglion from which two pairs of nerves arise to innervate their lophophores and the wall of the zooid's body, the latter having a diffuse neural network. The nerves running from the cerebral ganglion form the circumoral ring. Radial nerves arise from the lophophore ring along the base of each pair of tentacles. Serotonin was found in the cerebral ganglion and lophophores of each species with cell bodies occurring at the base of the tentacles. The location of the serotonergic cell bodies varied between the three species. FMRFamide was found in all three species, occurring in the cerebral ganglion, lophophore, neural network of the cell body and in the case of *C. mucedo*, in the neural network associated with the sole musculature. In *C. mucedo*, a group of several dozen FMRFamide-containing cell bodies occurred in the basal part of the ganglion. Dr. Starunov concluded that serotonin and FMRFamide are widespread in the nervous system of freshwater bryozoans with serotonin regulating lophophore function. FMRFamide is probably used in the regulation of muscular components of the lophophore, body wall of zooids and sole of *C. mucedo*. Dr. Starunov also presented a poster which provided details about a new open access project to provide information on nervous and muscular systems, sensory structures and neurosecretory cells in a range of animals, mainly invertebrates. There will be detailed descriptions in Russian and English, and emphasis will be placed on neurobiology, developmental biology, ecology and bioindicators. Dr. Ivan Iakovlev (Institute of Systematics and Ecology of Animals, Russian Academy Sciences, Novosibirsk, Russia) reported on his work studying the effect of octopamine on aggressive behaviour in the ant, *Formica aquilonia*. Aggression is an innate key feature of ants where workers specialize in specific roles (Iakovlev 2013). Groups of ants, either in the

presence or absence of a queen, were fed a sucrose solution containing octopamine for up to 2 weeks and then tested for their behaviour towards a beetle, *Pterostichus oblongopunctatus*. Feeding octopamine for 1 week in the absence of a queen increased ants' aggression towards the beetle, compared with controls which had only been fed sugar solution. This effect became more obvious after 2 weeks. In contrast, this increased aggression was not seen in octopamine-treated ants maintained in the presence of a queen. Dr. Iakovlev also found that in his experiments octopamine did not influence locomotion in *F. aquilonia*. The final talk in this section was given by Dr. Olga Zaitseva and Dr. Alexander Shumeev (Zoological Institute of the Russian Academy Sciences and St Petersburg State University, St Petersburg, Russia) who, using confocal microscopy, histochemistry and immunocytochemistry, investigated the structure and formation of the neuromuscular and nervous system of a dorid, *Cadlina laevis*, and the distribution of catecholamines, acetylcholine transferase, substance P and FMRFamide in whole mounts of juveniles of this species. Juveniles have well-formed nervous and digestive systems and all the sensory organs of adults (Zaitseva et al. 2015). The brain is composed of cerebral, pleural, pedal and buccal ganglia together with small gastroesophageal ganglia. Catecholamine-containing neurons and fibres occur in all ganglia, in the neuropil, commissures, connectives and all the major nerves of the central nervous system. Catecholamine-containing neurons also occur in the digestive tract, sole of the foot and fibres which innervate the body wall, foot and pharynx. A large number of epidermal receptor cells, together with nerve fibres innervating the body wall musculature, show acetylcholine transferase immunoreactivity. FMRFamide and substance P are found in central ganglia and the digestive system. It is clear that in juvenile *C. laevis* catecholamines, acetylcholine and neuropeptides play a key role in the physiology of the animal.

The first plenary lecture was given by Professor Ákos Vértés (George Washington University, Washington, USA), on high-performance methods for metabolomic and lipidomic analysis of *Lymnaea stagnalis* embryonic development and central nervous system. He described two techniques for the analysis of single cells or small groups of cell, viz. laser ablation electrospray ionization (LAESI) (Nemes and Vertes 2007) and capillary microsampling combined with electrospray ionization (Zhang and Vertes 2015) for the analysis of single cells by ion mobility separation (IMS) and mass spectrometry (MS). LAESI-IMS-MS spectra from the central nervous system of *L. stagnalis* showed hundreds of peaks matching metabolites, peptides and lipids. Using this method, organ-specific peptides can be identified in salivary glands, heart, central nervous system and buccal ganglia of *L. stagnalis*. IMS-MS of

single hepatocytes resulted in 22 metabolites and 54 lipids. Using rotenone-treated hepatocytes, the adenylate energy charge can be calculated which provides a gauge of cell health. It is also possible to follow the metabolomics and lipid composition of the cell during development using *L. stagnalis* embryos. LAESI is useful for the identification of new peptides.

The second day of the symposium began with a plenary lecture by Professor Adrian Horridge (Australian National University, Canberra, Australia) on vision in the honeybee, *Apis mellifera*. An entirely new understanding of colour vision in insects is emerging from recent work by Professor Horridge which was summarized in his plenary lecture. Earlier work on bee vision was incorrectly interpreted in terms of human-like trichromatic colour vision. Each facet of the compound eye has three types of receptor cell: one for UV, one for blue and six for green. These receptors in the retina detect intensity, while the second-order neurons in the lamina detect change. Pattern discrimination is achieved by locating and measuring blue content and green modulation, with less preference for blue modulation (Horridge 2015c). Modulation is the receptor response to contrast and is summed over the length of a contrasting vertical edge which also provides a measure of angular width between outer vertical edges (Horridge 2015b). There are no bee colours, only shades of blue, and there is no achromatic vision of grey or white. Horridge found that bees locate the average position of blue relative to a landmark of green contrast (Horridge 2015a). Bees use green modulation alone or blue modulation alone to compare the widths of two single bars or the outside widths of two groups of bars. In the foraging behaviour, bees have an order of preference for learning and recognition. In addition to the three references above, readers are also referred to the following for detailed information on this research (Horridge 2014). Professor Horridge also presented his work as a poster. The two other talks in this session were on gastropod molluscs. The first talk was by Professor Ryota Matsuo (Fukuoka Women's University, Fukuoka, Japan) on ocular and non-ocular photosensing in the slug, *Limax valentianus*, which shows negative phototaxis behaviour. Slugs can show tropotaxis, movement towards or away from a stimulus as the slug compares sensory inputs from the paired receptors on each side of the head, moving to the darker side. Another taxis model is klinotaxis, a locomotory mode based on a sampling of external stimuli sequentially during movement (Matsuo et al. 2014). If one of the superior eye tentacles is removed, the slug shows circling behaviour in the direction of the tentacle that was removed. When the cerebral commissure is cut, the slug moves straight forwards, showing that the cerebral commissures are involved in circling. Information about light intensity is transmitted to the other side of the brain

