Title

Audition and Auditory Homeostasis in *Drosophila melanogaster*

Course

MRes Brain Sciences

Module

BRNF0003 Research Project

Candidate Number

SLZT6

Word Count

6594 (excl. references)

Contents

“Am not I

A fly like thee?

Or art not thou

A man like me?”

– *The Fly,* William Blake[1]

1. Introduction

1.1 Outline

This review describes the auditory system of *Drosophila melanogaster*, contextualising it as a model for audition. *Drosophila*'s hearing is appraised from an anatomical, biomechanical, and molecular perspective. No system can be understood independent of function; thus, behavioural relevance of hearing in *Drosophila* is addressed. Corresponding to a general organising principle in sensory systems, auditory homeostasis in *Drosophila* may be under circadian control, effecting circadian changes in hearing. Thus, the relevant chronobiology is discussed, establishing the precise mechanism of this circadian modulation as an open question in auditory neuroscience.

1.2 *Drosophila Melanogaster* as a Model for Audition

Much of our knowledge of the fundaments of living cells comes from the study of model organisms: working images of a scientific object[2]. Notable examples include the bacterium *Escherichia coli* and its bacteriophage viruses, brewer's yeast *Saccharomyces cerevisiae*, the mustard plant *Arabidopsis thaliana*, the nematode worm *Caenorhabditis elegans*, the zebrafish *Danio rerio*, the frog *Xenopus laevis,* the mouse *Mus musculus*, and the fruit fly *Drosophila melanogaster* (hereafter '*Drosophila'*, or 'the fly', Figure 1)[3]. In all cases, the image is effectively less complex than the object, either by virtue of reduced biological complexity (e.g. 250,000 neurons in *Drosophila* vs. 100,000,000,000 in humans[4]) or increased scientific expediency (e.g. the well-elucidated and highly tractable *Drosophila* genome[5]).

Drosophila has been fundamental to the elaboration of human biology by virtue of conservation at various levels of organisation. The genome of *Drosophila* is ~60% homologous to the human genome, less redundant, and possesses homologues for ~65% of human disease-causing genes[6]. This similarity is reflected in the conservation of numerous metabolic and cellular signalling pathways between both species[7]. Finally, there is increasing evidence for conservation at the level of behaviour and its molecular underpinnings. This has been convincingly shown for circadian rhythms, learning and memory, and sleep[7].

This expediency as a model system is no less true for the study of auditory development, function, and disease. Fruit flies and vertebrates exhibit striking similarities in their audition. These include functionally interchangeable key developmental genes (e.g. proneural transcription factors from the basic helix-loop-helix (bHLH) family of transcription factors), biophysical mechanisms (e.g. direct coupling and mechanical adaptation of mechanoelectrical transduction channels), and low-resolution organisation of auditory processing (e.g. afferent tonotopy and early-stage separation of vestibular and auditory pathways)[4].

2. Hearing in Drosophila

2.1 Anatomical Dissection of Hearing

The detection of sound is a special case of mechanosensation that requires high sensitivity. Sound represents "a mechanical disturbance from a state of equilibrium that propagates through a material medium[8]" as an acoustic wave (i.e. energy propagation by means of adiabatic compression and decompression)[9]. Acoustic waves with frequencies between 20Hz–20kHz elicit an auditory percept in humans[10]. This range varies depending partly on species-specific trade-offs between information salience and cost of processing[11]. In flies this range is ~4–700Hz[12 13].

A general principle of sensation is transduction: the conversion of variation in some environmental parameter into changes in membrane potential difference[14] [15]. Ion channels of sensory receptors are responsible for the electrical currents that represent the cellular response to excitation. In photoreceptors, olfactory receptors, and a fraction of gustatory receptors, these channels report outcomes of precursory events in a chemical reaction cascade[16]. By contrast, the mechanoelectrical transduction channels of auditory receptors are directly coupled to the stimulus[17].

Drosophila mechanoreceptors fall into two groups, type I or II. Type I sensory neurons innervate the sense organs they are related to by lineage[18]. These organs likely derive from a single ectodermal precursor which produces at least one monodendritic neuron alongside a number of support cells. Type I organs are subclassified into 1. *es organs*: mechano- or chemosensory organs that possess cuticular external sensory structures (e.g., bristles, campaniform sensilla), and 2. *ch organs*: chordotonal organs – internally located stretch receptors. The larval peripheral nervous system possesses many type II neurons that have multiple dendrites (*md neurons*) that (generally) do not associate with support cells[19].

Mechanotransduction of sound is facilitated by the fly's antennal ear (Figure 2). The arista and the third antennal segment (a3) together constitute the sound receiver. By virtue of the arista's surface area, the sound receiver captures the particle velocity component to oscillate sympathetically with acoustic stimulation. This rotatory oscillation about the a3's longitudinal axis is transmitted via a pivot joint to the second antennal segment (a2)[20]. Stretch receptors ('scolopidia') projecting from the a2 housing perpendicularly attach to the a3 shaft in two topographical arrays, deforming in a reciprocal manner depending on the direction of rotation[21] (Figure 3). Together they constitute Johnston's Organ (JO), the fly's largest chordotonal organ. The a3 also functions as the primary olfactory organ with hundreds of olfactory sensilla[22].

Scolopidia are self-contained sensory units containing up to three sensory neurons (Figure 4). JO scolopidia contain cholinergic[23] bipolar JO neurons (JONs) with two cytoplasmic projections extending from the soma. The apical dendrite attaches to cap cells which anchor the scolopidium under tension beneath the cuticular plate. Scolopale cells also contribute to this cap, and form a sealed capsule around the sensory cilia ('scolopale space')[13]. The basal JON axon projects to the antennal mechanosensory and motor centre (AMMC) in the ventral deutocerebrum of the fly's brain[24].

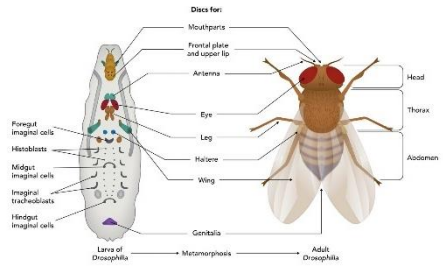

Figure 1: Drosophila anatomy (ref.[18]).

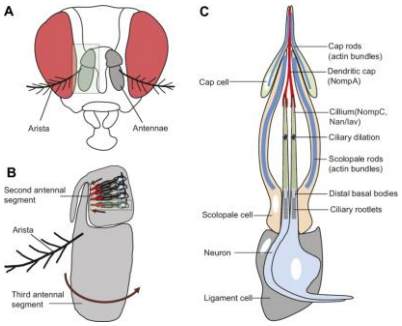

Figure 2: a, b. Antenna, c. Scolopidium (ref.[18]).

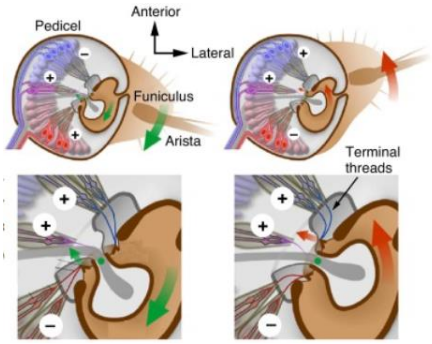

Figure 3: Scolopidia arrangement (ref.[23]).

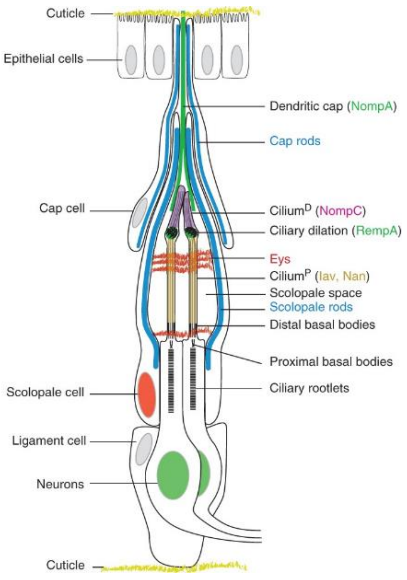

Figure 4: Scolopidium (ref. [13]).

The ciliary endings of these neurons pass through the scolopale space which contains an endolymph low in calcium (Ca^{2+}) and high in potassium (K^+) relative to the JON distal cilium – an ionic gradient regulated by scolopale cells[25] (and resembling the composition of mammalian cochlear endolymph). The short auditory transduction latency suggests that JONs are directly gated rather than relying on a second messenger, with mechanical stimulation directly opening cation channels and the subsequent (assumed) K^+ influx depolarising scolopidial neurons[26].

~225 scolopidia comprise ~500 JONs in JO. JO responds to two types of mechanical stimulation – vibratory stimuli (e.g., near-field sound relevant to courtship), and slower, more sustained stimuli (associated with its roles as a gravity[27] and wind sensor[28], and its mediation of air current feedback for flight control[29]). These mechanosensory submodalities reflect a heterogenous population of JONs, identified based on distinct response mechanics[30], molecular composition, and AMMC synapse patterns[31]. JONs project via the antennal nerve to the AMMC. Five different mechanosensory subpopulations (A-E) are recognised, based on spatially distinct AMMC synapse formation (Figure 5) and spectro-temporally distinct stimulus response behaviours. Class AB JONs (~200) preferentially respond to sound-induced antennal vibrations whereas class CE JONs (~250) prefer maintained antennal deflections, serving gravity and wind detection[32]. Class D JONs (~50) respond to both types of stimuli[33], possibly to monitor flight-generated wind and wing-beat sounds[29], serving a proprioceptive role.

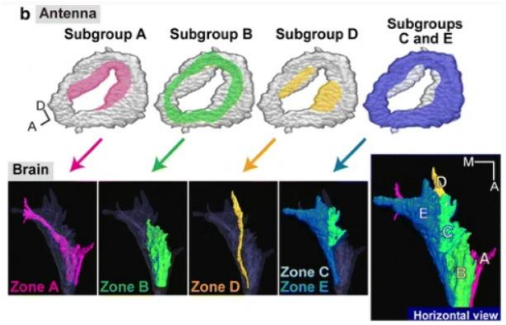

Figure 5: JO cell-body distribution (ref.[32]).

2.2 Biomechanical Dissection of Hearing

In the vertebrate cochlea, basilar membrane motion manifests a frequency-specific, level-dependent compressive nonlinearity. Responses to low-amplitude, characteristic frequency stimuli are sensitive and sharply tuned, and responses to high-amplitude stimuli are insensitive and broadly tuned[34 35]. This occurs as a result of essential (and saturating) nonlinearities of the mechanotransduction process and positive mechanical feedback (termed 'the cochlear amplifier[36]').

Outer hair cell prestin-mediated[37] cell-body contractions (in mammals[38]) or active hair bundle twitches (in lower tetrapods[39]) occur in phase with sound-induced vibrations, reinforcing them ('negative damping'). This feedback preferentially occurs at low intensities, nonlinearly altering mechanical tuning as stimulus intensity changes, consequently optimising the detection of low-amplitude sounds. In fact, in quiet environments, excess feedback can produce spontaneous outer hair cell oscillations, which transmit via the endolymph to actuate the ossicular

impedance-matching mechanism, causing the cochlea to emit sound[40] (putatively, though spontaneous otoacoustic emissions (SOAEs) may arise by two alternative mechanisms[41 42]).

As in vertebrates, *Drosophila* auditory neurons possess an active, force-generating apparatus that improves the ear's sensitivity to sound[43]. As Drosophilid auditory systems are much more experimentally tractable compared to their vertebrate counterparts[44], this expedites them as models for the elucidation of this mechanical feedback amplification.

