Project Proposal

-

Investigating intracellular mechanisms of sleep in Drosophila Melanogaster

Gergo Bohner

September 14, 2017

Abstract

Sleep is a vital function for most animals, and as such it has been investigated on many levels, including behaviour, neural networks, as well as genetics. Recent studies in Drosophila Melanogaster - a widely used animal model for human sleep disorders - have pinpointed a small number of neurons in the dorsal fan-shaped body (dFB), where sleep pressure and wake signals seem to converge.

In addition to dFB neurons' high degree of control over the fly's sleep-wake behaviour, they do so via a noteworthy mechanism, by operating as a binary switch. During sleep they are part of a central pattern generator network emitting spikes periodically, but given a short dopamine wake signal they dramatically change their membrane properties and stay completely silent for long periods of time - while the animal is awake.

I propose to further investigate the dFB neurons in vivo to gain insight into this crucial system. Using sleep assays followed by electrophysiology, one needs to establish in more detail how cells to change their electrical signal transmission behaviours in response to neuromodulatory inputs, as well as how those changes materialise in terms of membrane properties - with particular attention to the time courses of the effects. By sketching up a set of plausible pathways based on previous literature, we can find the intracellular actors that may mediate the effects, then follow or selectively disable them using the vast genetic, pharmacological and imaging tools already available for drosophilae.

These set of studies would provide new molecular targets for sleep disorder drugs, or even enable us to repurpose existing ones by understanding the correct dosage and administration time scales. Furthermore, the added insight into how living neurons store the effects of short signals for extended periods could aid us in designing novel and more biologically plausible artificial ones to be used in neural networks.

1 Introduction

The Drosophila Melanogaster sleep-wake cycle is under scrupulous investigation, both to provide a deeper understanding of the ubiquitous circadian rhythm, and to find new molecular targets in an animal model of human sleep disorders [1][2]. Dopamine's crucial role as an arousal signal has been established over the past decade, using pharmacology [3][4], genetics [5][6] and imaging [7]. Recent advances have located a small set of neurons in the dorsal fan-shaped body (dFB), where sleep pressure and dopaminergic wake signals converge [8][9]. These neurons single-handedly control the animal's sleeping behaviour, their artificial activation induces sleep, while reduced excitability leads to insomnia [5]. Physiologically, evidence shows that their excitability is modulated by the activity of upstream dopaminergic neurons, and that this effect is mediated by DopR receptors expressed on the surface of dFB neurons [7].

The main behavioural and cellular responses to artificial dopamine release at the dendritic tuft of dFB neurons are summarised in Figure 1. During sleep, the neurons are the output arm of a central pattern generator network, emitting spikes periodically. Given a dopamine wake signal, the membrane resistance and time constant drop over several minutes, and the cells are no longer firing spikes, even when severely depolarised [9]. The neuron then remains in this quiet state for substantial periods. Eventually the cells return to their periodic firing and the animal sleeps. Although the signals mediating the sleep pressure and the time courses of cellular changes during falling asleep are largely unknown, the data available for this process is consistent with the arousal process: sleep deprivation increases the dFB membrane resistance and time constant in wild type animals [8].

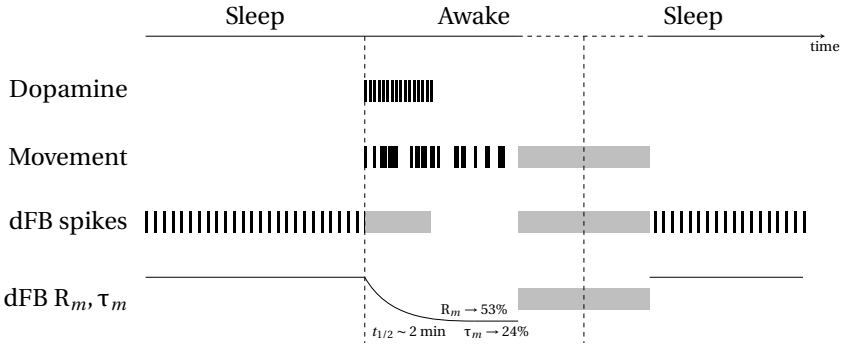

Figure 1: **Schematic responses to dopamine arousal signal.** Graphical summary of known experimental facts. A single large dopamine pulse immediately wakes the animal up; a few minutes of smaller, sustained, high frequency dopamine ejections change the dFB membrane properties, turning off the neuronal spiking for a substantial amount of time. Vertical lines indicate detected event times, grey rectangles are missing/inconclusive data.