through the cerebral commissures. Professor Matsuo presented evidence that circling behaviour is triggered by an imbalance between the light intensities of the eyes. There is also evidence that slugs can avoid light in the absence of sight, providing evidence for extraocular detection of light. Blinded slugs can still reach a dark place though they take longer. There are three visual pigment genes found in slugs, opsin 5, retinochrome and melanopsin, which are expressed in the eye and brain. Slug eyes are sensitive to blue light and probably also to UV light. It was found that 400 nm was the best light to show slug avoidance behaviour. When the eyes are present, slugs avoid light in a topotactic mode, but when eyes are absent, they avoid light in a klinotactic mode. It is suggested that there are photosensory brain neurons. In the second talk, Professor Alexander Sidorov (Belarusian State University, Minsk, Belarus) discussed age-related changes in the electrical properties of neuron RPeD1 and respiratory network function in the snail, *Lymnaea stagnalis*. Snails were divided into three groups based on their body weight, shell length and age, viz. junior, 32 ± 2 weeks; senior, 46 ± 4 weeks; and old, 53 ± 4 weeks. Three parameters were measured, viz. number of respiratory cycles per hour; activity of the giant dopamine-containing neuron, RPeD1; and activity of superoxide dismutase (SOD). The number of respiratory cycles increased with age, being greater in old and senior snails compared with junior snails. Professor Sidorov found that as snails aged so the resting membrane potential became more depolarized which was accompanied by an increased action potential (AP) frequency. The AP duration also increased with age; however, AP threshold and undershoot amplitude did not change significantly. There was a decrease in SOD activity in the central nervous system with age, the drop being particularly noticeable between junior and senior snails. Professor Sidorov concluded that his results supported an age-dependent increase in excitability of the respiratory central pattern generator. He has written a review on the evolution of cell-to-cell communication and chemical signal transduction (Sidorov 2012).

The third plenary lecture was delivered by Professor Markus Knaden (Max Planck Institute for Chemical Ecology, Jena, Germany) on olfaction in the vinegar fly, *Drosophila melanogaster*. His talk centred on the problem of finding a mate and avoiding enemies; in this case, a parasitoid wasp. *D. melanogaster* smells rotten fruit through a complex olfactory system. When rotten fruit is infected with mould, the latter releases a chemical, geosmin, which activates a specific circuit in flies (Stensmyr et al. 2012). Geosmin only activates a single type of sensory neuron which expresses the receptor Or56a. Activation of this receptor inhibits oviposition and feeding in *D. melanogaster*. The behaviour of *D. melanogaster* to a wide

range of odours has also been investigated (Knaden et al. 2012). Parasitoid wasps of the genus *Leptopilina* parasitize fly larvae where up to 80 % of larvae are affected. Flies will not lay eggs on plates with wasp odour, and larvae avoid the odour. Iridomyrmecin, a wasp pheromone, is a constituent of this odour. *D. melanogaster* olfactory sensory neurons which coexpress the receptors Or49a and Or85f are very sensitive to iridomyrmecin. Professor Knaden and his group have identified methyl laurate as the first clear sex pheromone in *D. melanogaster* (Dweck et al. 2015). Olfactory sensory neurons expressing the receptors Or47b and Or88a detect methyl laurate, together with methyl myristate and methyl palmitate. Or47b-expressing neurons only detect methyl laurate, and this compound is required for optimal male copulation, while Or88a-expressing neurons detect all three compounds and this receptor is required for attraction. For further information on olfaction in *D. melanogaster*, readers are referred to the following; (Halle and Carlson 2006; Steck et al. 2012). The next lecture was given by Dr. Elena Savvateeva-Popova (Pavlov Institute of Physiology, St Petersburg, Russia) on the use of *Drosophila* mutants as models in the study of miRNA biomarkers of neurodegenerative diseases (Savvateeva-Popova et al. 2015). miRNAs are small non-coding RNAs that control gene expression, and their levels can change with disease. For example, there is evidence for the involvement of miRNAs in the pathogenesis of neurodegenerative diseases, viz. Alzheimer's disease, Parkinson's disease, Huntington's disease, where the disease may result from dysregulation of miRNAs (Maciotta et al. 2013) and miRNAs have also been implicated in memory formation in *D. melanogaster* (Busto et al. 2015). Dr. Savvateeva-Popova described the miRNA biomarkers for each disease, their equivalents in, and their roles in *D. melanogaster*. The biomarker for Huntington's disease, miR-9, is altered in the *D. melanogaster* mutant *agn^{ts3}*. Levels of let-7 and miR-9 are reduced in this mutant, while the level of miR-34 is increased. Heat shock induces marked defects in learning and memory in *agn^{ts3}*. The reader is referred to the following papers for further information: (Medvedeva et al. 2010; Nikitina et al. 2014; Savvateeva-Popova et al. 2007, 2008). In the final lecture of this session, Dr. Gabor Maász (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) described the neuroprotective function of PACAP (pituitary adenylate cyclase-activating polypeptide) in dopamine-based neurodegeneration. PACAP is a member of the growth hormone-releasing factor family, and its N-terminal domain is highly conserved through the phyla (Kiss and Pirger 2013). In humans, it has 38 amino acids and has three receptors, viz. PAC-1, VPAC-1 and VPAC-2. In invertebrates, it has a number of roles, including feeding, active-inactive state, learning and memory and ageing. In his lecture, Dr. Maász