The fly sound receiver can be modelled as a moderately damped simple harmonic oscillator where sound constitutes a periodic external driving force[45]. Assuming the sound frequency and intensity are constant, the motion about equilibrium is a function of time t:

$$x(t) = x_0 \cos(\omega t + \phi)$$

The motion is periodic, repeating sinusoidally with constant amplitude x_0. x_0 is itself a function of the driving angular frequency ω (i.e., sound frequency):

$$x_0(\omega) = \frac{F_0/m}{((b/m)^2\omega^2 + (\omega_0^2 - \omega^2)^2)^{\frac{1}{2}}}$$

where F_0 is the driving amplitude (maximum force imparted by the sound wave). Phase constant ϕ is also a function of ω:

$$\phi\,(\omega) = \tan^{-1}\frac{(b/m)\omega}{\omega_0^2 - \omega^2}$$

In the equations above, b is a proportionality constant (the viscous damping coefficient) that relates the damping force to the velocity of the mass, to which it is proportional. ω_0 is the natural angular frequency of the undriven, undamped oscillator, and is equivalent to:

$$\omega_0 = \sqrt{\frac{k}{m}}$$

where k is the spring constant, and m is the oscillator effective mass[46 47 48 49].

In vertebrates, nonlinear cochlear response characteristics are introduced by negative damping[50]. Flies appear to introduce nonlinearities in their antennal responses by actively modulating receiver stiffness. Laser-Doppler Vibrometry (LDV) measurements showed that the resonant frequency of the antennal receiver decreases markedly from ~800Hz to ~400Hz as stimulus particle velocity is decreased (Figure 6). This intensity-dependent shift implies that auditory system components vary in compliance as stimulus intensity declines. This observation is further supported by transition to a linear regime in both post-mortem and (transiently) in anaesthetised flies (Figure 6 & Figure 7), where resonant frequency remains ~800Hz regardless of stimulus intensity. This frequency value solely reflects the stiffness of the mechanical coupling of the anatomical components in the hearing organ[51]. These findings may suggest that the resonant frequency downshift is an active process. More refined experiments establish an even greater range for this compressive nonlinearity (~800-170Hz[52]).

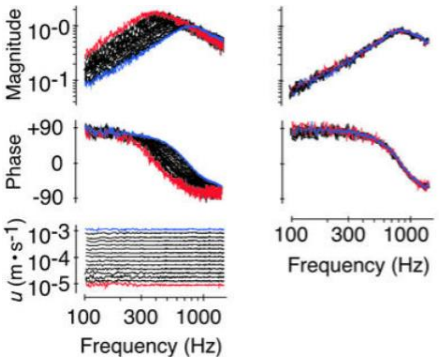

Figure 6: Response magnitude (top) and phase response (middle) vs. different stimulus particle velocities (bottom), live (left) and dead (right). Response magnitudes given as ratio between vibration velocity and stimulus particle velocity (m·s−1/m·s−1), (ref.[50]).

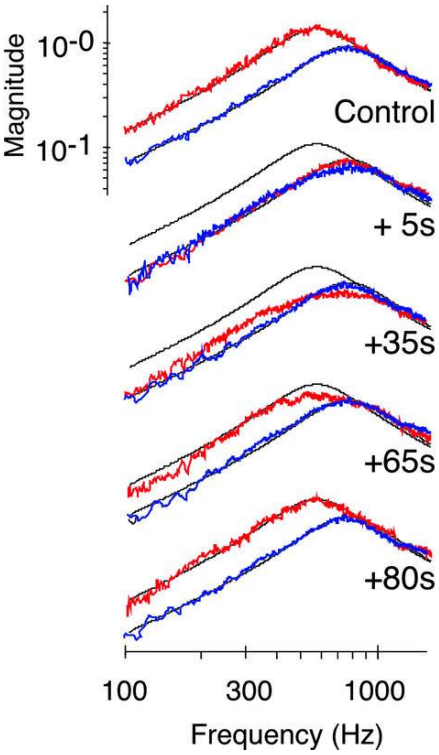

Figure 7: Transient linearisation of receiver response after temporary anaesthesia, colour conventions as above (ref.[50]).

Further evidence for active amplification is the observation that the sound receiver twitches spontaneously in the absence of sound (evocative of vertebrate SOAEs). These oscillations are nonsinusoidal but significantly comprise frequency components in the fly song frequency range. Complex oscillations consisting of both rapid strokes and slower components, as well as augmentation by a local analgesic, both support the existence of an active push-pull mechanism rather than movement due to Brownian motion. Mechanosensory mutant experiments showed that the motion is generated by mechanosensory neurons[51]. This physiological mechanism may serve the purpose of mating; low stimulus intensities shift the receiver's resonant frequency towards frequencies that dominate the fly's mating song (160-210Hz)[53]. This mechanism appears to be peripherally rather than centrally mediated; mechanical, electrophysiological and immunohistochemical analyses found that the antenna has no peripheral synapses or efferent innervations[54]. This contrasts with the cochlear amplifier, which relies on efferent feedback[55].

This amplificatory response is not the only nonlinearity observed in the fly auditory system. LDV assays of the sound receiver's steady-state mechanics following electrostatic step-like deflections showed that for small displacements (max. ~2000nm from equilibrium) the antenna behaves like an ideal

Hookean spring, i.e., displacement is linearly proportional to force (Figure 8). Simultaneous extracellular compound action potential (CAP) recordings found that nerve responses occur transiently with the onset and offset of 'force-steps'. These transient responses coincide with transient nonlinearities in the receiver's mechanics, where the receiver stiffness decreases. Following a positive force step, the receiver displaces markedly in the direction of the force (overshooting in comparison to a purely passive system) before rapidly recoiling (undershooting) and then displacing positively to a steady-state position away from equilibrium. This steady-state position is consistently linearly proportional to the magnitude of the applied force. The reverse is seen for an equivalent negative force[26]. These dynamics closely resemble those observed in sensory hair bundles of vertebrate inner-ear hair cells[56 57], reflecting the direct opening (initial overshoot) and ensuing adaptation (recoil to steady-state) of transducer channels, as elucidated by the gating-spring model of mechanotransduction[16]. This explains the transience of the nerve currents observed.

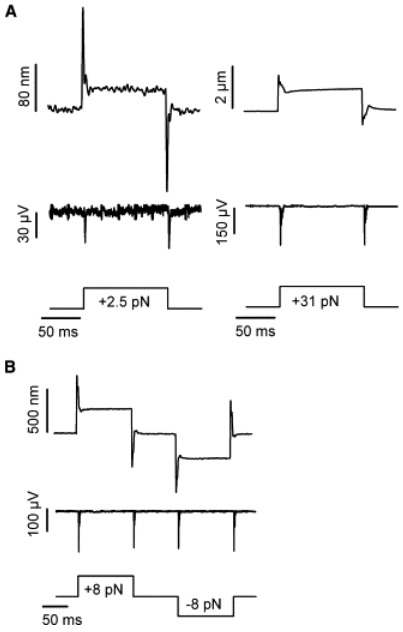

Figure 8a & b: Receiver displacement (top) and simultaneously recorded CAPs (middle) versus different force steps (bottom) (ref.[54]).

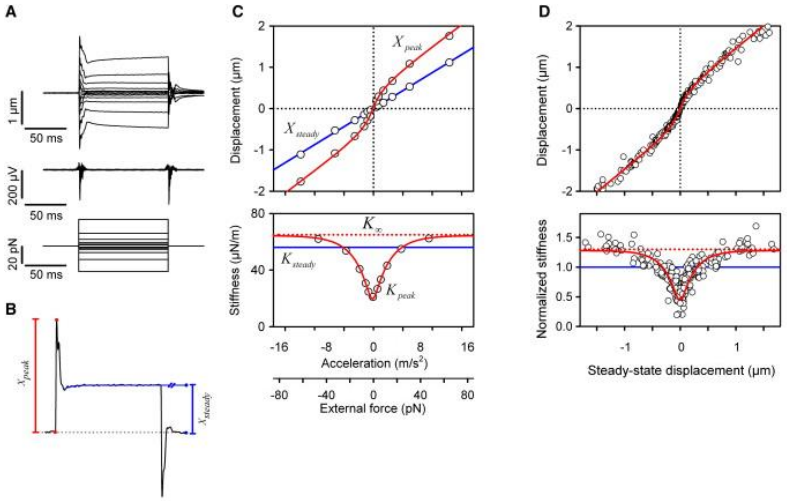

Figure 9 (left):

a. Convention as per Figure 8,

b.Displacement after individual force step and colour legend,

c. Xpeak and Xsteady (top) and Kpeak and Ksteady (bottom) vs. acceleration and external force,

d. Pooled responses of 20 receivers.

Solid lines are fits of the gating-spring model equation reviewed (ref.[54]).

The gating-spring model propounds that in hair cells and JONs alike, mechanotransducers are mechanically actuated via elastic elements ('gating springs') that pull on the channel's gates. Because the gating springs relax as the channels open, this leads to a reduced stiffness over the range of forces the channels gate, resulting in a nonlinear gating compliance[58]. The steady-state displacement of the receiver (i.e., following prolonged forcing, as described), X_{steady}, linearly scales with applied force, yielding a constant stiffness (K_{steady}) of ~50 µN/m. However, the initial displacement peak (X_{peak}) exhibits a nonlinear force-displacement relationship (Figure 9b & c), where the corresponding dynamic receiver stiffness (K_{peak}) is

lowest for small forcing amplitudes (−50 to +50 pN), minimising for small stimuli at the equilibrium position.

This nonlinear behaviour aligns with a two-state gating-spring model[16], where force F required to displace the sound receiver by distance X is given by

$$F = K_\infty X - p_0(X)Nz + F_0$$

where K_∞ is the asymptotic receiver stiffness (the dynamic stiffness when all channels are either open or closed; dotted red line, Figure 9c). The open probability is a function of X and is defined as

$$p_0(X) = \frac{1}{1 + e^{\frac{-z(X - X_0)}{k_b T}}}$$

where N is the number of channels, $z = \Delta F$ in a single gating spring as the channel opens, F_0 is a constant offset, X_0 is the displacement at which $p_0 = \frac{1}{2}$ (i.e., half the channels are open), k_b is the Boltzmann constant, and T is the absolute temperature in °K (see ref.[26] for further discussion). This model is validated by its accurate prediction of nerve response[26].

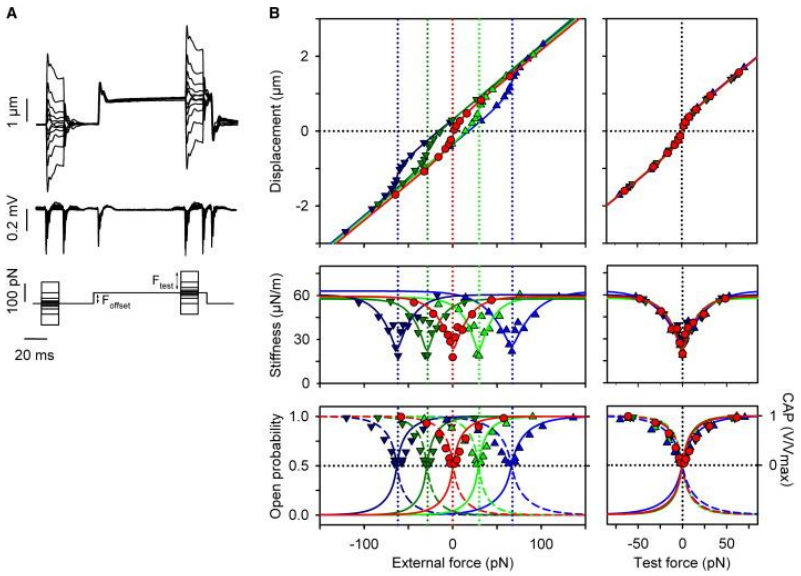

Figure 10:

a. Receiver displacement (top) and CAP responses (middle) to test stimuli before/after a sustained receiver deflection (bottom).

b. Xpeak (top), Kpeak (middle), and CAP responses (bottom) as a function of external force for five different offset forces.