There is little known of the intracellular actors translating the dopamine signal into long-term excitability changes. The individual actors, especially ones identified to be specific to wakefulness, may serve as drug targets. Furthermore, this process is of particular interest from a computational point of view too, as the deeper understanding of such internal state dynamics could inform our artificial neuron models and lead to better LSTM cells [10].

Using primarily gene knockdown experiments, a few facts have been established. The G-protein coupled dopamine receptor Dop1R2 is needed for both immediate arousal and long-term wakefulness. Blocking exocytosis or knocking down the potassium leak channel *Sandman* disable the quiet dFB state and thus increase daily sleep [9]. Translating sleep pressure into membrane property changes require the Rho-GTPase-activating protein encoded by the *crossveinless-c* gene [8].

In this proposal I wish to take a systematic approach and dissect this fascinating cellular system. I start by establishing the plausible pathways that translate a dopamine signal into lasting intracellular changes (Section 2), then suggest a set of experimental methods that examine the importance of individual components, as well as sketch up a timeline of changes (Section 3). Once the key actors have been identified, one can follow their time courses to fill in the gaps of knowledge during the recommencement of sleep, which could lead to identifying the main protein partners and upstream signals that mediate sleep pressure. These comprehensive set of studies would provide new molecular targets for sleep disorder drugs and deepen our insight into the dynamics of the internal cell state, to spawn future research both in biology and artificial neural networks.

2 Intracellular pathways

As learned from previous experiments, dopamine release at the dendritic tuft of dFB neurons cause the cells' membrane properties to change dramatically within minutes, and remain in their modified state for longer bouts, even with no further dopamine input. This change requires the G-protein coupled dopamine receptor, *Dop1R2*, as well as membrane transport that changes the ion channel distribution of the membrane. What are the pathways that may translate a ligand-bound Dop1R2 receptor to sustained reorganisation of the membrane?

The membrane's properties primarily depend on the type, number and exact amino acid sequence of the embedded ion channels. At any time point, only a low percentage of the total ensemble of functional transmembrane proteins available to the cell are expressed on the outer membrane, with the majority residing on the surface of endosomes or in other intracellular stores. The strongly regulated membrane trafficking system keeps a dynamic balance between the stored and expressed ion channels and receptors, with transmembrane protein dwelling rates varying from seconds to minutes, based on species [11][12].