considered the protective role of PACAP in the snail, *Lymnaea stagnalis*, and the rat, using them as models for Parkinson's disease. The protective effect of PACAP was assessed following treatment with rotenone in the snail and 6-OH-dopamine in the rat, in terms of changes in dopamine and serotonin levels in the brain and changes in dopamine metabolic enzymes. Rotenone inhibited motility and biting activity in *L. stagnalis*. PACAP reduced by about 50 % the rotenone and 6-OH-dopamine effects in both models. Rotenone decreased dopamine and serotonin levels in both models but PACAP only restored the level of dopamine. Proteomic analysis showed that of 95 proteins only the Parkinsonism-associated protein, PARK-7 (DJ-1), was present in controls but absent in both 6-OH-dopamine and 6-OH-dopamine + PACAP-treated groups. Changes in metabolic enzymes were also investigated. Both rotenone and 6-OH-dopamine reduced levels of soluble-catechol-O-methyltransferase in both animal models, and this effect was not reversed with PACAP. Monoamineoxidase-B (MAO-B) was decreased by 6-OH-dopamine which was reversed with PACAP. MAO-B was absent from the brain of *L. stagnalis*. The authors concluded that PACAP was neuroprotective in dopamine-associated neurodegenerative diseases and that rotenone was suitable as an agent for developing and testing models for these diseases.

The Janos Salánki memorial lecture was given by Professor Roger Croll (Dalhousie University, Halifax, Nova Scotia, Canada) on the origins of the nervous system. Professor Croll began by paying tribute to the contribution of Professor Salánki to invertebrate neurobiology. He then gave a historical view of the evolution of the nervous system, stating that there are different ways of investigating the nervous system, starting with morphological characteristics and leading on to molecular genetics. Using information from comparative studies, what can be learnt about the urbilaterian nervous system? Sponges do not have a nervous system with the first nervous system occurring as a diffuse system or nerve net as seen in cnidarians although some medusa have an elaborate nerve ring associated with their manubrium (central opening) (Arendt et al. 2008). Flatworms have been used as a model for early bilaterian protostomes as they have a ventral nerve cord and specialized nervous system. Professor Croll supported the hypothesis that the last common bilaterian ancestor, Urbilateria, possessed a central nervous system which used many of the same developmental mechanisms still important to metazoans. Hox genes play an important role in determining anterior–posterior patterning in vertebrates and invertebrates. There are similarities between the eyes of vertebrates and invertebrates where, for example, Pax-6, which directs expression of the opsin gene, occurs in both vertebrates and invertebrates which argues for relative sophistication of the nervous system before the

split of the protostomes and deuterostomes. There is also the early occurrence of small molecule transmitters, such as acetylcholine, dopamine, serotonin, glutamate and GABA, which are similar throughout the phyla though their functions may differ. Professor Croll considers the nervous system existed before the split resulting in protostomes and deuterostomes, and this common nervous system was constructed using the same genetic networks. Invertebrates have complex life cycles with each stage often possessing a characteristic nervous system (Croll 2016). Professor Croll concluded by focusing on the development and function of the gastropod nervous system from trochophore to veliger to adult to provide novel insights into the degree of sophistication that might have been manifested in the urbilaterian nervous system (Croll 2009; Voronezhskaya and Croll 2015). Professor Croll also presented a poster on his studies to reveal the extent of the peripheral nervous system of molluscs using antibodies. It is clear that there is an extensive peripheral nervous system consisting of numerous neurons and plexuses of axons in larvae and adults of gastropods, bivalves and cephalopods. Using antibodies, the poster described the distribution of FMRFamide, histamine, tyrosine hydroxylase, nitric oxide synthase and GABA, the latter using antibodies against a vesicular GABA transporter. The diversity and number of peripheral neurons mean that we must re-evaluate the role of the central nervous system in molluscs. This session continued with three papers, the first by Professor Pavel Balaban (Institute of Higher Nervous Activity and Neurophysiology, Russian Academy Sciences, Moscow, Russia) on stability and plasticity of memory in the snail, *Helix lucorum*, using a network underlying aversive learning. The study involved the retrieval of memory followed by reconsolidation or extinction of memory performance. Previous studies have shown that inhibition of PMK ζ eliminates long-term memory and a specific form of long-term synaptic plasticity that underlies learning in *H. lucorum* (Balaban et al. 2015). The serotonin system is required for learning but not for the maintenance or retrieval of memory. The present investigation tested whether “reinforcement” serotonin-containing neurons act as the gate condition for choosing between extinction/reconsolidation triggered by memory retrieval. A snail was tethered by its shell on a rotating ball and received electric shocks via its foot. Mechanical stimuli were applied via a hair and tentacle withdrawal recorded. A semi-intact preparation allowed electrophysiological recordings from interneurons involved in feeding and withdrawal behaviour and from the serotonin-containing “reinforcing” neurons. Simultaneously, a drop of juice could be applied to the lip. Following food-aversion training, the activity of the serotonin-containing cerebral interneuron involved in feeding did not change in response to food, while the premotor

FMRFamide-containing interneurons involved in triggering head and foot withdrawal became active to previously subthreshold food stimuli. The possibility that after associate training serotonin-containing pedal neurons may begin to respond during retrieval to previously ineffective stimuli, and thus, triggering reconsolidation was also investigated. Professor Balaban’s group also presented a poster testing a histochemical method for detecting newly formed RNA (Jao and Salic 2008) in the nervous system of juvenile and adult *H. lucorum*. Results showed that RNA synthesis, transition from nuclei to cytoplasm, and its degradation are slow processes in molluscs. Staining reaction occurred faster in the isolated central nervous system of juvenile than in adult snails. There was evidence that the dynamics of RNA transcription and turnover is different in isolated and intact central nervous systems. It is concluded that the low sensitivity and long time for tag identification does not make this a good method for detecting fast events in the nervous system. The next presentation was by Dr. Varvara Dyakonova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) on the impact of enhanced motor activity on brain and behaviour in the cricket, *Gryllus bimaculatus*, and the snail, *Lymnaea stagnalis*. Since the benefits of physical exercise on the nervous system are well known in mammals, the authors decided to extend the investigation to invertebrates to analyse the cellular and molecular mechanisms involved. Both *G. bimaculatus* and *L. stagnalis* increased motor activity altered behaviour and changes in monoamine systems (Dykonova 2014). Intense locomotion in *L. stagnalis* resulted in long-term changes in activity of identified serotonin-containing neurons and their responses to neurotransmitters. Interestingly, when isolated, the enhanced activity persisted in these neurons. Dr. Dyakonova speculated on the possible mechanism involved. In her poster, Dr. Dyakonova presented evidence that isolated neurons from *L. stagnalis* can retain memory of recent behavioural states (Alania et al. 2004; Dykonova et al. 2015). Two behavioural states were used, food deprivation for 24–40 h and forced terrestrial locomotion for 2 h, and intracellular recordings made from serotonin-containing A (PeA) cluster pedal neurons. An increased rate of firing and enhanced depolarization of their membrane potential was seen in neurons isolated from starved snails. After 2 h of increased locomotory activity, neuronal firing rate was also increased but to an even greater degree. These results can be correlated with the nutritional state of the animal and the role of serotonin in food searching and terrestrial locomotion. In summary, an isolated neuron can retain the memory of a recent behaviour. Food deprivation also caused a rise in serotonin but not dopamine levels in the pedal and buccal ganglia. The final talk in this session was by Ms Katleen Peymen (Department of Biology,