Right panels: superposition of above functions for comparison (ref.[54]).

Solid lines: individual fits of the gating-spring model equation reviewed.

This model is further reinforced by evidence for adaptation. During maintained stimulation, adapting transducers recognisably shift their operating range to restore channels' open probabilities to prestimulus levels. This is supported by three observations. Firstly, identical CAP responses resulted from a test stimulus both before and 50ms after imposition of a maintained receiver deflection (Figure 10a). Secondly, the functions relating X_{peak} and K_{peak} to external force remained

identical at five different offset forces (Figure 10b, top). Thirdly, the operating range of the CAP response is translated identically about each offset force (Figure 10b, bottom)[26].

The shifts in gating compliance during sustained deflections imply that transducer adaptation results from a recalibration of the gating spring tension. This recalibration may be facilitated by adaptation motors, as in hair cells[59]. This gating spring arrangement may serve three purposes. Firstly, as in hair cells, gating spring motors may promote active amplification[59]. Secondly, as gating springs store kinetic energy and use it to modulate channels' open probabilities, this may ensure an electrical response proportionate to stimulus intensity[60]. Thirdly, transducer adaptation to deflections on relatively large timescales (as induced by wind/gravity) preserves their mechanotransductive capacity with respect to deflections on small timescales – i.e., sound. This allows them to both adapt to a new equilibrium position during a sustained deflection, and to high-pass filter incoming sound signals.

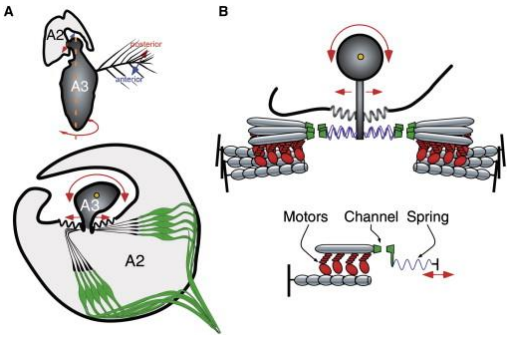

Figure 11: a. Antenna (top), cross-section showing perpendicularly connected transduction modules (bottom), b. Transduction module schematic (ref.[42]).

Integrating these findings, the fly auditory system can be characterised as two opposed populations of transducers coupled to a simple harmonic oscillator (the sound receiver, (Figure 11a)). Both transducer populations consist of an equal number of parallel transduction modules. Each module conforms to the gating-spring model, consisting of an ion channel, a set of adaptation motors, and a gating spring[43] (Figure 11b).

2.3 Molecular Dissection of Hearing

In mammals and flies, many neural and non-neural mechanosensitive signalling processes rely on transient receptor potential (TRP) channels[61]. Nine TRP channels are implicated in JO function[62], but only three are recognised as crucial molecular components of the transduction module. The TRP channel TRPN1 or NompC (encoded by *nompC*)[63] and the two TRP vanilloid (TRPV) channels Nanchung (*nan*) and Inactive (*iav*)[64] all play a role in mechanotransduction. NompC localises to the distal end of mechanosensory cilia[65], whereas Nanchung[66] and Inactive[64] localise to the proximal cilium (Figure 12). As both proteins are each undetectable in the cilia of mutants lacking the other protein, it has been concluded that both contribute to a heteromultimeric (putatively, a heterodimeric[67]) transduction channel *in vivo*: Nan/Iav[64]. This reflects the variation in sensory function determined by the combinatorial expression of TRPV channel proteins[68].

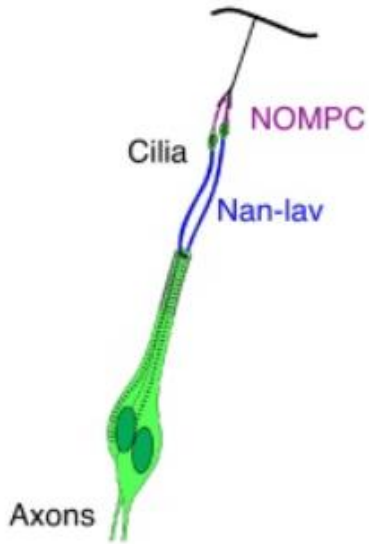

Figure 12 (ref.[66]).

As reviewed, flies combine transduction and amplification in a single molecular module. The close interplay between

transduction and amplification implies that some of the transduction module's constituent proteins may also be required for amplification[43], although this is disputed[69] due to the mixed picture presented by loss-of-function mutation experiments. Loss of Nan/Iav completely attenuates CAP responses in the antennal nerve[66 64] but upregulates auditory feedback amplification[70]. Conversely, loss of NompC significantly attenuates CAP responses, but essentially eliminates mechanical, transducer-based amplification[70 71]. Given the increased auditory amplification observed in *nan/iav* null mutants and the near perfect loss of auditory feedback amplification in *nompC* mutants, it was posited that NompC may constitute (wholly or partly) the mechanotransducer channel in the auditory sensory neuron subset of JONs, whereas Nan/Iav channels play a further downstream role in action potential generation and mechanical gain control.

This theory is consistent with the observation that loss of NompC reproduces a characteristic decrement in gating compliance seen after ablation of JO auditory sensory neurons[63]. This NompC-based model of JO auditory transduction was challenged by two findings. Firstly, sound-evoked activation of the giant fiber neuron (GFn) remains present in NompC null flies. Secondly, loss of Nan/Iav completely ablates sound-evoked GFn potentials[69]. It was therefore proposed that Nan/Iav – not NompC – may directly mediate mechanosensory transduction in auditory-sensory-subset JONs. However, this hypothesis is contested by (1) the demonstration that NompC can in fact form a mechanotransducer channel (or at least a channel subunit) in *Drosophila* touch sensitive neurons[72], and (2) the recognition that a uniquely long ankyrin-repeat domain conveys force to mechanically gate the NompC channel[73]. Nonetheless, further clarifications are necessary to disambiguate the identity of the JO auditory transduction channel, whether it be NompC, Nan/Iav, or a currently unknown ion channel. The NompC and Nan/Iav models are summarised in Figure 13.

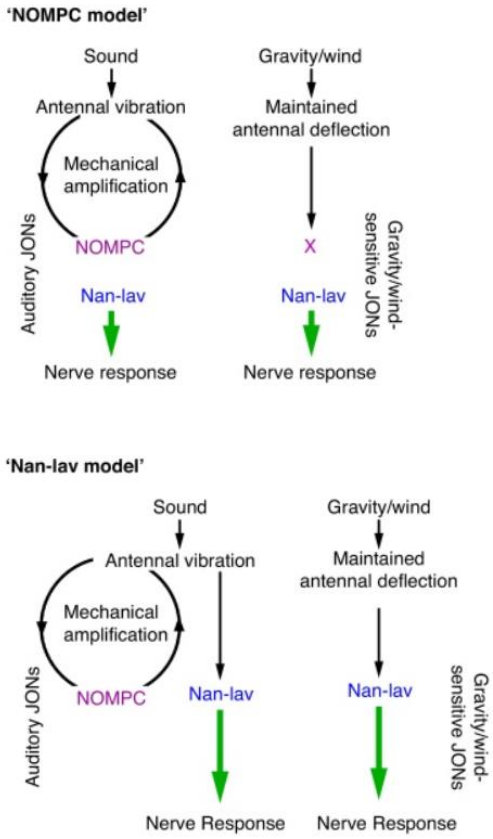

Figure 13 (ref.[66]).

With respect to the molecular identity of the other transduction module constituents, NompC's N-terminal ankyrin spring may function as the gating spring, which, if confirmed, would establish NompC as the transduction module ion channel (notably, this domain has also been proposed as the gating spring in vertebrate hair cells[74]). This domain colocalises with membrane-microtubule connecting filaments, which have also been suggested as the gating spring due to their compliance[75]. No candidate has been proposed for the molecular motor, but as in hair cells (which may utilise a Ca^{2+}-dependent myosin-superfamily motor protein[76]) the motor-

spring linkage may be Ca^{2+}-mediated: disrupting Ca^{2+}-transmissive channels in *Drosophila*'s auditory neurons modifies the amplificatory gain[70].

3. Behavioural Relevance of Hearing

3.1 Sound-induced Behaviours

Neural networks in biological organisms are hierarchically organised to facilitate serial extraction of behaviourally relevant features from sensory information to produce behaviour[77]: an organism's actions in response to its environment[78 79]. *Drosophila* is a remarkably useful model for both simple and complex behaviours. Regarding complex behaviours, flies recapitulate foraging behaviour, demonstrating two larval phenotypes with bimodally distributed foraging trail lengths explicable by variation in a single gene[80]. Furthermore, flies demonstrate all the basic fundaments of mammalian learning and memory. An aversive olfactory association is encoded as either a short-lasting (short-term memory) or long-lasting (long-term memory) hyperexcitability of a mushroom body neuron in the olfactory pathway – an association which disappears on ablation of this neuron[81]. Flies also exhibit exquisitely complex courtship behaviour, reliant on the integration of information from at least three sensory modalities – vision, audition, and olfaction[82].

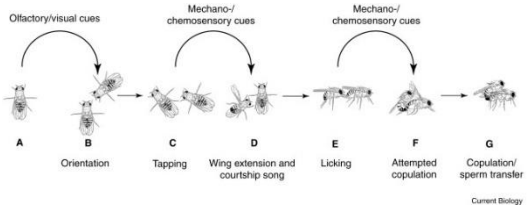

Figure 14 (ref.[81]).

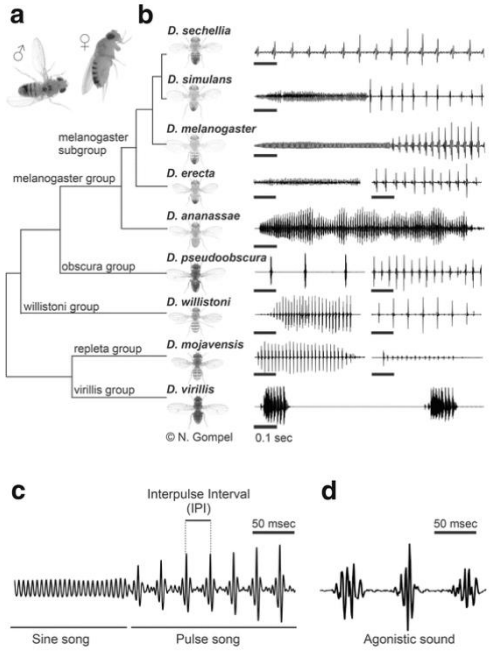

Figure 15 (ref.[81]).

The fly's courting ritual is described in detail by Manning[83] and outlined in Figure 14. The ritual begins with the male tapping the female and orienting himself adjacently. He then produces a courtship song by extending and vibrating a single wing. This song comprises two components: sine and pulse modes (Figure 15c). The pulse train is characterised by a constant inter-pulse interval (IPI) and a constant inter-pulse frequency (IPF). There is variation in these parameters in the *Drosophila* genus, with the IPI significantly differing between species and hence conferring species-specificity (Figure 15b). The *melanogaster* IPI is ~35ms[84]. Presentation of a pulse train to *melanogaster* with this IPI increases courtship behaviour in males and increases female receptivity[85]. As interactions directly preceded by the pulse train predominantly end in copulation, it may act as a trigger[86].