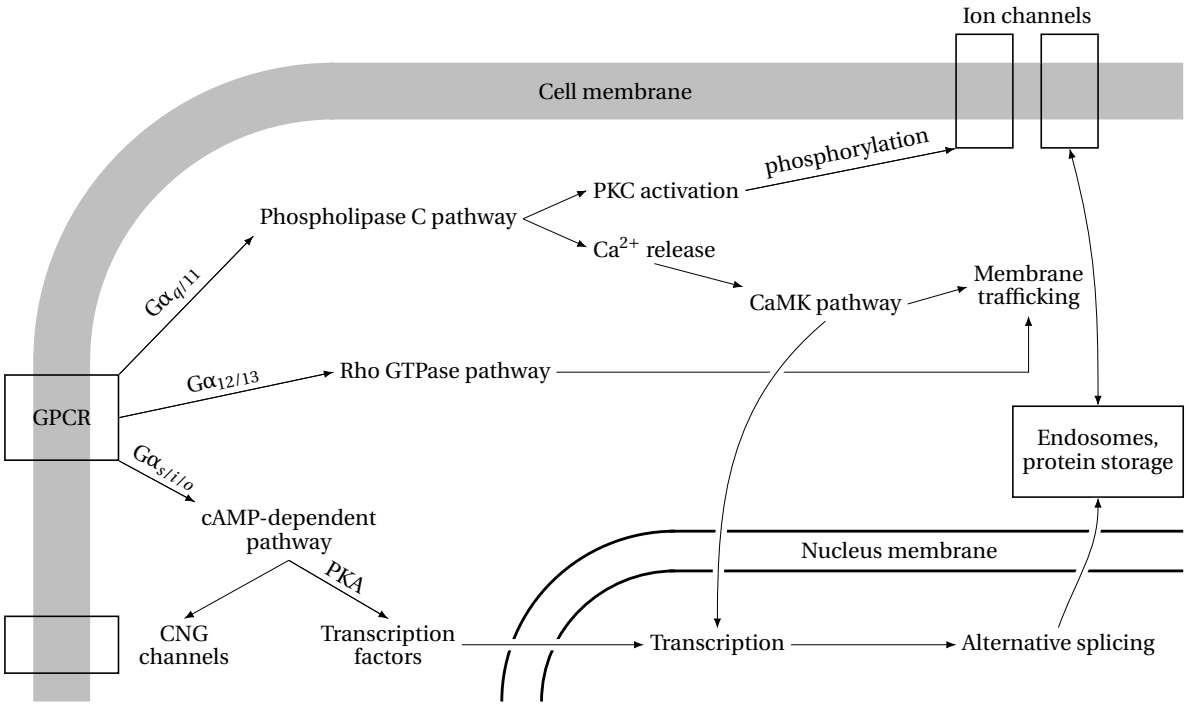

Figure 2: **Intracellular pathways.** The plausible ways of extracellular dopamine binding to a GPCR affecting the membrane properties.

The main ways of modulating membrane composition are to 1. modify or mark particular ion channel species for removal of the membrane via phosphorylation, 2. regulate the proteins involved in trafficking to change the dynamic balance, or 3. adapt the makeup of the total protein ensemble; ordered by the increasing time to their effect (Figure 2, right).

Extracellular dopamine binding to a G-protein coupled receptor (GPCR) is capable of affecting each of the above, depending on the type of the Gα subunit of the bound G-protein that the receptor activates [13]. Generally a given GPCR binds extremely preferentially to a particular subunit type, but unfortunately *Dop1R2* has been experimentally shown to bind all major types [14][15], and thus we can not disregard any of the initiated pathways. As shown on the left side of Figure 2, there are three major downstream pathways from *Dop1R2*, roughly corresponding to the three ways of modulating the membrane composition:

1. Phosphorylation of membrane-embedded proteins

The G$\alpha_{q/11}$ subunits activate phospholipase C (Figure 2, top), which cleaves membrane embedded phospholipids at the phosphate group. Through a molecular cascade this results in the release of calcium ions from intracellular stores into the cytosol, causing an up to

3-fold increase in Ca^{2+} concentration within minutes [16]. Additionally, it leads to the activation of protein kinase C (PKC) as well as calcium and calmodulin dependent kinases (CaMK). Phosphorylation by these kinases may directly affect ion channel properties [17][18] or mark integral membrane proteins for internalisation via employing the arrestin - adaptor protein cascade for clathrin-mediated endocytosis [19], providing the fastest routes to change the membrane properties in response to dopamine binding.

A further long-lasting effect of GPCR activation may involve the ligand-bound GPCR's migration from cell membrane to the surface of endosomes via autophosphorylation. Interestingly, blocking specifically GPCR endocytosis has no effect on the immediate GPCR signalling, however it does affect longer term activity of various kinases [16], as the ligand enclosed in the small vesicle have a high probability of binding to and thus keeping the endosomal GPCR active. This suggests a role for internalised GPCRs in mediating long-lasting effects of GPCR activation.

2. Modulation of trafficking proteins

The GPCR may directly or via a $G\alpha_{12/13}$ subunit activate small monomeric GTPases (Figure 2, middle), most importantly from the Rho family [20]. Two notable members, RhoA and Cdc42, play a role in clathrin-independent endocytosis [19], whose role is corroborated by the fact that the Rho-GTPase-activating protein encoded by the *Cv-c* gene also affects sleep [8]. On a wider scope - outside of sleep - a number of studies show that trafficking is essential for homeostatis, and involves various ion-channels [21][19][22][23]. Endocytotic regulatory elements were discussed above, exocytosis of certain ion channels depends on 14-3-3 adaptor proteins [24]. These are phosphorylated by CaMKII [25], a kinase activated by the first pathway discussed. The activation of proteins involved in trafficking shifts the dynamic balance between expressed and stored ion channels and thus changes membrane makeup.

