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EDITORIAL

Ligand-gated ion channels: from genes to behaviour

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The study of ligand-gated ion-channels was born out of a marriage of convenience between early pharmacologists intent on developing a drug-receptor theory (Colquhoun, 2006) and pioneering neurophysiologists wishing to map out the many neurotransmitter pathways of the nervous system (Krnjevic, 1974, 2010). Investigators gravitated towards the cholinergic synapse of the neuromuscular junction in the late 1950s (Colquhoun & Sakmann, 1998) and produced our first serious treatise of agonist behaviour (Del Castillo & Katz, 1957), analysis at the single-channel level (Neher & Sakmann, 1976; Colquhoun & Sakmann, 1985) and ultimately the cloning of the nicotinic acetylcholine receptor (nAChR) (Sakmann *et al.* 1985). *The Journal of Physiology* played an integral role in many of these advances and has continued this tradition as the study of ligand-gated ion channels has broadened ever further over the decades to include investigators from many other disciplines.

It was therefore fitting that when the GEPROM (Groupe d'Étude des Protéines Membranaires) research group in Montréal decided to organize a two-day scientific symposium last June to focus on ligand-gated ion-channels, it did so in partnership with the *Journal of Physiology*. The symposium drew upon the expertise of 15 internationally recognized speakers from different disciplines (Fig. 1) to each give their unique perspective on 'Ligand-gated ion channels: from genes to behaviour'. This issue of *The Journal of Physiology* collects the symposium reviews from eight of these invited speakers (Auerbach, 2012; Bowie, 2012; Diaz, 2012; Furukawa, 2012; Krishnaswamy & Cooper, 2012; Liu & Savtchouk, 2012; Popescu, 2012; Yan & Tomita, 2012).

The first session on 'Gene transcription and translation' was presented by Matthew Jones from the University of Wisconsin in Madison and Siqiong June Liu from Louisiana State University. The Jones lab has been working on defective genes in patients with idiopathic generalized epilepsy (IGE) that often encode inhibitory $GABA_A$ receptor subunits. The GABA $\gamma 2$ (R43Q) mutation has been linked to absence seizures in humans with IGE (Goldschen-Ohm *et al.* 2010). Dr Jones explained that the consequence of this particular amino acid residue is to disrupt intersubunit interfaces involved in binding of GABA and therapeutic agents, such as benzodiazepines. How alterations in excitatory neurotransmission are influenced by experience was elegantly portrayed by Siqiong June Liu (Liu & Savtchouk, 2012). A single fear-inducing stimulus profoundly affects both glutamatergic transmission and the excitability of inhibitory stellate cells of the cerebellum (Liu *et al.* 2010; Savtchouk & Liu, 2011). Teaming up with Suzanne Zukin from the Albert Einstein College of Medicine in New York, she showed that fear upregulates GluA2 AMPA-type ionotropic glutamate receptor (iGluR) gene which affects synaptic efficacy over the next several hours and suggests that the GluA2 gene is a molecular switch tuned to respond to changes in an individual's emotional state.

In the second session on 'Signalling complex assemblies', Susumu Tomita from Yale University and Elva Diaz from the University of California at Davis each presented their work on NETO1/2 and SynDIG1 auxiliary proteins that bind to kainate- (KAR) and AMPA-type iGluRs, respectively (Diaz, 2012; Yan & Tomita, 2012). The results with NETO1/2 explain the long-standing conundrum as to why native receptors have slow channel kinetics compared to recombinant KARs (Zhang *et al.* 2009; Straub *et al.* 2011) and how heteromeric KARs have a more tardy response to neurotransmitter (Barberis *et al.* 2008). Examination of another auxiliary protein family, namely the transmembrane AMPA receptor regulatory proteins or TARPs (Straub & Tomita, 2011). Dr Tomita has provided some defining criteria that are unique to auxiliary proteins (Yan & Tomita, 2012). Elva Diaz presented an enthralling account of how SynDIG1 was plucked out of the cerebellum by a DNA microarray approach (Diaz *et al.* 2002). SynDIG1 colocalizes with AMPARs at glutamatergic synapses, regulating their number (Kalashnikova *et al.* 2010) and providing a molecular anchor that may be critical for the recruitment of AMPARs to nascent synapses during synaptogenesis.