Females are additionally responsive to long-timescale song-structure, to the magnitude of tens of seconds. Whereas short-timescale features confer species identity, long-timescale features appear to demonstrate fitness and allow females to distinguish between conspecifics[87]. The main indicator of female receptivity is slowing of locomotor activity[88], although

females often send 'rejection signals[89]'. However, without successful acoustic courtship, males are rarely accepted[90].

The production, and likewise detection of species-specific mating signals are both neurally mediated. Much work has gone towards identifying the neuronal classes active in the male fly courtship song circuit: from neurons that integrate social cues[91], to descending fibres that activate pulse production[92], to thoracic interneurons that may be involved in the pattern generator(s) that compose the pulse song[93]. In contrast to other species, where courtship song structure varies considerably between renditions as a result of noise within pattern-generating circuits, male flies pattern sine/pulse transitions depending on fast modulations in visual signals and self-motion information[94]. These courtship-related neuronal circuits are sexually dimorphic, although females remain understudied[95]. Females have recently been found to sing a characteristic song during copulation. This song may determine the male's ejaculate allocation which may influence postcopulatory mate choice[96].

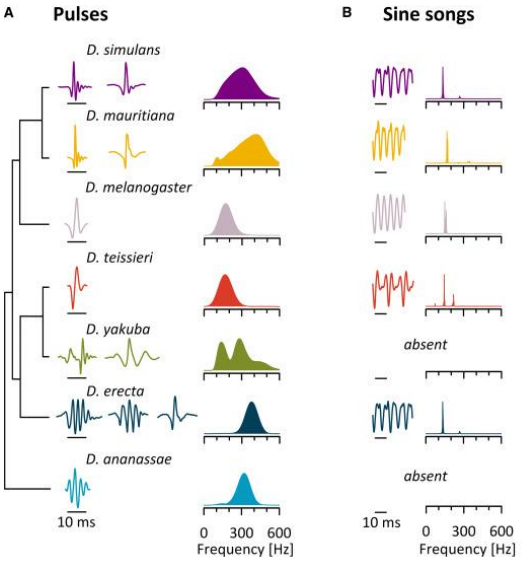

Figure 16: Pulses vs. sines for different subspecies (ref.[94]).

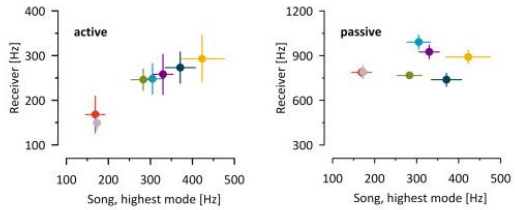

Figure 17: Song frequencies for different subspecies (colour legend as above) vs. respective antennal best frequency before anaesthesia (left), correlated, and after (right), uncorrelated (ref.[94]).

Finally, this neurally-facilitated conspecific sensitivity extends to the auditory system. Antennal best frequencies of each *melanogaster* subspecies are tuned to the conspecific pulse frequency (Figure 16 & Figure 17). This species-specific tuning depends on amplificatory mechanical feedback from auditory neurons. Due to the intensity-dependence of this feedback, it is particularly useful for sensitising flies to low-intensity stimuli such as conspecific song pulses, but becomes negligible for high-intensity stimuli[52]. This suggests that species-specific audibility ranges may assist in maintaining reproductive isolation in Drosophilid flies, particularly where they are sympatric.

Acoustic signals also play a crucial role in aggression. Aggression is a universal complex social behaviour important for resource and mate acquisition. Male flies acquire and defend territories to attract conspecifics for courtship. The agonistic signal, like the courtship signal, is highly stereotyped and has an identical IPF, but is temporally distinct, featuring pulse components and no sine components. Additionally, the agonistic signal features a longer IPI, a longer pulse duration, and is produced with both wings[97] (Figure 15d). Bilateral removal of aristae in males reduces aggressive encounters by ~40%. Hence, acoustic inputs are an important, but not exclusive, mediator of aggression, acting in concert with chemosensory inputs. Contrastingly, exposure to the courtship signal ameliorates aggressive behaviours[98].

Additionally, JO has a significant role in the GFn escape circuit (an important model for parallel processing[99]), which mediates a reflexive escape response on detection of a looming predator. A combination of rapid air movement (detected by JO) and fast visual looming evoke an action potential in the GFn, which descends to the thorax to synapse with the tergotrochanteral motor neuron (TTMn) of the TTM jump muscle, eliciting an escape jump[100].

The reviewed finding that courtship song structure can be predicted entirely from sensory inputs[94] was enabled by simultaneously observing flies' sensory environment and behavioural output at high temporal resolution[101]. This encapsulates the value conferred by *Drosophila's* superlative experimental tractability. The application of similarly detailed analyses to natural behaviours can distil ostensibly unpredictable complex behaviours into simple algorithms and sensorimotor transformations.

3.2 Chronobiology of Hearing

Natural environments exist in a state of constant flux. The sun's electromagnetic energy, the major energy source for terrestrial life, is made available to organisms in the form of chemical energy via photosynthesis. However, the earth's rotation periodises the availability of this energy source. Consequently, most terrestrial organisms exist in twenty-four-hour cycles of environmental change (with respect to brightness, temperature, humidity, food availability, predation, and so on). Hence, it becomes necessary to anticipate these rhythms in order to temporally structure physiological processes in an energetically optimal manner.

Accordingly, the periodicity introduced by the day-night cycle reverberates throughout the levels of biological organisation, from subcellular processes, to specific tissue functions, to behaviour (and beyond the individual organism, extending to the ecological levels)[102]. A biological rhythm that has a period of ~1 day and subsists under constant conditions is called 'circadian' (circa-, 'around,' -dian, 'day'). Circadian rhythms rely on central and peripheral biological 'clocks.' These clocks entrain to environmental rhythms via numerous sensory inputs ('zeitgebers', German for 'time-givers'), generating circadian behaviour which often persists when environmental rhythms are removed[103].

Light cycles are the major zeitgeber in all organisms[104]. Light entrainment is highly redundant in flies, which have at least three light-input pathways including the compound eyes and an extraocular 'eyelet.' However, the most sensitive pathway is the

molecular blue-light photoreceptor *cryptochrome* (*cry*), later reviewed. Although synergistically influencing the circadian system in different ways, each pathway is independently sufficient for entrainment[104]. The clock can also be entrained by temperature cycles, unless they conflict with light cycles, in which case light is preferred (hinting at a Bayesian integration process reflective of a wider pattern of probabilistic sensory learning[105]). Even vibration/silence cycles can serve as zeitgebers, mediated by chordotonal organs[106].

Interestingly, circadian behavioural outputs feed back to the input: circadian rhythms gate sensory perception. This has been documented across the taxa. Olfactory sensitivity varies with respect to circadian time in *Drosophila*[107], cockroaches[108 109], moths[110], mice[111], and rats[112]. Circadian rhythms characterise most visual systems, including in *Drosophila*[113 114], extending from proximal centres in the visual pathway to the retina. This includes the daily synthesis of rhodopsin (fish, toads, and mice) and the shedding of rod disc membranes (frogs and rats) and cone disc membranes (chicks and squirrels), serving a homeostatic role[115]. In humans, chronotype modulates circadian variation in contrast sensitivity, colour discrimination, and object recognition[116]. This circadian gating extends even to somatosensation[117]. Less is known about circadian gating in the auditory system. Frogs display frequency-dependent differences in auditory brainstem responses[118]. Mice[119 120] and rats[121 122] exhibit diurnal variation of hearing sensitivity and otoprotection. In fact, the murine cochlea demonstrates a topographic differential phase arrangement of cellular circadian clocks along its tonotopic axis[123]. This may regulate time-dependent frequency sensitivity, as found for SOAEs in humans[124]. It may also distribute energy expenses throughout the cochlea in a time-dependent manner to avoid metabolic exhaustion. An explanation for this circadian variation in function across sensory modalities is that homeostatic repair programmes are under circadian control and may cluster around phases of sensory quiescence[125].

Key parameters of *Drosophila*'s auditory system vary with circadian time. LDV measurements reveal that power gain and tuning sharpness of the antennal receiver are at their highest during the morning hours. Additionally, CAP responses in the antennal nerve are significantly greater in the morning than in the evening[126]. This indicates that the mechanical amplification and neural sensitivity of the auditory system are under circadian control. As courtship relies on acoustic signalling, this auditory circadian gating may underlie *melanogasters*'s diurnal mating pattern, where mating frequency is highest during the

early hours[127]. This pattern is imposed by females[128] and is independent of circadian locomotor behaviour[129].

These system-level circadian patterns emerge from temporal computations in individual cells, where molecular clocks are instantiated by autonomous oscillations of 'clock genes.' These oscillators are maintained by interdependent molecular transcriptional/translational feedback loops (TTFLs) where constituent genes autoregulate their own expression[106] (Figure 18). First discovered in *Drosophila,* these TTFLs are highly conserved in other animals (reviewed by Hardin[130]).

Firstly, the transcription factors CLOCK (CLK)[131] and CYCLE (CYC)[132] bind to the E-box regions in the gene promoters of the primary TTFL genes *per* and *tim*, activating their transcription[133]. Transcription peaks between ZT4–ZT18 (24h zeitgeiber time, where ZT0 and ZT12 are lights on and off respectively). Translation of the respective mRNAs produces the proteins PERIOD (PER) and TIMELESS (TIM). PER is innately unstable and hence undergoes proteasomal degradation after targeting by the kinase DOUBLETIME (DBT)[134 135 136]. However, the PER-DBT dimer stabilises on complexation with TIM. Consequently, the PER-TIM-DBT complex accumulates in the cytosol 6-8h following *per* and *tim* transcription activation (~ZT12)[137].

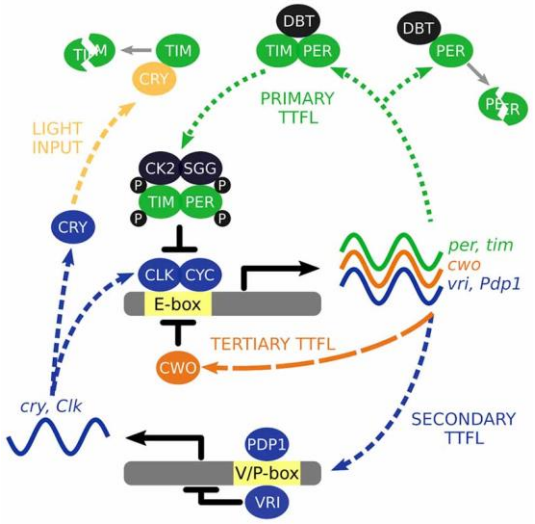

Figure 18: The circadian molecular clock, reviewed in-text (ref.[104]).

The kinases CK2[138] and SSG[139] phosphorylate PER and TIM, causing them to accumulate in the nucleus[140]. Within the nucleus, the active form of PER supresses CLK-CYC initiation of *per* and *tim* transcription between ZT18–ZT4[141]. This reduces cytosolic concentrations of PER and TIM, subsequently reducing their nuclear concentration, ultimately lifting CLK-CYC inhibition. This restarts the molecular cycle ~24h later.

The primary TTFL is fine-tuned by secondary and tertiary loops. In the secondary TTFL, CLK-CYC additionally activates *vrille* and *Pdp1ε/δ* transcription between ZT4–ZT6. VRILLE (VRI) concentration peaks at ~ZT14 compared to ~ZT18 for PDP1ε/δ. Both proteins act at VRI/PDP1-boxes to regulate transcription of *Clk* and *cry*[142 143]. PER-TIM-DBT from the

primary TTFL supresses activity of CLK-CYC, linking the two loops. CRY, once photoactivated, promotes TIM degradation, hence functioning as a light-reset switch for the primary TTFL[144]. In the tertiary TTFL, CLK-CYC promotes *clockwork orange* expression which binds competitively to the same E-box regions to downregulate CLK-CYK-mediated transcription[145].