3. Nuclear regulation of protein synthesis

The $G\alpha_{s/i/o}$ subunit family regulates adenylyl cyclase activity (Figure 2, bottom), vastly increasing the intracellular cAMP concentration, up to 140-fold in minutes [16]. This in turn elevates cAMP-dependent kinase (PKA) levels. PKA is a major intracellular actor that phosphorylates various transcription factors, just like PKC and CaMK. These activated kinases thus regulate the de-novo protein synthesis process within the nucleus. The resulting change in the makeup of the total protein ensemble available to the cell affects it on longer timescales - possibly lasting hours - as predicted by measured protein half-lives [11].

Intranuclear regulatory networks are largely unexplored. A recent comprehensive study identified nearly 1000 genes, 7% of the fly genome, that encode proteins directly influencing transcription within the nucleus [26]. Relevant examples include changing neural excitability via dynamic DNA methylation of ion channel genes [27], affecting Drosophila sleep via transcription factors in a cAMP dependent manner (CREB [28]) or modulating the activity of endocytosis pathway protein coding genes (tfAP2 [29]). Furthermore, the

properties of newly synthesised ion channels depend on their exact amino acid sequence, which can be modulated in an activity dependent manner during RNA splicing (Pasilla and Pumilio [30]).

There is little data regarding the precise time courses of these pathways. Phosphorylation kinetics have been studied extensively, but mostly theoretically or in simplified in vitro assays [31]. It has been observed in an eukaryotic cell model, that in response to environmental stress, certain regulatory elements are phosphorylated within 3 minutes, which then led to a peak transcription of stress-responsive genes into mRNA in 10 minutes and functional proteins within 30 minutes [32]. In neurons, de novo ion channel synthesis affects membrane composition within 25 minutes in response to a glutamate receptor agonist [11].

3 Experimental methods

I wish to find out the importance of the individual pathways detailed above and sketch up both the time courses from the arousal stimulus to observable changes, as well as the return to baseline activity for each key component. This would lead to identifying molecular targets for sleep disorder drugs, understanding how cells lastingly shift their internal state in response to a short signal, and elucidate some characteristics of the sleep pressure signal.

As drosophilae are a workhorse of neuroscience, a large number of genetic lines, experimental assays and analytical techniques are commercially available. Here I shortly describe a number of increasingly complex experimental tools (Figure 3) and how they will aid us in answering the questions.

A. Sleep assay for pre-screening
Considering the number of proteins potentially involved and tested, it is indispensable to have a high-throughput screening method to drive the experimental focus towards the interesting candidates. The 32 tube automated Trikinetics Drosophila Activity Monitor[1] system (Figure 3A) is capable of simultaneously assessing the sleep-wake status of individual flies by monitoring movement via infra-red beam crossings. Amended by a temperature- and light-controlled box to set the natural circadian rhythm, as well as a vibrating desk to sleep-deprive groups of flies, we can quickly assess various behavioural impacts of an experimental modification using a control and a modified group of flies.

B. Targeted and inducible genetics
The simplest type of modification is the localised and inducible knockdown of suspected proteins. There are 7000 established transgenic Drosophila driver lines that express GAL4