Katholieke Universiteit Leuven, Leuven, Belgium) on long-term associative memory assays in the nematode, *Caenorhabditis elegans*. The Leuven group is interested in studying the roles of neuropeptides in short- and long-term memory and consider *C. elegans* an ideal animal to use in the analysis of the underlying cellular and molecular mechanisms associated with memory. Two assays were used, a butanone assay and a high salt assay. Nematocin, related to mammalian vasopressin and oxytocin, and its receptor, NTR-1, are involved in modifying salt chemotaxis in the light of recent experiences, which can be designated a form of short-term gustatory associative learning (Beets et al. 2012). Nematocin (CFLNSCPYRRYamide) is produced by AVK interneurons and activates the NTR-1 receptor located on ASEL neuron which may contribute to the ASE-derived sensitization signal (Beets et al. 2013).

After lunch, Professor Tibor Kiss (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) gave the fourth plenary lecture on spatial odour sensation in stylommatophoran gastropods, using *Helix pomatia* as his model. Snail tentacles are highly flexible with some movements exploring the environment, while other movements sense the environment. Until recently, both large-scale and patterned movements were attributed to the concerted movements of tentacle retractor muscle (TRM) and muscles of tegumentum. Recently, Hernádi and Teyke (2012) have shown the presence of three novel muscles, designated as, M1, M2 and M3 (or flexor muscles, FM), in the posterior tentacles of *H. pomatia*. The tentacle flexor muscles have a resting length of 2–3 mm and are capable of contracting and are extremely tensile. Ultrastructure studies show that these are smooth muscles with a rich and extensive collagen system and sarcoplasmic protrusions, allowing extreme extensibility during olfaction orientation. The flexor muscles also have mitochondria which function as internal calcium stores. Professor Kiss described the anatomy of these muscles which originate from the ventral side of the sensory pad and are anchored at different sites at the base of the tentacle stem. The anchoring point arrangement of FMs at the base of the stem determines the three axes of space and along with separate or synchronous contraction of muscles are able to move tentacles in space. These muscles play a key role in movements, such as twitching, quivering and tentacle bending during olfactory orientation. The innervation of these muscles has been investigated using anterograde neurobiotin tracing via the olfactory and peritentacular nerves (Hernádi and Teyke 2013). There are also peripheral motoneurons in the posterior tentacles of the snail whereby an olfactory stimulus activates a local motoneuron response which generates local lateral movements of the tentacle tip, that is, a quiver (Hernádi et al. 2014). Both acetylcholine (ACh) and glutamate excite tentacular flexor

muscles, while both choline acetyltransferase and vesicular glutamate transporter occur in axons innervating the flexor muscles (Krajcs et al. 2013). Both nerve and transmitter-evoked responses were antagonized by glutamate and ACh antagonists, supporting the idea that both act as endogenous transmitters. Pharmacological studies demonstrated that the ACh receptors are nicotinic slowly desensitizing α BgTx-sensitive responses obtained from flexor muscles and contain α 7-like subunit (Kiss et al. 2014). This is the first demonstration of an obligatory role for a functional α 7-like nAChR at the molluscan periphery. The ACh receptor subunit, α 4, only occurs presynaptically. An appropriate movement of tentacles for environmental cues is important for adjusting the whole organism to its environment. The study showed that monoamine- (5-HT/DA) and/or neuropeptide-mediated (FMRFamide and PACAP) presynaptic facilitation or inhibition provides a mechanism to modulate large-scale and specific tentacles movements. Dopamine decreased electrically evoked contractions of M1/M2 while increasing those of M3 while serotonin potentiated the amplitude of nerve and ACh-induced contractions in all three muscles. FMRFamide attenuated both stimulus and ACh-evoked contractions. PACAP and its receptor occur in nerves innervating the flexor muscle but not in the muscle (Krajcs et al. 2015). PACAP acts both pre- and postsynaptically at the cholinergic synapse, presynaptically via the adenylate cyclase-cAMP-PKA pathway to increase ACh release and postsynaptically by a PKC-mediated pathway to increase muscle contractions by increasing the release of calcium from intracellular stores. The experiments demonstrated that in order to place protracted tentacles and thereby olfactory receptors in an appropriate position for optimal perception of odour stimuli, extraordinary complex movements are required. The three-dimensional scanning movements of the tentacles during exploration of the environment are important to gain information concerning the occurrence and spatial location of the actual odour source. It is concluded that flexor muscles may exclusively be responsible for the tentacle movement around the basal pivot. There then followed three papers on *L. stagnalis*, the first by Professor György Kemenes (University of Sussex, Brighton, UK) on decision-making in the feeding network in relation to searching for food in *L. stagnalis*. There are two types of decision, perceptual-based decisions and value-based decisions, the latter having both internal and external factors. Using *L. stagnalis*, it is possible to study decision-making at a single cell level in a well-defined circuit (Benjamin 2012, 2015). Professor Kemenes used both whole animal behaviour and in vitro preparations containing identified circuits. Snails were starved for up to 4 days prior to experimentation, and the longer the animals were starved so the number of appetitive bites increased. Mechanosensation is the key