Molecular clocks exist in only ~150 neurons in the fly's brain. These neurons comprise the 'central clock': a circadian pacemaker neural network (Figure 19), and perhaps the best-understood CNS network governing behaviour in *Drosophila*[146]. Subsets of this network coordinate different aspects of circadian behaviour, including locomotor activity. The network is divided into small (sLNv) and large ventral lateral neurons (lLNv), dorsal lateral neurons (LNd), three populations of dorsal neurons (DN1-3), and the lateral posterior neurons (LPN)[147].

In LD conditions (12h light/12h dark cycle, cf. LL (24h light) and DD (24h dark)), flies are more active during the light phase (Figure 20). There are two locomotor activity peaks: a morning (M) peak, and an evening (E) peak, respectively associated with light-to-dark and dark-to-light transitions. Activity increases ahead of these transitions – termed 'anticipation'. The LNv mediate morning anticipation and are thus termed M-cells. The LNd/DN1 mediate evening anticipation and are thus termed E-cells. The ~20 M-cells mediate their effects via the neuropeptide pigment-dispersing factor, whereas E-cells do not.

If flies are LD-entrained and then released into DD conditions devoid of temporal information, circadian locomotor patterns persist, but become unimodal, as M and E peaks merge. Notably, this unimodal peak begins to drift. This may continually align the clock to the light-dark environment, resulting in greater precision[148]. If flies are LD-entrained and released into LL conditions, the locomotor pattern becomes arrhythmic, likely due to the clock being continually reset by light[149].

As well as mediating locomotor rhythms, the central clock temporally structures eclosion rhythms[150] and metabolic processes[151]. Additionally, many tissue-autonomous circadian rhythms are observed (e.g., chemosensory sensilla[107], mechanoreceptive bristles[152]), where oscillations of clock genes indicate the presence of peripheral molecular clocks[153]. Robust circadian oscillations of the core clock genes *per*, *tim*, and *clk* are documented in a2, indicating the presence of a peripheral JO molecular clock[154]. As reviewed, JO lacks efferent synapses, and pan-neural inhibition of synaptic transmission does not alter afferent sensory neuron activity or mechanical feedback gain[54]. Together, these findings suggest that auditory circadian

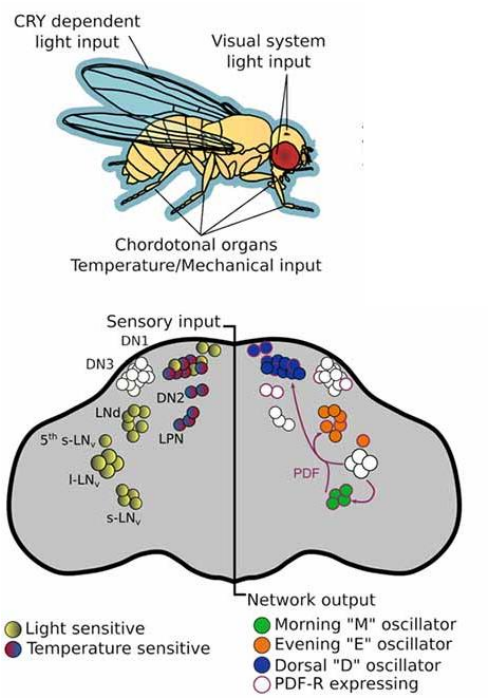

Figure 19: The central clock, reviewed in-text, and cell/zeitgeber-modality correspondences (ref.[104]).

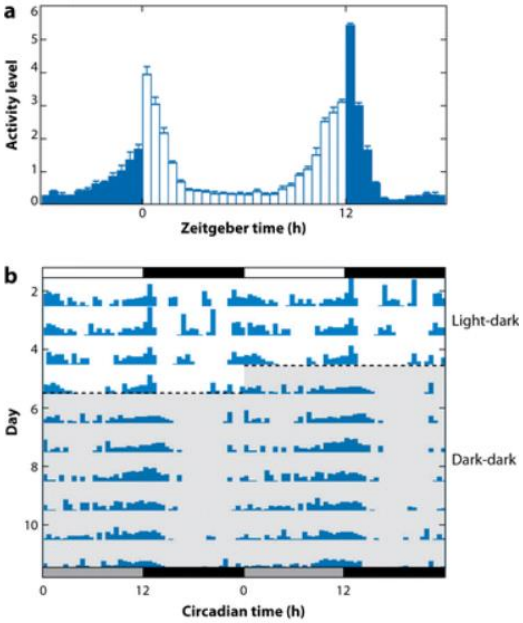

Figure 20: a. Averaged locomotor activity vs. time in wild-type flies, light/dark bars correspond to light/dark respectively, b. Plot for one fly over 12d with each row's second day double-plotted on the next row's first day (ref.[146]).

patterns in the fly are peripherally mediated downstream of the JO molecular clock.

These patterns may be a signature of functional homeostatic repair[125]. In mammals, brain-derived neurotrophic factor (BDNF) is a major neurotrophin implicated in cochlear neuro- and synaptogenesis, and triggers synaptic regeneration following excitotoxic injury. Circadian control of BDNF expression is recognised as a circadian repair mechanism after noise damage[119]. This homeostatic logic may be recapitulated in JO. Repair processes may target points in the circadian day where hearing is relatively less important, in antiphase with patterns of mating which critically rely on bidirectional acoustic communication.

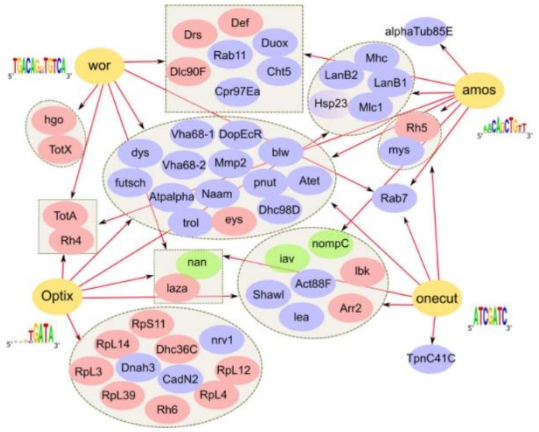

Figure 221: iRegulon-predicted upstream master regulators (yellow) for age-variable JO target genes with arrows leading to their anticipated targets. Targets are grouped, up and down-regulated genes respectively shown in blue and red. Mechanosensory ion channels are shown in green (ref.[157]).

NompC mechanotransducers undergo constant replacement with estimated turnover times of ~9hr, but any membrane fluctuations appear too modest to effect the auditory circadian rhythm[155]. Nevertheless, Nan/Iav turnover or post-translational modifications of both ion channels cannot be excluded as rhythm effectors. Peripheral clocks mediate tissue-specific expression patterns mostly via post-transcriptional regulation. However, they also influence expression patterns by circadian regulation of transcription factors which control vast transcriptional programs[156]. Four such master regulatory transcription factors[157] are implicated in a study of JO's time- and age-variable transcriptome[158]. These master regulators are *wor, Optix, amos* and *onecut*. They intersect on a cluster of genes (Figure 21), one of which is *Naam*. *Naam* RNA products appear to cycle with circadian time (Figure 22), suggesting clock-mediated transcription.

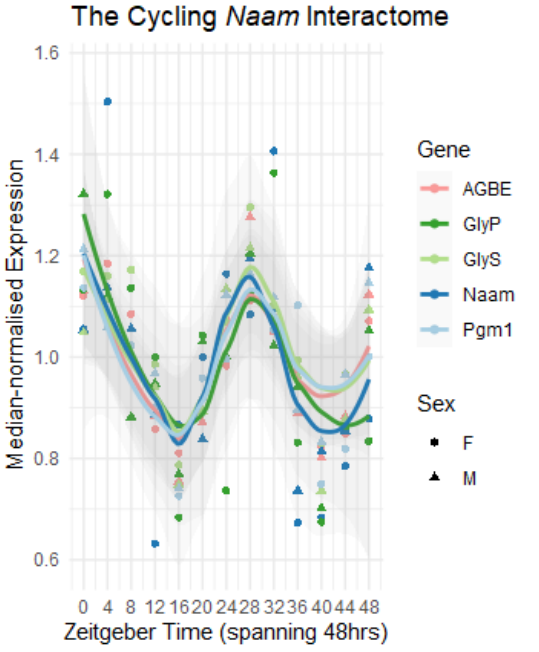

Figure 212: Unpublished data: RNA sequencing of JO at 4-hour intervals (each data point is the mean of a biological triplicate of 280 JOs) in the circadian day across 48h reveals oscillatory concentrations of Naam *RNA products, indicating circadian-regulated transcription of* Naam. AGBE, GlyP, GlyS, Pgm1, *and* Rpl32 *were identified as Naam protein-interaction partners using the STRING protein association network database[158].*

Naam encodes nicotinamidase, which catalyses the decomposition of nicotinamide into nicotinate and ammonia, contributing to the salvage pathway of nicotinamide adenine dinucleotide (NAD+). NAD+/NADH ratio is an indicator of cellular reduction potential and is modulated by small changes in NAD+ concentration[159]. NAD+ is the central electron carrier in cellular metabolism, including respiration. NAD is also consumed in many non-redox processes such as ADP-ribosylation, cyclic-ADP-ribose production, and by NAD-dependent histone deacetylases (sirtuins: Sir2 homologues). NAD+/NADH ratio shifts towards NADH are pro-senescent; NADH competitively inhibits sirtuins and facilitates reactive oxygen species (ROS) formation[160]. Mitochondria generate ROS during cellular respiration. ROS are normally cleared by antioxidant scavengers. The mammalian cochlea is especially sensitive to oxidative stress due to hair cells' high metabolic demand. As protective antioxidant mechanisms degenerate

with age, ROS overproduction is implicated in cochlear senescence. Age-related sensory hair cell loss is exacerbated in *Sod1* mutant mice, a key antioxidant protective against oxidative stress[161]. Circumstantial evidence indicates that similar processes may occur in *Drosophila*. Flies, like mammals, succumb to age-related hearing loss (ARHL), entering a phase of rapid terminal auditory decline during which the active energy injection process progressively fails. During this phase, *Naam* is downregulated[158], implying a role in functional auditory homeostasis, as *Naam* is differentially expressed in JO[162] and embryonic chordotonal organs[163]. Additionally, flies responding to oxidative stress show a six-fold increase in *Naam* mRNA transcription and translation, and *Naam*-overexpressing flies exhibit a Sir2-dependent increased mean lifespan of ~30%[164]. Overexpression using *elavGal4* implicates neurons as the key mediator cell for *Naam*'s lifespan-extending effects.

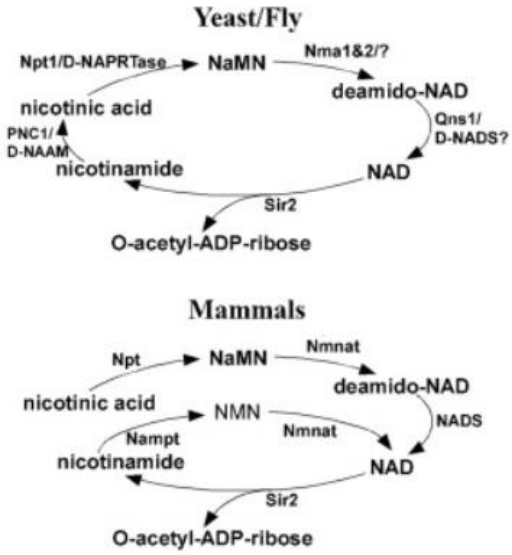

Figure 23: NAD salvage pathways in mammals and yeast/flies (ref.[162]).