[1] http://www.trikinetics.com/

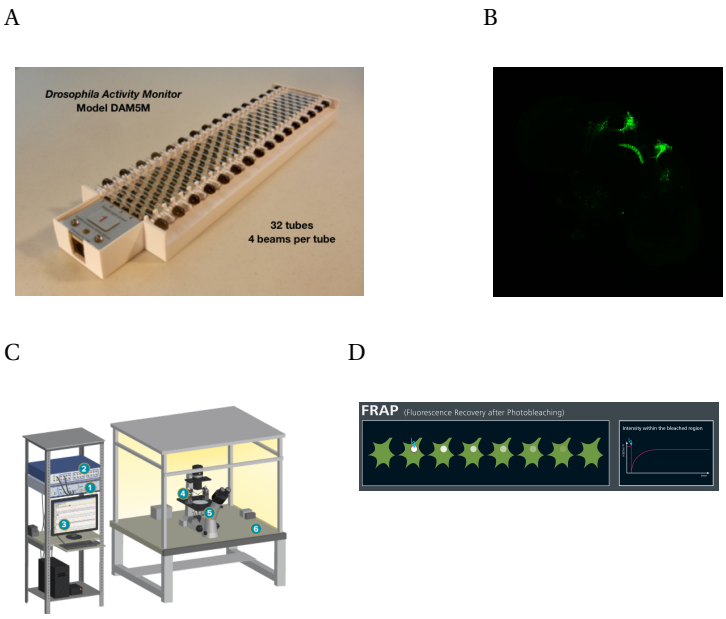

Figure 3: **Experimental techniques.** A. Trikinetics Drosophila Activity Monitor B. Gfp expressed via the GAL4/UAS system using the R23E10-GAL4 driver line shows specific expression in dFB neurons C. Molecular Devices patch clamp rig for imaging and electrophysiology in head-fixed flies D. Flourescence recovery after photobleaching[4] to assess the trafficking time courses

in various parts of the CNS [33], one of which (R23E10-GAL4) is specific to dFB[2] (Figure 3B). GAL4 is a DNA binding protein that binds to a specific enhancer sequence UAS and facilitates transcription of genes downstream from UAS. Expressing transgenic RNAi constructs driven by UAS-Dcr2 enables the cell line specific knockout of select genes [34]. GAL80 is a repressor of the GAL4/UAS system, and importantly has a temperature-sensitive mutant, GAL80^{ts}, that is active at low, permissive temperatures (18 ^{o}C), but enables the UAS-coupled gene expressions at higher ones (29 ^{o}C) [35]. This leads to an on-demand inducible knockdown of an arbitrary[3] gene's expression, specific to dFB neurons, that has no effect on development. One danger of the temperature-inducible knockdown is of course that the activating heat shock changes natural pathways in both the test and the control animals, which necessitates further temperature control groups.

After fusing all the required lines, the resulting adult individuals (along with aptly chosen control ones), will be placed in the sleep assay at high temperatures to induce the knockdown, and sleep patterns will be automatically monitored.

[2]http://flweb.janelia.org/cgi-bin/view_flew_imagery.cgi?line=R23E10
[3]Easy to purchase, covers 91% of fly genome: http://stockcenter.vdrc.at/control/library_rnai
[4]https://www.flickr.com/photos/zeissmicro/10690270154

Particular gene knockdowns of interest, with commercially available transformants[5] would be: RhoA and *cv-c* that affect clathrin-independent endocytosis; AP-2 for clathrin-mediated endocytosis; Gα_s to diminish the adenylyl cyclase activation and thus limit the contribution of the cAMP pathway; and Gprk-2, as G-protein coupled receptor kinase, that is possibly responsible for internalisation of Dop1R2 receptors after binding.

C. Patch clamp and imaging rig

Constructs that show change in sleep behavior will then be chosen for electrophysiological measurements of dFB neuron properties. These flies will be head-fixed via thermoplastic wax and placed on a spherical treadmill to monitor movement (Figure 3C).

Using micromanipulators, a dFB neuron will be patch-clamped at multiple locations (to be visually guided via an UAS-GFP construct expressed in dFB) and dopamine will be administered at the dendritic tuft extracellularly. The multiple locations enable us to compare membrane properties spatially, as well as to judge the associated changes in electrotonic length more precisely, and thus argue about the physiological role of the membrane changes, rather than only observing the lack of response to artificially administered currents.

The rig itself needs to be light and temperature controlled, and recordings will be held through natural recommencement of sleep, to fill the gaps in our knowledge of the process. Once the time course of the reverse membrane changes are known, one may look for extracellular inputs that complement those time scales in the search for the elusive sleep pressure signal. Furthermore, carrying out a similar literature review could elucidate pathways involved in sleep signalling.