modality for judging the presence of food. Professor Kemenes found that the core decision-making system for feeding in *L. stagnalis*, consisted of just two neurons. A command-like neuron, vTN (ventral trigger neuron) in the buccal ganglion, responds to tactile stimulation of the radula and initiates fictive feeding cycles via monosynaptic connections with a feeding central pattern generator (CPG) interneuron, N1 M (N1medial). A dual role, CPG and intrinsic modulatory interneuron, N3t (N3 tonic) provides variable levels of inhibition to N1 M, depending on the satiety state of the animals (Staras et al. 2003). Thus, vTN reports the presence of food, while N3t encodes motivational state and acts as a gain controller for adaptive behaviour in the absence of food. In the second *Lymnaea* talk, Dr. Ildikó Kemenes (University of Sussex, Brighton, UK) discussed lapses during memory consolidation (Marra et al. 2013). Temporary amnesia has been reported during memory consolidation, but its function is difficult to study because its exact timing is unknown. Dr. Kemenes set out to investigate this using a single-trial conditioning paradigm in *L. stagnalis*. Following conditioning, two lapse periods were identified: one after 30 min and a second after 2 h. External stimuli can only disrupt long-term memory consolidation during these two periods. These time points of memory lapse relate to different phases of memory and are associated with different molecular events. The 30-min lapse coincides with the transition from short-term memory at 10 min to intermediate-term memory at 1 h. The 10-min memory trace does not require either *de novo* protein or RNA synthesis, while the 1-, 2- and 3-h memory traces require protein synthesis but not *de novo* RNA synthesis. Dr. Kemenes investigated the possibility that the 2-h point marked another transition between two forms of intermediate memory. The 1-, 2- and 3-h time points were PKA activity dependent. PKC is only required for the 1- and 2-h periods, while memory at 1, 2 and 3 h was dependent on PKM. These results suggest the memory lapse at 2 h is due to a transition from PKC-/PKM-dependent phase of intermediate memory to a PKM-dependent phase (Marra et al. 2013). Interestingly, long-term memory at 4 and 24 h is dependent on neither PKC nor PKM. When a mildly aversive stimulus is used to cause disturbance, disturbance at 30 min only affects memory 4 h after training, while disturbance at 2 h only affects the 24 h memory. It is concluded that during periods of molecular transition memory recall is weakened which allows new sensory events to block long-term memory consolidation (Marra et al. 2013). The final talk in this session was by Professor Etsuro Ito (Tokushima Bunri University, Sanuki, Japan) on the effect of food deprivation on memory acquisition using a conditioned taste aversion (CTA) paradigm (Ito et al. 2015). When *L. stagnalis* are food-deprived for 5 days, learning and memory fail to develop, while optimal CTA is

obtained following starvation for 1 day. Why does this occur? 5-day food-deprived snails were found to learn, but memory consolidation was blocked by severe food deprivation. The context in which snails were trained was important for consolidation of long-term memory. Snails do not express memory phenotype if they are extremely hungry or if they have recovered from lack of food but are tested in a food-satiated state. Memory expression is context dependent and can only be expressed after resolving the conflict between the drive to eat and the memory of learning not to eat. Professor Ito also reported on the importance of insulin for the acquisition of CTA in snails (Mita et al. 2014). Injection of 100 nM insulin into 5-day food-deprived snails decreased haemolymph glucose but learning and memory of CTA occurred. When an insulin receptor antibody, 17.5 nM, was injected 60 min prior to insulin injection into day 5 snails, learning was formed but memory failed to occur. Serotonin plays a role in both feeding and regulation of heart rate in *L. stagnalis*, and its effect on food-deprived snails has been investigated (Yamagishi et al. 2015). Serotonin increased heart rate in food-deprived snails but not in food-satiated snails and improved memory in 5-day food-deprived snails.

Following a poster presentation, there were three talks on nematodes: the first on the potato cyst nematode, *Globodera pallida*, and two talks on the free-living nematode, *C. elegans*. Dr. Anna Crisford (University of Southampton, Southampton, UK) gave a talk on *G. pallida* in which she described work to characterize serotonin neuronal signalling and serotonin receptors in an attempt to develop novel targets for its control. The *G. pallida* stylet is a hollow lance-like structure which can extend from its mouth and is essential for hatching, root invasion, establishing the feeding site and feeding inside the root. Application of serotonin stimulates stylet thrusting in juvenile *G. pallida*. A recent genomic sequence for *G. pallida* has enabled the identification of orthologues of genes encoding serotonin receptors and its synthetic enzymes (Cotton et al. 2014). For example, orthologues for the synthetic enzyme for serotonin, tryptophan hydroxylase, *tph-1*, and a serotonin receptor, *ser-7*, have been identified. *G. pallida tph-1* has around 70 % identity with *C. elegans tph-1*, while *G. pallida ser-7* has around 45 % identity with *ser-7* receptors identified from *C. elegans*. Dr. Crisford described experiments designed to rescue *C. elegans* mutants deficient in *tph-1* and *ser-7* with the orthologues from *G. pallida*. The *C. elegans tph-1(mg280)* was completely rescued in terms of pharyngeal pumping following injection of *G. pallida tph-1*. In contrast, *G. pallida ser-7* only partially rescued the pumping phenotype of *C. elegans*. The next talk was by Professor Lindy Holden-Dye (University of Southampton, Southampton, UK) who described her work on modelling alcohol intoxication and