Sirtuins are linked to cellular stress resistance and extended lifespan, regulating genes involved in mitochondrial function, inflammation, apoptosis, and many aspects of aging[165]. Sirtuins can rapidly deplete cellular NAD, resulting in increased concentrations of nicotinamide, which acts as a potent feedback inhibitor[164]. Therefore, NAAM is critical to the salvage pathway that both replenishes NAD and reduces nicotinamide negative feedback (Figure 233). The NAD salvage pathway differs in mammals, incorporating a functional equivalent of *Naam*: *Nampt* (nicotinamide phosphoribosyltransferase)[166]. NAD salvage may play a major role in auditory homeostasis in both vertebrates and invertebrates.

The *Drosophila* evidence for *Naam* function comes from non-auditory-specific overexpression studies and does not directly address whether *Naam* is part of a normal homeostatic mechanism. Nevertheless, there is enough evidence to indicate that manipulating *Naam* may disrupt the putative circadian patterning of homeostatic repair, potentially influencing circadian auditory rhythms.

4. Conclusion

In summary, the antennal ear relies on active signal amplification. The power gain of this amplifier varies with circadian time, as a potential consequence of the temporal patterning of auditory homeostatic repair. This assumed patterning is in near-antiphase to circadian mating rhythms, optimising the circadian auditory sensitivity profile to the acoustic communication demands of morning mating behaviour. Circadian homeostatic orchestration likely relies on a peripheral molecular clock in the antennal ear. The clock's homeostatic molecular control arms are poorly understood. *Naam*, encoding nicotinamidase, is one of a cluster of genes that may be the missing link between the molecular clock and circadian auditory homeostasis. Manipulation of *Naam* may reveal key principles of this presumptive circadian organisation.

Like all living systems, sensory homeostasis is a product of multiple dynamic equilibria producing the illusion of stability in an entropic universe[167]. As Richard Howard concludes *Like Most Revelations*[168]:

> "to this mortal process of continuing,
>
> it is the movement that creates the form."

When Blake (above) analogised man to the fly, he invoked the fly in a literary sense, where its symbolisations are legion. In ancient Babylonian mythology[169] [170] [171], as in Aeschylus's *Prometheus Bound*[172] and the Book of Exodus[173], the fly embodies divine expression – chiefly wrath. However, Blake was more likely to have been referencing Shakespeare's usage. In *King Lear*[174], the Duke of Gloucester reflects:

> "As flies to wanton boys are we to the gods;
>
> They kill us for their sport." (Act 4 Sc i: 38-9)

Emily Dickinson's poetic reflection on death[175] may use the fly to spurn these theological diatribes. Curiously, all these representations use the fly to explore man's relationship with a presumptive demiurge. As did the ancients, we moderns use the fly to explore our relationship with the cosmos, but in a less pious sense. At the gates of understanding the sapient neocortex – the most complex system in the known universe, the fly proves invaluable to elucidating the deep logic of neural organisation[176], even recapitulating basic emotions as neuromodulatory states[177]. So much of the fly's behaviour is explained by simple responses such as gravitaxis[27], chemotaxis[178], and phonotaxis[179]. Concerning complex behaviours, the algorithmic dissection of mating song composition[94] gives hope that all behavioural paradigms will be understood as deterministic systems – first, in flies, and one day, in man. Should there be a higher power, as the ancients conceived, it is somewhat startling to consider that they might see us in the same deterministic way that we see flies.

5. References

1. Blake, W. *Songs of Innocence and Songs of Experience*. (Courier Corporation, 2012).
2. Katz, P. S. 'Model organisms' in the light of evolution. *Current Biology* **26**, R649–R650 (2016).
3. Fields, S. & Johnston, M. Whither Model Organism Research? *Science* **307**, 1885–1886 (2005).
4. Albert, J. T., Jarman, A. P., Kamikouchi, A. & Keder, A. 2.50 - Drosophila as a Model for Hearing and Deafness. in *The Senses: A Comprehensive Reference (Second Edition)* (ed. Fritzsch, B.) 985–1004 (Elsevier, 2020). doi:10.1016/B978-0-12-809324-5.24216-8.
5. Hales, K. G., Korey, C. A., Larracuente, A. M. & Roberts, D. M. Genetics on the Fly: A Primer on the Drosophila Model System. *Genetics* **201**, 815 (2015).
6. Ugur, B., Chen, K. & Bellen, H. J. Drosophila tools and assays for the study of human diseases. *Disease Models & Mechanisms* **9**, 235–244 (2016).
7. Greenspan, R. J. & Dierick, H. A. 'Am not I a fly like thee?' From genes in fruit flies to behavior in humans. *Human Molecular Genetics* **13**, R267–R273 (2004).
8. sound | Properties, Types, & Facts | Britannica. https://www.britannica.com/science/sound-physics.
9. Mahbub, M. *Fundamentals of Telecommunications (Second Edition)-Roger L. Freeman*.
10. Purves, D. *et al.* The Audible Spectrum. *Neuroscience. 2nd edition* (2001).
11. Gridi-Papp, M. & Narins, P. M. 3.04 - Sensory Ecology of Hearing. in *The Senses: A Comprehensive Reference* (eds. Masland, R. H. et al.) 61–74 (Academic Press, 2008). doi:10.1016/B978-012370880-9.00011-6.
12. Patella, P. & Wilson, R. I. Functional Maps of Mechanosensory Features in the Drosophila Brain. *Current Biology* **28**, 1189-1203.e5 (2018).
13. Boekhoff-Falk, G. & Eberl, D. F. The Drosophila auditory system. *WIREs Developmental Biology* **3**, 179–191 (2014).
14. Henley, C. *Foundations of Neuroscience*. (Michigan State University Libraries, 2021).
15. Westheimer, G. 1.01 - The Visual System and Its Stimuli. in *The Senses: A Comprehensive Reference* (eds. Masland, R. H. et al.) 1–7 (Academic Press, 2008). doi:10.1016/B978-012370880-9.00250-4.
16. Hudspeth, A. J., Choe, Y., Mehta, A. D. & Martin, P. Putting ion channels to work: Mechanoelectrical transduction, adaptation, and amplification by hair cells. *Proceedings of the National Academy of Sciences* **97**, 11765–11772 (2000).
17. Hudspeth, A. J. How the ear's works work. *Nature* **341**, 397–404 (1989).
18. Bodmer, R., Carretto, R. & Jan, Y. N. Neurogenesis of the peripheral nervous system in Drosophila embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21–32 (1989).
19. Brewster, R. & Bodmer, R. Origin and specification of type II sensory neurons in Drosophila. *Development* **121**, 2923–2936 (1995).
20. Göpfert, M. C. & Robert, D. Turning the key on Drosophila audition. *Nature* **411**, 908–908 (2001).
21. Kamikouchi, A., Shimada, T. & Ito, K. Comprehensive classification of the auditory sensory projections in the brain of the fruit fly Drosophila melanogaster. *J Comp Neurol* **499**, 317–356 (2006).
22. Carlson, J. R. Olfaction in Drosophila: from odor to behavior. *Trends in Genetics* **12**, 175–180 (1996).
23. Ishikawa, Y., Okamoto, N., Nakamura, M., Kim, H. & Kamikouchi, A. Anatomic and Physiologic Heterogeneity of Subgroup-A Auditory Sensory Neurons in Fruit Flies. *Frontiers in Neural Circuits* **11**, (2017).

24. Nadrowski, B., Effertz, T., Senthilan, P. R. & Göpfert, M. C. Antennal hearing in insects–new findings, new questions. *Hear Res* **273**, 7–13 (2011).
25. Field, L. H. & Matheson, T. Chordotonal Organs of Insects. (1998) doi:10.1016/S0065-2806(08)60013-2.
26. Albert, J. T., Nadrowski, B. & Göpfert, M. C. Mechanical signatures of transducer gating in the Drosophila ear. *Curr Biol* **17**, 1000–1006 (2007).
27. Armstrong, J. D., Texada, M. J., Munjaal, R., Baker, D. A. & Beckingham, K. M. Gravitaxis in Drosophila melanogaster: a forward genetic screen. *Genes Brain Behav* **5**, 222–239 (2006).
28. Yorozu, S. *et al.* Distinct sensory representations of wind and near-field sound in the Drosophila brain. *Nature* **458**, 201–205 (2009).
29. Mamiya, A., Straw, A. D., Tómasson, E. & Dickinson, M. H. Active and Passive Antennal Movements during Visually Guided Steering in Flying Drosophila. *J. Neurosci.* **31**, 6900–6914 (2011).
30. Kernan, M. J. Mechanotransduction and auditory transduction in Drosophila. *Pflugers Arch* **454**, 703–720 (2007).
31. Tootoonian, S., Coen, P., Kawai, R. & Murthy, M. Neural Representations of Courtship Song in the Drosophila Brain. *J. Neurosci.* **32**, 787–798 (2012).
32. Kamikouchi, A. *et al.* The neural basis of Drosophila gravity-sensing and hearing. *Nature* **458**, 165–171 (2009).
33. Matsuo, E. *et al.* Identification of novel vibration- and deflection-sensitive neuronal subgroups in Johnston's organ of the fruit fly. *Frontiers in Physiology* **5**, (2014).
34. LeMasurier, M. & Gillespie, P. G. Hair-Cell Mechanotransduction and Cochlear Amplification. *Neuron* **48**, 403–415 (2005).
35. Lukashkin, A. N., Walling, M. N. & Russell, I. J. Power Amplification in the Mammalian Cochlea. *Current Biology* **17**, 1340–1344 (2007).
36. Robles, L. & Ruggero, M. A. Mechanics of the Mammalian Cochlea. *Physiological Reviews* **81**, 1305–1352 (2001).
37. Xia, A. *et al.* Prestin Regulation and Function in Residual Outer Hair Cells after Noise-Induced Hearing Loss. *PLoS One* **8**, e82602 (2013).
38. Dallos, P. The active cochlea. *J. Neurosci.* **12**, 4575–4585 (1992).
39. Hudspeth, A. Mechanical amplification of stimuli by hair cells. *Current Opinion in Neurobiology* **7**, 480–486 (1997).
40. R, P. Otoacoustic emission : An Overview. New aspect of cochlear mechanics and inner ear pathophysiology. *Advances in Oto-Rhino-Laryngology* (1990).
41. Shera, C. A. Mammalian spontaneous otoacoustic emissions are amplitude-stabilized cochlear standing waves. *The Journal of the Acoustical Society of America* **114**, 244–262 (2003).
42. Hudspeth, A. J. Making an effort to listen: mechanical amplification in the ear. *Neuron* **59**, 530–545 (2008).
43. Nadrowski, B., Albert, J. T. & Göpfert, M. C. Transducer-Based Force Generation Explains Active Process in Drosophila Hearing. *Current Biology* **18**, 1365–1372 (2008).
44. He, D. Z. Z., Jia, S. & Dallas, P. Mechanoelectrical transduction of adult outer hair cells studied in a gerbil hemicochlea. *Nature* **429**, 766–770 (2004).
45. Göpfert, M. C., Humphris, A. D. L., Albert, J. T., Robert, D. & Hendrich, O. Power gain exhibited by motile mechanosensory neurons in Drosophila ears. *Proceedings of the National Academy of Sciences* **102**, 325–330 (2005).