Additional experimental modifications are also available during this stage via the careful dialysis of individual neurons, adding drugs or toxins, changing the intracellular concentration of ions, or introducing small proteins of interest. One could possibly aim for measuring individual intracellular protein concentration changes by introducing fluorescent antibodies tagging either the proteins themselves, or even the phosphorylation state of them.

D. Identifying trafficking time courses

Due to the quick turnover of integral membrane proteins, and the dynamic balance between expressed and stored transmembrane protein ensembles, assessing small changes in trafficking over long time scales is difficult. Given a membrane protein of interest, we can preferentially express a GFP tagged version of it using genetic tools, and then we can quickly deplete the membrane of it using a short pulse of high intensity light, absorbed by the GFP tagged protein, which then breaks down. Monitoring the flourescence recovery after photobleaching (FRAP, Figure 3D), we can precisely measure the time courses of the artificially shifted dynamic balance. Carrying this out for transmembrane protein species

[5]The Vienna Drosophila Resource Center codes for the genes are: RhoA (12734GD), *cv-c* (16786GD), AP-2 (34148GD), Gα_s (24958GD), Gprk-2 (1835GD)

of interest in flies at various stages of their circadian rhythm reveals even small shifts in dynamic balance of trafficking associated with changes of cellular state.

4 Summary

I wish to investigate the intracellular mechanisms of the Drosophila Melanogaster sleep-wake system within the dorsal fan-shaped body neurons, where arousal and sleep pressure signals converge. Recent studies established dopamine as an arousal signal, and hinted at its lasting intracellular effect being a change in membrane composition. Little is known of molecular actors or time courses of the recommencement of sleep.

I propose to fill in these gaps in knowledge via a systematic approach, testing all potential pathways operating on a range of time scales, using novel combinations of commercially available experimental tools and established methods.

These comprehensive set of studies would provide new molecular targets for sleep disorder drugs and deepen our insight into the dynamics of the internal cell state, to spawn future research both in biology and artificial neural networks.

References

[1] C. Cirelli, "The genetic and molecular regulation of sleep: from fruit flies to humans.," *Nat. Rev. Neurosci.*, vol. 10, pp. 549–60, aug 2009.

[2] U. B. Pandey and C. D. Nichols, "Human Disease Models in Drosophila melanogaster and the Role of the Fly in Therapeutic Drug Discovery," *Drug Deliv.*, vol. 63, no. 2, pp. 411–436, 2011.

[3] R. Andretic, B. Van Swinderen, and R. J. Greenspan, "Dopaminergic modulation of arousal in Drosophila," *Curr. Biol.*, vol. 15, no. 13, pp. 1165–1175, 2005.

[4] K. Kume, S. Kume, S. K. Park, J. Hirsh, and F. R. Jackson, "Cellular/Molecular Dopamine Is a Regulator of Arousal in the Fruit Fly," *J. Neurosci.*, vol. 25, no. 32, pp. 7377–7384, 2005.

[5] J. M. Donlea, M. S. Thimgan, Y. Suzuki, L. Gottschalk, and P. J. Shaw, "Inducing sleep by remote control facilitates memory consolidation in Drosophila," *Science*, vol. 332, no. April 2017, pp. 1571–1576, 2011.

[6] T. Ueno, J. Tomita, H. Tanimoto, K. Endo, K. Ito, S. Kume, and K. Kume, "Identification of a dopamine pathway that regulates sleep and arousal in Drosophila," *Nat. Neurosci.*, vol. 15, no. 11, pp. 1516–1523, 2012.

[7] Q. Liu, S. Liu, L. Kodama, M. R. Driscoll, and M. N. Wu, "Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila," *Curr. Biol.*, vol. 22, no. 22, pp. 2114–2123, 2012.

[8] J. M. Donlea, D. Pimentel, and G. Miesenböck, "Neuronal machinery of sleep homeostasis in Drosophila," *Neuron*, vol. 81, no. 4, pp. 860–872, 2014.

[9] D. Pimentel, J. M. Donlea, C. B. Talbot, S. M. Song, A. J. F. Thurston, and G. Miesenböck, "Operation of a homeostatic sleep switch.," *Nature*, vol. 536, no. 7616, pp. 333–337, 2016.