withdrawal using as a model system, *C. elegans* (Mitchell et al. 2007, 2010). Ethanol can modulate many transmitter functions, including glutamate, GABA, serotonin, dopamine and opioids. Ethanol can also modify voltage-gated channels and intracellular signalling cascades. Chronic ethanol can lead to tolerance and its removal, to withdrawal. *C. elegans* can be used to investigate the mode of action of ethanol at all levels, viz. gene, molecule, single neuron, neuronal circuits and behaviour. Her talk focused on the importance of the concentration dependence of the effect of ethanol on animal behaviour (Dillon et al. 2013) and observations supporting the contention that ethanol rapidly equilibrates across the *C. elegans* cuticle (Mitchell et al. 2007). The final talk in this group was by Dr. Elien Van Sinay (Katholieke Universiteit Leuven, Leuven, Belgium) on the identification of evolutionary conserved neuropeptide systems in *C. elegans* (Frooninckx et al. 2012); <https://worm.peptide-gpcr.org>. Dr. Van Sinay presented details of a project to deorphanize G protein-coupled receptors (GPCRs) associated with neuropeptides in *C. elegans*. Neuropeptides have a range of roles in the nervous system, viz. fast-acting neurotransmitters, neuromodulators or as neurohormones, and play roles in feeding, locomotion, reproduction, social behaviour and learning and memory formation. There are at least 120 precursor genes encoding for over 250 neuropeptides in *C. elegans*. The majority of neuropeptides bind to GPCRs of which about 150 receptor genes have been predicted in *C. elegans*. The aim of the peptide-GPCR project is to link all GPCRs with their respective neuropeptides. Orphan receptors are heterologously expressed in cells and screened using over 300 peptides. Using this approach, the project has identified several evolutionary conserved neuropeptide systems, including one related to mammalian neuropeptide Y (NPY) and insect neuropeptide F (NPF). Expression patterns of the NPY/F precursor and receptor genes suggest a role for NPY/F signalling in the regulation of *C. elegans* feeding behaviour. There was also a poster presented by Dr. Charline Borghgraef from the same group on vitellogenesis in *C. elegans*. The *C. elegans* genome contains 6 *vit* genes, viz. *vit-1* to *vit-6*, which encode types of 3 yolk proteins (vitellogenins), viz. YP170A/B, YP115 and YP88. During these studies, a novel regulator of yolk protein, VRP-1, was identified.

On the final day of the meeting, there were four talks, three on worms and one on a snail. The first talk was by Dr. Tatiana Mayorova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) who discussed a paradoxical effect of serotonin in the regulation of ciliary locomotion in two species of dinophilid polychaete, *Dinophilus gyrocolius* and *Dinophilus taeniatus* (Fofanova et al. 2014). This family is a unique group among the lophotrochozoa, and the two species have very different

life cycles. Adult *D. gyrocolius* are all females, while males are dwarf and occur only at the embryonic stage within a cocoon where they transfer sperm into female embryos and then die. By contrast, *D. taeniatus* have adults of both sexes. The distribution of serotonin in the nervous system was identified in whole mounts using serotonin antibodies. Since the structures making up the serotonergic systems are different in the two species (but quite similar in juveniles and adults of each species) (Fofanova 2014), Dr. Mayorova investigated the effects of serotonin and related compounds on juveniles and adults of the two species. Serotonin increased the gliding locomotion in juveniles of both species, but the same concentration slowed or stopped locomotion in adults of both species. 5-Hydroxytryptophan (5-HTP) had the same effect as serotonin, and when animals were incubated with 5-HTP, the levels of endogenous serotonin in the nervous system increased. It was concluded that the likely explanation for the differences in response to serotonin between juveniles and adults was due to activation of different serotonin receptors. In addition, the differences in morphology of the serotonin systems in the two species did not correlate with its physiological actions. This talk was complemented by a poster on neurogenesis in *D. gyrocolius* and *D. taeniatus*. The first nervous elements were identified using anti-tubulin antibodies, but these early elements failed to show any immunoreactivity to serotonin, FMRFamide or tyrosine hydroxylase though later stages showed immunoreactivity to all three. This observation implies a unique strategy of early neurogenesis of dinophilids among other Lophotrochozoa. The second paper was presented by Dr. Viktor Starunov (St Petersburg State University, St Petersburg, Russia) on the innervation of the annelid pygidium and its evolutionary significance (Starunov et al. 2015; Starunov and Lavrova 2013). There is great diversity in the most posterior part of the annelid body, the pygidium, but relatively little is known about it. The general structure of the pygidium of *Alitta virens* was investigated using antibodies against α -tubulin. The pygidium contains a coelom-like cavity which lacks efferent ducts. The nervous system consists of two tapering nerve trunks which are connected by a thin commissure. In addition, two pairs of nerves arise from these trunks: one pair innervates the rear area of the pygidium, and the other pair innervates the dorsal pygidium. While there are FMRFamide- and serotonin-positive nerve fibres in the pygidium, positive staining cell bodies are absent. Serotonin-positive immunoreactivity was also observed in the commissures and circumpygidial nerve ring. It is suggested that serotonin may act as a sensory transmitter in this structure, while FMRFamide may act at the interneuron level. Dr. Starunov concluded that the annelid pygidium is a far more complex structure than originally thought. The third presentation was by Dr. Elena

Temereva (Moscow State University, Moscow, Russia) on the development of the phoronid nervous system and who presented new data regarding their relationship with protostomes and deuterostomes (Temereva and Tsitrin 2014a, b; Temereva and Wanninger 2012). She studied the development of the nervous system of *Phoronopsis harmeri* using a combination of immunocytochemistry, laser confocal microscopy and transmission electron microscopy. Larvae of *P. harmeri* have a very complex nervous system with an apical organ composed of 4 different neural cell types which include serotonin- and FMRFamide-like immunoreactive components. The digestive system receives both serotonin- and FMRFamide-immunoreactive innervation. Overall organization of the phoronid larval nervous system has greater similarity with the deuterostome than with the protostome nervous system. Dr. Temereva described the remodelling of the nervous system during metamorphosis. The nervous system of all adult phoronids has a similar cytological organization and stratified structure. Adult *P. harmeri* neurons and their projections have serotonin- and FMRFamide-like immunoreactive tracts occurring in the tentacle, tentacular nerve ring, dorsal ganglion, trunk nerve plexus and oesophagus, but the giant nerve fibre lacks such immunoreactivity. Dr. Temereva concluded that the larval phoronid nervous system, due to its complexity, was inherited from the last common bilateral ancestor and then became simplified to accommodate the adult sessile way of life. Dr. Temereva also presented a poster on the innervation of the lophophore and tentacles of brachiopods, using *Lingula anatine* and *Hemithyris psittacea* (Temereva and Tsitrin 2015). The final presentation was by Dr. Zoltán Serfőző (Balaton Limnological Institute, MTA Centre Ecology Research, Tihany, Hungary) on the putative molecular events associated with nitric oxide (NO) release in the olfactory lobe of the procerebrum of the snail, *Helix pomatia* (Nacsa et al. 2012, 2015). Glutamate is an important transmitter in the snail nervous system, and it is likely that glutamate, through activation of an NMDA receptor, stimulates the release of NO. NO-producing neurons in the procerebrum were isolated and labelled using an NO-scavenging fluorescent probe, DAF-2, and analysed using fluorescence microscopy and flow cytometry. DAF-2 labelling was found in both small, 5–8 µm, and larger, 12–15 µm, neurons, and NOS substrates increased the number of labelled neurons, while a NOS or NMDA receptor antagonist decreased the number of labelled neurons. Both NO and odour increased levels of cGMP during eating, while incubation with an NO donor increased the number of neurons showing cGMP immunoreactivity. Evidence suggested that PKG is linked to the NO-cGMP cascade in only limited areas of the procerebrum and may be less involved in NO transmitted

olfactory information in the snail compared with other kinases, such as AKt/PKB.