46. 23.6: Forced Damped Oscillator. *Physics LibreTexts* https://phys.libretexts.org/Bookshelves/Classical_Mechanics/Classical_Mechanics_(Dourmashkin)/23%3A_Simple_Harmonic_Motion/23.06%3A_Forced_Damped_Oscillator (2020).
47. 15.4: Damped and Driven Oscillations. *Physics LibreTexts* https://phys.libretexts.org/Bookshelves/University_Physics/Book%3A_Physics_(Boundless)/15%3A_Waves_and_Vibrations/15.4%3A_Damped_and_Driven_Oscillations (2018).
48. Ground Motion. https://www.desy.de/~sahoo/WebPage/Ground%20Motion.htm.
49. Damped Driven Oscillator. https://galileo.phys.virginia.edu/classes/152.mf1i.spring02/Oscillations4.htm.
50. Göpfert, M. C. & Robert, D. The mechanical basis of Drosophila audition. *Journal of Experimental Biology* **205**, 1199–1208 (2002).
51. Göpfert, M. C. & Robert, D. Motion generation by Drosophila mechanosensory neurons. *Proceedings of the National Academy of Sciences* **100**, 5514–5519 (2003).
52. Riabinina, O., Dai, M., Duke, T. & Albert, J. T. Active process mediates species-specific tuning of Drosophila ears. *Curr Biol* **21**, 658–664 (2011).
53. Bennet-Clark, H. C. Acoustics of Insect Song. *Nature* **234**, 255–259 (1971).
54. Kamikouchi, A., Albert, J. T. & Göpfert, M. C. Mechanical feedback amplification in Drosophila hearing is independent of synaptic transmission. *Eur J Neurosci* **31**, 697–703 (2010).
55. Liberman, M. C., Liberman, L. D. & Maison, S. F. Efferent Feedback Slows Cochlear Aging. *J. Neurosci.* **34**, 4599–4607 (2014).
56. Howard, J. & Hudspeth, A. J. Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the Bullfrog's saccular hair cell. *Neuron* **1**, 189–199 (1988).
57. Kennedy, H. J., Crawford, A. C. & Fettiplace, R. Force generation by mammalian hair bundles supports a role in cochlear amplification. *Nature* **433**, 880–883 (2005).
58. Corey, D. P. & Hudspeth, A. J. Kinetics of the receptor current in bullfrog saccular hair cells. *J. Neurosci.* **3**, 962–976 (1983).
59. Eatock, R. A. Adaptation in hair cells. *Annual Review of Neuroscience* **23**, 285–314 (2000).
60. Bartsch, T. F. *et al.* Elasticity of individual protocadherin 15 molecules implicates tip links as the gating springs for hearing. *Proceedings of the National Academy of Sciences* **116**, 11048–11056 (2019).
61. Christensen, A. P. & Corey, D. P. TRP channels in mechanosensation: direct or indirect activation? *Nat Rev Neurosci* **8**, 510–521 (2007).
62. Zanini, D. & Göpfert, M. C. Mechanosensation: Tethered Ion Channels. *Current Biology* **23**, R349–R351 (2013).
63. Effertz, T., Nadrowski, B., Piepenbrock, D., Albert, J. T. & Göpfert, M. C. Direct gating and mechanical integrity of Drosophila auditory transducers require TRPN1. *Nat Neurosci* **15**, 1198–1200 (2012).
64. Gong, Z. *et al.* Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in Drosophila. *J Neurosci* **24**, 9059–9066 (2004).
65. Lee, J., Moon, S., Cha, Y. & Chung, Y. D. Drosophila TRPN(= NOMPC) Channel Localizes to the Distal End of Mechanosensory Cilia. *PLOS ONE* **5**, e11012 (2010).
66. Kim, J. *et al.* A TRPV family ion channel required for hearing in Drosophila. *Nature* **424**, 81–84 (2003).
67. Nesterov, A. *et al.* TRP Channels in Insect Stretch Receptors as Insecticide Targets. *Neuron* **86**, 665–671 (2015).

68. Tobin, D. M. *et al.* Combinatorial Expression of TRPV Channel Proteins Defines Their Sensory Functions and Subcellular Localization in C. elegans Neurons. *Neuron* **35**, 307–318 (2002).
69. Lehnert, B. P., Baker, A. E., Gaudry, Q., Chiang, A.-S. & Wilson, R. I. Distinct roles of TRP channels in auditory transduction and amplification in Drosophila. *Neuron* **77**, 115–128 (2013).
70. Göpfert, M. C., Albert, J. T., Nadrowski, B. & Kamikouchi, A. Specification of auditory sensitivity by Drosophila TRP channels. *Nat Neurosci* **9**, 999–1000 (2006).
71. Effertz, T., Wiek, R. & Göpfert, M. C. NompC TRP channel is essential for Drosophila sound receptor function. *Curr Biol* **21**, 592–597 (2011).
72. Yan, Z. *et al.* Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature* **493**, 221–225 (2013).
73. Zhang, W. *et al.* Ankyrin Repeats Convey Force to Gate the NOMPC Mechanotransduction Channel. *Cell* **162**, 1391–1403 (2015).
74. Sotomayor, M., Corey, D. P. & Schulten, K. In search of the hair-cell gating spring elastic properties of ankyrin and cadherin repeats. *Structure* **13**, 669–682 (2005).
75. Liang, X. *et al.* A NOMPC-dependent membrane-microtubule connector is a candidate for the gating spring in fly mechanoreceptors. *Curr Biol* **23**, 755–763 (2013).
76. Peng, A. W., Effertz, T. & Ricci, A. J. Adaptation of mammalian auditory hair cell mechanotransduction is independent of calcium entry. *Neuron* **80**, 960–972 (2013).
77. Busse, L. *et al.* Sensation during Active Behaviors. *J. Neurosci.* **37**, 10826–10834 (2017).
78. Schlinger, H. D. Behavior analysis and behavioral neuroscience. *Frontiers in Human Neuroscience* **9**, (2015).
79. Engel, B. T. & Schneiderman, N. Operant conditioning and the modulation of cardiovascular function. *Annu Rev Physiol* **46**, 199–210 (1984).
80. de Belle, J. S., Hilliker, A. J. & Sokolowski, M. B. Genetic localization of foraging (for): a major gene for larval behavior in Drosophila melanogaster. *Genetics* **123**, 157–163 (1989).
81. Connolly, J. B. *et al.* Associative Learning Disrupted by Impaired Gs Signaling in Drosophila Mushroom Bodies. *Science* **274**, 2104–2107 (1996).
82. Sokolowski, M. B. Drosophila: Genetics meets behaviour. *Nat Rev Genet* **2**, 879–890 (2001).
83. Bastock, M. & Manning, A. The Courtship of Drosophila Melanogaster. *Behaviour* **8**, 85–110 (1955).
84. Stanley, R. A. & Kyriacou, C. P. A latitudinal cline in a courtship song character of Drosophila melanogaster. *J Neurogenet* **35**, 333–340 (2021).
85. Moulin, B., Aubin, T. & Jallon, J.-M. Why there is a one-way crossability between D. melanogaster and D. simulans? An ontogenic explanation. *Genetica* **120**, 285–292 (2004).
86. von Schilcher, F. The behavior of cacophony, a courtship song mutant in Drosophila melanogaster. *Behavioral Biology* **17**, 187–196 (1976).
87. Clemens, J. *et al.* Connecting Neural Codes with Behavior in the Auditory System of Drosophila. *Neuron* **87**, 1332–1343 (2015).
88. Spieth, H. T. Courtship behavior in Drosophila. *Annu Rev Entomol* **19**, 385–405 (1974).
89. Paillette, M., Ikeda, H. & Jallon, J.-M. A new acoustic signal of the fruit-flies Drosophila simulans and D. melanogaster. *Bioacoustics* **3**, 247–254 (1991).
90. Pavlou, H. J. & Goodwin, S. F. Courtship behavior in Drosophila melanogaster: towards a 'courtship connectome'. *Curr Opin Neurobiol* **23**, 76–83 (2013).

91. Pan, Y., Meissner, G. W. & Baker, B. S. Joint control of Drosophila male courtship behavior by motion cues and activation of male-specific P1 neurons. *Proc Natl Acad Sci U S A* **109**, 10065–10070 (2012).
92. von Philipsborn, A. C. *et al.* Neuronal control of Drosophila courtship song. *Neuron* **69**, 509–522 (2011).
93. Clyne, J. D. & Miesenböck, G. Sex-specific control and tuning of the pattern generator for courtship song in Drosophila. *Cell* **133**, 354–363 (2008).
94. Coen, P. *et al.* Dynamic sensory cues shape song structure in Drosophila. *Nature* **507**, 233–237 (2014).
95. Shirangi, T. R., Stern, D. L. & Truman, J. W. Motor control of Drosophila courtship song. *Cell Rep* **5**, 678–686 (2013).
96. Kerwin, P. & von Philipsborn, A. C. Copulation Song in Drosophila: Do Females Sing to Change Male Ejaculate Allocation and Incite Postcopulatory Mate Choice? *BioEssays* **42**, 2000109 (2020).
97. Jonsson, T., Kravitz, E. A. & Heinrich, R. Sound production during agonistic behavior of male Drosophila melanogaster. *Fly (Austin)* **5**, 29–38 (2011).
98. Versteven, M. *et al.* Hearing regulates Drosophila aggression. *Proceedings of the National Academy of Sciences* **114**, 1958–1963 (2017).
99. Pézier, A. P., Jezzini, S. H., Bacon, J. P. & Blagburn, J. M. Shaking B Mediates Synaptic Coupling between Auditory Sensory Neurons and the Giant Fiber of Drosophila melanogaster. *PLOS ONE* **11**, e0152211 (2016).
100. Blagburn, J. M. A new method of recording from the giant fiber of Drosophila melanogaster shows that the strength of its auditory inputs remains constant with age. *PLoS One* **15**, e0224057 (2020).
101. Ölveczky, B. P. Ordered randomness in fly love songs. *Nature* **507**, 178–178 (2014).
102. Aviram, R., Adamovich, Y. & Asher, G. Circadian Organelles: Rhythms at All Scales. *Cells* **10**, 2447 (2021).
103. Ishida, N., Kaneko, M. & Allada, R. Biological clocks. *Proceedings of the National Academy of Sciences* **96**, 8819–8820 (1999).
104. Bolwig's Organ and Ocelli. https://www.sdbonline.org/sites/fly/aimorph/eye3.htm#Schlichting.
105. Körding, K. P. & Wolpert, D. M. Bayesian integration in sensorimotor learning. *Nature* **427**, 244–247 (2004).
106. Somers, J., Harper, R. E. F. & Albert, J. T. How Many Clocks, How Many Times? On the Sensory Basis and Computational Challenges of Circadian Systems. *Frontiers in Behavioral Neuroscience* **12**, (2018).
107. Krishnan, B., Dryer, S. E. & Hardin, P. E. Circadian rhythms in olfactory responses of Drosophila melanogaster. *Nature* **400**, 375–378 (1999).
108. Page, T. L. & Koelling, E. Circadian rhythm in olfactory response in the antennae controlled by the optic lobe in the cockroach. *J Insect Physiol* **49**, 697–707 (2003).
109. Rymer, J., Bauernfeind, A. L., Brown, S. & Page, T. L. Circadian rhythms in the mating behavior of the cockroach, Leucophaea maderae. *J Biol Rhythms* **22**, 43–57 (2007).
110. Silvegren, G., Löfstedt, C. & Qi Rosén, W. Circadian mating activity and effect of pheromone pre-exposure on pheromone response rhythms in the moth Spodoptera littoralis. *J Insect Physiol* **51**, 277–286 (2005).
111. Granados-Fuentes, D., Tseng, A. & Herzog, E. D. A circadian clock in the olfactory bulb controls olfactory responsivity. *J Neurosci* **26**, 12219–12225 (2006).