[10] D. Hassabis, D. Kumaran, C. Summerfield, and M. Botvinick, "Neuroscience-Inspired Artificial Intelligence," *Neuron*, vol. 95, pp. 245–258, sep 2017.

[11] C. Hanus and E. M. Schuman, "Proteostasis in complex dendrites," *Nat. Rev. Neurosci.*, vol. 14, no. 9, pp. 638–648, 2013.

[12] A. Triller and D. Choquet, "Surface trafficking of receptors between synaptic and extrasynaptic membranes: And yet they do move!," *Trends Neurosci.*, vol. 28, no. 3, pp. 133–139, 2005.

[13] C. D. Hanlon and D. J. Andrew, "Outside-in signaling - a brief review of GPCR signaling with a focus on the Drosophila GPCR family," *J. Cell Sci.*, pp. 3533–3542, 2015.

[14] G. Feng, F. Hannan, V. Reale, Y. Y. Hon, C. T. Kousky, P. D. Evans, and L. M. Hall, "Cloning and functional characterization of a novel dopamine receptor from Drosophila melanogaster.," *J. Neurosci.*, vol. 16, no. 12, pp. 3925–3933, 1996.

[15] R. Cohn, I. Morantte, and V. Ruta, "Coordinated and Compartmentalized Neuromodulation Shapes Sensory Processing in Drosophila.," *Cell*, vol. 163, no. 7, pp. 1742–1755, 2015.

[16] V. May and R. L. Parsons, "G Protein-Coupled Receptor Endosomal Signaling and Regulation of Neuronal Excitability and Stress Responses: Signaling Options and Lessons From the PAC1 Receptor.," *J. Cell. Physiol.*, vol. 232, no. 4, pp. 698–706, 2017.

[17] I. I. Ismailov and D. J. Benos, "Effects of phosphorylation on ion channel function.," *Kidney Int.*, vol. 48, no. 4, pp. 1167–1179, 1995.

[18] R. A. Baines, "Postsynaptic Protein Kinase A Reduces Neuronal Excitability in Response to Increased Synaptic Excitation in the Drosophila CNS," *J. Neurosci.*, vol. 23, no. 25, pp. 8664–8672, 2003.

[19] I. O???Kelly, "Endocytosis as a mode to regulate functional expression of two-pore domain potassium (K2P) channels," *Pflugers Arch. Eur. J. Physiol.*, vol. 1, pp. 1133–1142, 2014.

[20] V. P. Sah, T. M. Seasholtz, S. A. Sagi, Brown, and J. Heller, "THE ROLE OF RHO IN GPROTEIN-COUPLED RECEPTOR SIGNAL TRANSDUCTION," *Annu. Rev. Pharmacol. Toxicol.*, vol. 40, no. 1, pp. 459–89, 2000.

[21] Y. Chen-Izu, R. M. Shaw, G. S. Pitt, V. Yarov-Yarovoy, J. T. Sack, H. Abriel, R. W. Aldrich, L. Belardinelli, M. B. Cannell, W. A. Catterall, W. J. Chazin, N. Chiamvimonvat, I. Deschenes, E. Grandi, T. J. Hund, L. T. Izu, L. S. Maier, V. A. Maltsev, C. Marionneau, P. J. Mohler, S. Rajamani, R. L. Rasmusson, E. A. Sobie, C. E. Clancy, and D. M. Bers, "Na+ channel function, regulation, structure, trafficking and sequestration," *J Physiol*, vol. 5936, no. 6, pp. 1347–1360, 2015.

[22] M. Zuzarte, K. Heusser, V. Renigunta, G. Schlichthörl, S. Rinné, E. Wischmeyer, J. Daut, B. Schwappach, and R. Preisig-Müller, "Intracellular traffic of the K+ channels TASK-1 and TASK-3: role of N- and C-terminal sorting signals and interaction with 14-3-3 proteins.," *J. Physiol.*, vol. 587, no. Pt 5, pp. 929–52, 2009.

[23] V. Renigunta, G. Schlichthörl, and J. Daut, "Much more than a leak: structure and function of K2P-channels," *Pflugers Arch. Eur. J. Physiol.*, pp. 867–894, 2015.