The poster communications were on display throughout the meeting, and those linked to speakers have already been reviewed. The other posters will now be reviewed briefly. Most of the posters were multi-authored, but only the first author and his/her institute will be given. Where possible, a reference will be included. Dr. Boris Osadchenko's (Lomonosov Moscow State University, Moscow, Russia) poster described the development of the nervous system of the hydrozoan, *Aglantha digitale*. The nervous system begins with the differentiation of two bipolar neurons, one associated with each tentacle. Later, the nerve ring forms which consists of multiple nerve processes, some with RFamide immunoreactivity. RF-amide immunoreactivity only occurred in neuron bodies associated with the tentacles and manubrium. Evidence indicated that the development of the nervous system of *A. digitale* was atypical of hydrozoan medusa. There were three posters on echinoderms. Dr. Tatiana Mayorova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) described the localization of transcripts encoding L-type (S1) and F-type (S2) SALMFamide precursors in brachiolaria larvae of *Asterias rubens* using in situ hybridization (Elphick et al. 2013). The transcripts encoding the L- and F-type precursors are expressed in two different populations of cells. Dr. Masao Migit (Shiga University, Otsu, Japan) presented a new model of righting behaviour in the starfish, *Asterina pectinifera*, in an attempt to analyse its complex righting behaviour. Dr. Alexandra Obukhova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) investigated the role of dopamine in the regulation of swimming behaviour in *Mesocentrotus (Strongylocentrotus) nudus* from early blastula up to 4-arm pluteus. Dopamine immunoreactivity was found in the Golgi apparatus at non-neuronal (blastula and gastrula) stages, which could be released to stimulate the cilia during hatching. Raising the level of dopamine induced rotational acceleration at all stages examined. Dopamine increased swimming speed at non-neuronal stages while inhibiting direct swimming at the neuronal stage of development. There were three posters on research using lamellibranchs. Mr Masahito Okutani (Tokyo Metropolitan University, Tokyo, Japan) used behavioural, morphological and pharmacological analyses to investigate the neuronal mechanism regulating the labial palps during suspension feeding in *Mytilus galloprovincialis*. Serotonin immunoreactivity occurred in the neural processes associated with the cilia of the labial palps, while both serotonin and dopamine excited most groups of cilia. Mr Okutani proposed that monoamines controlled the ciliary and muscular movements of the labial palps which function to select particles for ingestion or rejection. Dr. Izabella

Battonyai (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) described the serotonin and FMRFamide innervation of the foot and byssus retractor muscle of *Dreissena polymorpha* and *Dreissena bugensis*. In a second poster, Dr. Battonyai presented data on the development of the nervous system of the trochophore and veliger stages of *D. polymorpha* and the appearance of serotonin and FMRFamide immunoreactivity. Application of 5-hydroxytryptophan enhanced swimming activity, while p-chlorophenylalanine, a serotonin synthesis blocker, slowed swimming. Dr. Tatiana Korshunova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) examined how the balance of serotonin and dopamine determines the locomotor pattern of the central pattern generator in the pteropod mollusc, *Clione limacine* (Arshavsky et al. 1998). The regularity of interneuron I2 plateau potentials is determined by this serotonin/dopamine balance. In his poster, Dr. Makoto Kurokawa (Tokyo Metropolitan University, Tokyo, Japan) compared the structure and function of two species of Aplysiidae, *Bursatella leachii* and *Aplysia sp.*, and then compared the results with earlier work on *L. stagnalis* (Kurokawa et al. 2012). Ms Zita Zrinyi (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) investigated the action of synthetic steroid oral contraceptives (endocrine disruptors) which enter the aquatic environment on the embryonic development of *L. stagnalis* with particular reference to changes in lipid and peptide profiles of embryos. Mr Sándor Lovas, from the same Institute, presented a related poster on the effect of endocrine disruptors on the activity of identified neurons from *L. stagnalis*. The firing rate of cerebral giant neurons was decreased by steroids, and this effect was mediated by increased inward calcium currents, an effect not seen in another neuron, RPeD1. Dr. Dóra Petrovics (University of Pecs, Pecs, Hungary) presented evidence that B-myo-like protein plays a key role in embryonic development in *L. stagnalis*. Dr. Hiroshi Sunada's (Tokushima Bunri University, Sanuki, Shido, Japan) poster showed that traumatic stress caused long-lasting but reversible impairment in learning and memory in *L. stagnalis* and that deficiencies in memory due to traumatic stress involved the endocannabinoid system. Dr. Marina Khabarova (Institute of Developmental Biology, Russian Academy Sciences, Moscow, Russia) reported on the effect of a chemical factor emitted by adults on serotonin receptors in embryos of the snail, *Helisoma trivolvis* (Voronezhskaya et al. 2008). During 2 years, embryos were exposed to the chemical factor which was collected monthly and also to serotonin receptor agonists at two stages, premetamorphic and metamorphic. Evidence would suggest that different serotonin receptors are expressed through the year which may be linked to