The final session of the first day focused on 'Physiology and disease' with all three speakers coming from Montreal's oldest academic and teaching institution, McGill University. Ellis Cooper summarized a series of elegant studies linking the effects of reactive oxygen species (ROS) of neuronal nAChRs to dysautonomias in patients with diabetes (Campanucci *et al.* 2008, 2010). ROS are able to do this by cross-linking a ring of highly conserved cysteine residues in the cytoplasmic mouth of the pore. Curiously, muscle nAChRs lack these cysteine residues and thus skeletal muscle function is spared from the detrimental effects of ROS (Krishnaswamy & Cooper, 2012). Charles Bourque presented an exciting account of a predominant role for novel transient receptor potential (TRP) channels in osmo- and thermoregulation (Bourque, 2008; Sudbury *et al.* 2010) and future work which may lead to a new generation of centrally acting therapeutic agents to control hypertension. I presented the final talk on synapses lacking NMDA-type iGluRs and how they are able to actively recruit AMPARs during development. We have shown that sensory experience drives a switch in AMPAR properties in the developing mammalian retina (Osswald *et al.* 2007). Curiously, the calcium-permeable AMPARs that are expressed in the adult retina are resistant to the effect of polyamine-based compounds routinely used to block them. Other investigators have reported similar findings over the last two decades suggesting that a third, previously unappreciated class of AMPA-type iGluR may be expressed in the vertebrate CNS (Bowie, 2012).

The second day started in earnest with a session on 'Structure at atomic resolution'. Hiro Furkawa from Cold Spring Harbor Laboratories presented unpublished data on the unexpected structural properties of the amino-terminal domain (ATD) of NMDA-type iGluRs which is a key structural determinant of both the gating and allosteric properties of the NMDARs (Furukawa, 2012). Dr Furukawa left little opportunity for his findings to be scooped

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Figure 1. Photograph of the participants at the two-day GEPROM symposium on ligand-gated ion channels including invited speakers, student presenters and members of the organizing committee
From left to right, back row, A. Cottreau, R. Blunck, E. Cooper, G. Popescu, S. Tomita, H. Furukawa, R. Robert, C. Bourque, A. Auerbach, M. Jones, J.-Y. Lapointe; front row, D. Bowie, R. Gaudet, P. Brown, E. Diaz, S. J. Liu, M. Li, C. Czajkowski and S. Wall-Lacelle.

since at the end of his 30 minute talk, the data he presented had just appeared in that morning's issue of *Nature*! (Karakas *et al.* 2011). Rachelle Gaudet from Harvard University gave an equally compelling presentation describing the properties of ankyrin repeats in TRPV1 channels (Lishko *et al.* 2007) and the unappreciated role of this domain in pain sensation.

The morning concluded with a session on 'New approaches' with both Rikard Blunck, from the Université de Montréal, and Yves De Koninck, from the Université de Laval, presenting complementary approaches on how to ascertain the subunit copy number of a neurotransmitter receptor. Hugo McGuire in Rikard Blunck's lab working with Mark Aurousseau from my own lab have developed a method modified from Ehud Isacoff's lab (Ulbrich & Isacoff, 2007). In essence, they determine the number of subunits per receptor by counting bleaching steps of GFP fused to the protein of interest, using total internal reflection fluorescence (TIRF) microscopy. Working with Paul Wiseman at McGill, Dr De Konick's lab developed a more computationally demanding approach based on spatial intensity distribution analysis (or SpIDA) to directly measure oligomerization states using standard fluorescence microscopy (Godin *et al.* 2011).

The symposium concluded with a feast of presentations on the 'Gating mechanisms'. Gabriela Popescu from the State University of New York in Buffalo gave an absorbing talk on the nature of NMDAR modal gating. As discussed in her review article (Popescu, 2012), modal-gating offers an effective regulatory mechanism by which to control channel activation. Mufeng Li, a talented postdoctoral fellow from Kenton Swartz's lab at the US National Institutes of Health, presented data on the trimeric pore of Purinergic 2X receptor channels (Li *et al.* 2008, 2010). She described how the structure of the pore changes during gating by measuring the thiol modification of introduced cysteine residues and by engineering metal bridges. Her data point to a unique movement of the pore region during gating where the initial displacement of the external pore is accompanied by a significant narrowing of the inner pore (Li *et al.* 2010). Cynthia Czajkowski from the University of Wisconsin in Madison has been using site-directed spin labelling electron paramagnetic resonance spectroscopy (or SDSL-EPR) and voltage-clamp fluorometry to study the gating motions of the prokaryotic Cys-loop receptor, pGLIC. She showed a series of experiments demonstrating proof of principle that this technique can give a functional readout of the gating motions and so complement the static structures obtained by X-ray crystallography. The final speaker of the symposium was Anthony Auerbach also from the State University of New York in Buffalo. Over the last few decades, Dr Auerbach's team has pioneered a greater understanding of the most prototypical of all ligand-gated ion-channels, the nAChR. He gave a historical background to the seminal work by Pierre Changeux's lab (Monod *et al.* 1965) and how analysis of nAChR mutants continues to support and advance the original findings reported more than half century and argued that the formalism described by Monod, Wyman and Changeux may be as informative when applied to other ligand-gated ion-channels (Auerbach, 2012).

The ongoing challenge for the future will be to bring motion to the emerging structural models of ligand-gated ion-channel families. The two-day GEPROM symposium offered some insights in how to reach this goal. But clearly, much remains to be understood of the complex molecular events triggered by agonist binding to neurotransmitter receptors.

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