[24] M. Kilisch, O. Lytovchenko, B. Schwappach, V. Renigunta, and J. Daut, "The role of protein???protein interactions in the intracellular traffic of the potassium channels TASK-1 and TASK-3," *Pflugers Arch. Eur. J. Physiol.*, vol. 467, no. 5, pp. 1105–1120, 2015.

[25] Y. Zhou, W. M. Schopperle, H. Murrey, A. Jaramillo, D. Dagan, L. C. Griffith, and I. B. Levitan, "A Dynamically Regulated 14 3 3 , Slob , and Slowpoke Potassium Channel Complex in Drosophila Presynaptic Nerve Terminals," vol. 22, pp. 809–818, 1999.

[26] D. Y. Rhee, D.-y. Cho, D. Y. Rhee, D.-y. Cho, B. Zhai, M. Slattery, L. Ma, J. Mintseris, and C. Y. Wong, "Transcription Factor Networks in Drosophila melanogaster Transcription Factor Networks in Drosophila melanogaster," *Celrep*, vol. 8, no. 6, pp. 2031–2043, 2014.

[27] J. P. Meadows, M. C. Guzman-Karlsson, S. Phillips, J. A. Brown, S. K. Strange, J. D. Sweatt, and J. J. Hablitz, "Dynamic DNA methylation regulates neuronal intrinsic membrane excitability," *Sci. Signal.*, vol. 9, no. 442, pp. ra83–ra83, 2016.

[28] J. C. Hendricks, J. a. Williams, K. Panckeri, D. Kirk, M. Tello, J. C. Yin, and a. Sehgal, "A non-circadian role for cAMP signaling and CREB activity in Drosophila rest homeostasis.," *Nat. Neurosci.*, vol. 4, no. 11, pp. 1108–1115, 2001.

[29] M. M. Kucherenko, V. Ilangovan, B. Herzig, H. R. Shcherbata, and H. Bringmann, "TfAP-2 is required for night sleep in Drosophila.," *BMC Neurosci.*, vol. 17, no. 1, p. 72, 2016.

[30] W. H. Lin and R. A. Baines, "Regulation of membrane excitability: a convergence on voltage-gated sodium conductance," *Mol. Neurobiol.*, vol. 51, no. 1, pp. 57–67, 2015.

[31] Z.-X. Wang and J.-W. Wu, "Autophosphorylation kinetics of protein kinases," *Biochem. J.*, vol. 368, no. 3, pp. 947–952, 2002.

[32] E. de Nadal, G. Ammerer, and F. Posas, "Controlling gene expression in response to stress.," *Nat. Rev. Genet.*, vol. 12, no. 12, pp. 833–45, 2011.

[33] A. Jenett, G. M. Rubin, T. T. B. Ngo, D. Shepherd, C. Murphy, H. Dionne, B. D. Pfeiffer, A. Cavallaro, D. Hall, J. Jeter, N. Iyer, D. Fetter, J. H. Hausenfluck, H. Peng, E. T. Trautman, R. R. Svirskas, E. W. Myers, Z. R. Iwinski, Y. Aso, G. M. DePasquale, A. Enos, P. Hulamm, S. C. B. Lam, H. H. Li, T. R. Laverty, F. Long, L. Qu, S. D. Murphy, K. Rokicki, T. Safford, K. Shaw, J. H. Simpson, A. Sowell, S. Tae, Y. Yu, and C. T. Zugates, "A GAL4-Driver Line Resource for Drosophila Neurobiology," *Cell Rep.*, vol. 2, no. 4, pp. 991–1001, 2012.

[34] N. Perrimon, J.-q. Ni, L. Perkins, H. F. Noller, T. Volpe, and R. A. Martienssen, "In vivo RNAi : Today and Tomorrow In vivo RNAi : Today and Tomorrow," pp. 1–12, 2012.

[35] A. d. V. Rodríguez, D. Didiano, and C. Desplan, "Power tools for gene expression and clonal analysis in Drosophila," *Nat. Methods*, vol. 9, no. 1, pp. 47–55, 2012.