seasonal variations in the sensitivity of embryos to the chemical factor. Dr. Suguru Kobayashi (Tokushima Bunri University, Sanuki, Kagawa, Japan) reported on oscillatory neuronal networks formed from dispersed neurons in culture of *L. stagnalis* procerebrum. He proposed that ACh, acting through nicotinic receptors, can act as an excitatory transmitter in cultured procerebrum neuron networks. Dr. Natalia Vasilyeva (Moscow Lomonosov State University and Institute of Higher Nervous Activity and Neurophysiology, Russian Academy Sciences, Moscow, Russia) reported on the role of kinesin Eg5 and dynein proteins in short-term depression of acetylcholine-induced chloride currents in premotor interneurons of *Helix lucorum* (Vasil'yeva et al. 2015). A dynein inhibitor decreased the rate of ACh depression, while kinesin inhibitors reduced the initial rate of depression. Evidence was presented that dynein and kinesin are involved in recycling ACh receptors in these neurons. There is also evidence that reciprocal interactions between dyneins and kinesins, located on the same vesicle, can lead to reversal of their usual direction of transport. The poster of Dr. Aleksey Malyshev (Institute of Higher Nervous Activity and Neurophysiology, Russian Academy Sciences, Moscow, Russia) reported on the relationship of slow dynamics of action potential initiation and impaired encoding of *H. lucorum* neurons grown in culture in conditions which prevent cell branching. This work complements earlier research using rat neurons (Volgushev et al. 2008). From his experiments, Dr. Malyshev concluded that the tight relationship between dynamics of action potential initiation and encoding abilities of the neuron is universal across phyla. Dr. Alena Zuzina (Institute of Higher Nervous Activity and Neurophysiology, Russian Academy Sciences, Moscow, Russia) described an "artificial synapse" using parietal giant withdrawal neurons of *H. lucorum* (Balaban et al. 2004). Application of serotonin to this preparation resulted in long-term facilitation of glutamate-induced responses, while addition of ZIP (zeta inhibitory peptide, a protein kinase M ζ inhibitor) caused a short-term transient decrease in the glutamate response of this preparation. However, scrambled ZIP also has a similar effect, suggesting that the ZIP effect is a non-specific membrane effect on these neurons. There were two posters on neonicotinoids, the insect pesticides which have attracted a lot of research due to their effects on non-target insect pollinators. Dr. Ágnes Vehovszky (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) described the effects of neonicotinoids on a cholinergic synapse between identified neurons in *L. stagnalis* and *Helix aspersa* (Vehovszky et al. 2015). At low concentrations (<0.001 mg/ml), both acetamiprid and thiamethoxam acted as acetylcholine (ACh) agonists on *H. pomatia* ACh-evoked depolarizations, but at higher concentrations (0.001–1.0 mg/ml),

they acted as antagonists, both against applied ACh-evoked depolarizations in *H. pomatia* and synaptically evoked cholinergic excitatory postsynaptic potentials in *L. stagnalis*. Of a range of neonicotinoids tested, thiacloprid proved the most potent. In the second poster, Dr. János Gyóri (Balaton Limnological Institute, MTA Centre for Ecological Research, Tihany, Hungary) presented results on the effect of four commercial neonicotinoids on purified eel ACh esterase. Commercial neonicotinoids inhibited ACh esterase in a concentration-dependent manner where kinetic analysis of Michaelis–Menten plots showed a predominantly competitive mechanism. It was concluded that neuronal ACh esterase is likely to be one of the sites for neonicotinoid toxicity in insects though they may not act in a uniform manner regarding their antagonism. Dr. Alexey Polilov (Lomonosov Moscow State University, Moscow, Russia) presented two posters: one with Dr. A Makarova of the same university on the link between insect size and size of their neurons and one on insect connectomes (Polilov 2012). Microinsects not only have smaller neurons but also have fewer neurons. Microinsects tend to have neurons of a uniform size and nuclear–cytoplasm ratio. The second poster described 3D electron microscopy methods for studying the connectome of microinsects. Dr. Tohru Moriyama (Shinshu University, Ueda, Nagano, Japan) described experiments in which the antennae of the pill bug, *Armadillidium vulgare*, were extended using short Teflon tubes and tracked moving down a flight of stairs. Dr. Moriyama proposed that large (11.2 mm body length) pill bugs might use the Teflon tubes as tools to explore their environment to aid their descent (Moriyama et al. 2016). Dr. Takayuki Watanabe (Hokkaido University, Sapporo, Japan) presented two posters using the cricket, *Gryllus bimaculatus*. The first poster outlined reverse genetic techniques used to study neurogenetics in *G. bimaculatus*, while the second poster investigated sex determination genes linked to neuronal circuits underlying sex-specific behaviours (Watanabe and Aonuma 2014). Cricket homologues of *Drosophila* involved in sex determination were identified, viz. *sex-lethal (sxl)*, *transformer (tra)*, *transformer 2 (tra 2)*, *fruitless (fru)*, *doublesex (dsx)*, *intersex (ix)* and their expression in the central nervous system determined. Antibodies to *G. bimaculatus* FRU protein were used to compare its distribution in the central nervous system of male and female crickets. Dr. Ekaterina Nikitina (Pavlov Institute of Physiology, St Petersburg, Russia) introduced *Drosophila* as a model for determining the molecular basis of dementia (Savvateeva-Popova et al. 2007). The mutant *cardinal (cd)*, showing an excess of 3-hydroxykynurenine) demonstrated age-dependent memory loss together with synaptic pathology, changes in brain volume and Congo red-positive inclusions. Learning and long-term memory were assessed in *cd* mutants using a

conditioned courtship suppression paradigm following heat shock as a stress factor. Memory loss and loss of learning index were observed. The *cd* mutant was proposed as a model for Huntington's disease.

The final session of oral presentations was followed by a general assembly of ISIN at which the future of the society was discussed. There was a consensus that the 4 yearly conference organized by the ISIN continues to provide an excellent forum to bring together scientists from around the world who share a fascination with the neural basis of behaviour of a range of invertebrates. It serves an important role in facilitating international collaboration in this arena, and there was strong support for another meeting in 4 years.

The concluding remarks and thanks to the organizers were delivered by the outgoing president of the ISIN, Professor Etsuro Ito. Professor Elekes explained that due to new government legislation the ISIN would no longer formally exist as a society but that this would not present a bar to organising the next meeting at Tihany. If you would like to make suggestions for the next conference to be held in 2019, please contact Professor Elekes, or the corresponding author of this paper.

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Compliance with ethical standards

Conflict of interest None.

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