112. Granados-Fuentes, D., Saxena, M. T., Prolo, L. M., Aton, S. J. & Herzog, E. D. Olfactory bulb neurons express functional, entrainable circadian rhythms. *Eur J Neurosci* **19**, 898–906 (2004).
113. Nippe, O. M., Wade, A. R., Elliott, C. J. H. & Chawla, S. Circadian Rhythms in Visual Responsiveness in the Behaviorally Arrhythmic Drosophila Clock Mutant ClkJrk. *J Biol Rhythms* **32**, 583–592 (2017).
114. Mazzoni, E. O., Desplan, C. & Blau, J. Circadian pacemaker neurons transmit and modulate visual information to control a rapid behavioral response. *Neuron* **45**, 293–300 (2005).
115. Barlow, R. Chapter 35 Circadian and efferent modulation of visual sensitivity. in *Progress in Brain Research* vol. 131 487–503 (Elsevier, 2001).
116. Andrade, M. J. O. de. *Circadian Sensation and Visual Perception*. (IntechOpen, 2021). doi:10.5772/intechopen.100591.
117. Kwon, Y. H. & Nam, K. S. Circadian fluctuations in three types of sensory modules in healthy subjects. *Neural Regen Res* **9**, 436–439 (2014).
118. Zhu, B., Sun, X., Zhang, H., Tang, Y. & Cui, J. Circadian Rhythm and Intersexual Differences in Auditory Frequency Sensitivity in Emei Music Frogs. *Asian Herpetological Research* **13**, 43–52 (2022).
119. Basinou, V., Park, J., Cederroth, C. R. & Canlon, B. Circadian regulation of auditory function. *Hearing Research* **347**, 47–55 (2017).
120. Yang, C.-H. *et al.* Constant Light Dysregulates Cochlear Circadian Clock and Exacerbates Noise-Induced Hearing Loss. *International Journal of Molecular Sciences* **21**, 7535 (2020).
121. Chabot, C. C. & Taylor, D. H. Circadian modulation of the rat acoustic startle response. *Behavioral Neuroscience* **106**, 846–852 (1992).
122. Frankland, P. W. & Ralph, M. R. Circadian modulation in the rat acoustic startle circuit. *Behavioral Neuroscience* **109**, 43–48 (1995).
123. Park, J. *et al.* Differential Phase Arrangement of Cellular Clocks along the Tonotopic Axis of the Mouse Cochlea Ex Vivo. *Current Biology* **27**, 2623-2629.e2 (2017).
124. Haggerty, H. S., Lusted, H. S. & Morton, S. C. Statistical quantification of 24-hour and monthly variabilities of spontaneous otoacoustic emission frequency in humans. *Hearing Research* **70**, 31–49 (1993).
125. Alcedo, J., Maier, W. & Ch'ng, Q. *Sensory Influence on Homeostasis and Lifespan: Molecules and Circuits*. *Madame Curie Bioscience Database [Internet]* (Landes Bioscience, 2013).
126. Sudland, O. Circadian modulation in the antennal ear of Drosophila melanogaster. (University College London, 2021).
127. Nishinokubi, I., Shimoda, M. & Ishida, N. Mating rhythms of Drosophila: rescue of tim01 mutants by D. ananassae timeless. *J Circadian Rhythms* **4**, 4 (2006).
128. Sakai, T. & Ishida, N. Circadian rhythms of female mating activity governed by clock genes in Drosophila. *Proc Natl Acad Sci U S A* **98**, 9221–9225 (2001).
129. Hardeland, R. Species differences in the diurnal rhythmicity of courtship behaviour within the Melanogaster group of the genus Drosophila. *Animal Behaviour* **20**, 170–174 (1972).
130. Hardin, P. E. Chapter 5 - Molecular Genetic Analysis of Circadian Timekeeping in Drosophila. in *Advances in Genetics* (ed. Brody, S.) vol. 74 141–173 (Academic Press, 2011).
131. Allada, R., White, N. E., So, W. V., Hall, J. C. & Rosbash, M. A mutant Drosophila homolog of mammalian Clock disrupts circadian rhythms and transcription of period and timeless. *Cell* **93**, 791–804 (1998).
132. Hao, H., Allen, D. L. & Hardin, P. E. A circadian enhancer mediates PER-dependent mRNA cycling in Drosophila melanogaster. *Mol Cell Biol* **17**, 3687–3693 (1997).

133. Darlington, T. K. *et al.* Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. *Science* **280**, 1599–1603 (1998).
134. Kloss, B. *et al.* The Drosophila clock gene double-time encodes a protein closely related to human casein kinase Iepsilon. *Cell* **94**, 97–107 (1998).
135. Kloss, B., Rothenfluh, A., Young, M. W. & Saez, L. Phosphorylation of period is influenced by cycling physical associations of double-time, period, and timeless in the Drosophila clock. *Neuron* **30**, 699–706 (2001).
136. Price, J. L. *et al.* double-time Is a Novel Drosophila Clock Gene that Regulates PERIOD Protein Accumulation. *Cell* **94**, 83–95 (1998).
137. Zeng, H., Qian, Z., Myers, M. P. & Rosbash, M. A light-entrainment mechanism for the Drosophila circadian clock. *Nature* **380**, 129–135 (1996).
138. Akten, B. *et al.* A role for CK2 in the Drosophila circadian oscillator. *Nat Neurosci* **6**, 251–257 (2003).
139. Martinek, S., Inonog, S., Manoukian, A. S. & Young, M. W. A role for the segment polarity gene shaggy/GSK-3 in the Drosophila circadian clock. *Cell* **105**, 769–779 (2001).
140. Top, D., Harms, E., Syed, S., Adams, E. L. & Saez, L. GSK-3 and CK2 Kinases Converge on Timeless to Regulate the Master Clock. *Cell Rep* **16**, 357–367 (2016).
141. Menet, J. S., Abruzzi, K. C., Desrochers, J., Rodriguez, J. & Rosbash, M. Dynamic PER repression mechanisms in the Drosophila circadian clock: from on-DNA to off-DNA. *Genes Dev* **24**, 358–367 (2010).
142. Cyran, S. A. *et al.* vrille, Pdp1, and dClock form a second feedback loop in the Drosophila circadian clock. *Cell* **112**, 329–341 (2003).
143. Glossop, N. R. J. *et al.* VRILLE feeds back to control circadian transcription of Clock in the Drosophila circadian oscillator. *Neuron* **37**, 249–261 (2003).
144. Stanewsky, R. *et al.* The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. *Cell* **95**, 681–692 (1998).
145. Kadener, S., Stoleru, D., McDonald, M., Nawathean, P. & Rosbash, M. Clockwork Orange is a transcriptional repressor and a new Drosophila circadian pacemaker component. *Genes Dev* **21**, 1675–1686 (2007).
146. Allada, R. & Chung, B. Y. Circadian Organization of Behavior and Physiology in Drosophila. *Annual Review of Physiology* **72**, 605–624 (2010).
147. Collins, B. & Blau, J. Even a stopped clock tells the right time twice a day: circadian timekeeping in Drosophila. *Pflugers Arch - Eur J Physiol* **454**, 857–867 (2007).
148. Linford, N. J., Kuo, T.-H., Chan, T. P. & Pletcher, S. D. Sensory Perception and Aging in Model Systems: From the Outside In. *Annu Rev Cell Dev Biol* **27**, 759–785 (2011).
149. Kashkenbayeva, Assel. Modulators of Auditory Responses in Drosophila: From the Ear to Behaviour via the Clock? (University College London, 2020).
150. Myers, E. M., Yu, J. & Sehgal, A. Circadian control of eclosion: interaction between a central and peripheral clock in Drosophila melanogaster. *Curr Biol* **13**, 526–533 (2003).
151. Sehgal, A. Control of Metabolism by Central and Peripheral Clocks in Drosophila. in *A Time for Metabolism and Hormones* (eds. Sassone-Corsi, P. & Christen, Y.) (Springer, 2016).
152. Plautz, J. D., Kaneko, M., Hall, J. C. & Kay, S. A. Independent photoreceptive circadian clocks throughout Drosophila. *Science* **278**, 1632–1635 (1997).
153. Ito, C. & Tomioka, K. Heterogeneity of the Peripheral Circadian Systems in Drosophila melanogaster: A Review. *Front Physiol* **7**, 8 (2016).
154. Harper, R. E. F. 'Time Flies' Multisensory Processing by Circadian Clocks in Drosophila melanogaster. (University College London, 2017).

155. Boyd-Gibbins, N. *et al.* Turnover and activity-dependent transcriptional control of NompC in the Drosophila ear. *iScience* **24**, 102486 (2021).
156. Partch, C. L., Green, C. B. & Takahashi, J. S. Molecular Architecture of the Mammalian Circadian Clock. *Trends Cell Biol* **24**, 90–99 (2014).
157. Chan, S. S.-K. & Kyba, M. What is a Master Regulator? *J Stem Cell Res Ther* **3**, 114 (2013).
158. Keder, A. *et al.* Homeostatic maintenance and age-related functional decline in the Drosophila ear. *Sci Rep* **10**, 7431 (2020).
159. Lin, S.-J. & Guarente, L. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. *Current Opinion in Cell Biology* **15**, 241–246 (2003).
160. Poljsak, B. & Milisav, I. NAD+ as the Link Between Oxidative Stress, Inflammation, Caloric Restriction, Exercise, DNA Repair, Longevity, and Health Span. *Rejuvenation Research* **19**, 406–413 (2016).
161. Johnson, K. R. *et al.* Separate and combined effects of Sod1 and Cdh23 mutations on age-related hearing loss and cochlear pathology in C57BL/6J mice. *Hear Res* **268**, 85–92 (2010).
162. Senthilan, P. R. *et al.* Drosophila auditory organ genes and genetic hearing defects. *Cell* **150**, 1042–1054 (2012).
163. zur Lage, P., Newton, F. G. & Jarman, A. P. Survey of the Ciliary Motility Machinery of Drosophila Sperm and Ciliated Mechanosensory Neurons Reveals Unexpected Cell-Type Specific Variations: A Model for Motile Ciliopathies. *Frontiers in Genetics* **10**, (2019).
164. Balan, V. *et al.* Life Span Extension and Neuronal Cell Protection by Drosophila Nicotinamidase*. *Journal of Biological Chemistry* **283**, 27810–27819 (2008).
165. Frankel, S., Ziafazeli, T. & Rogina, B. dSir2 and longevity in Drosophila. *Experimental Gerontology* **46**, 391–396 (2011).
166. Yang, H., Lavu, S. & Sinclair, D. A. Nampt/PBEF/Visfatin: A regulator of mammalian health and longevity? *Exp Gerontol* **41**, 718–726 (2006).
167. Schoenheimer, R. The Dynamic State of Body Constituents. *The Dynamic State of Body Constituents.* (1942).
168. Poets, A. of A. Like Most Revelations by Richard Howard - Poems | Academy of American Poets. https://poets.org/poem/most-revelations.
169. *Encyclopedia of the Bible and Its Reception (EBR): Aaron - Animal Symbolism v. 1 (Encyclopedia of the Bible & Its Reception): 01*. (De Gruyter, 2009).
170. Black, J. A. & Green, A. *Gods, Demons and Symbols of Ancient Mesopotamia: An Illustrated Dictionary*. (British Museum Press, 1992).
171. Inanna's Descent: A Sumerian Tale of Injustice. *World History Encyclopedia* https://www.worldhistory.org/article/215/inannas-descent-a-sumerian-tale-of-injustice/.
172. AESCHYLUS, PROMETHEUS BOUND - Theoi Classical Texts Library. https://www.theoi.com/Text/AeschylusPrometheus.html.
173. Book of Exodus. *Wikipedia* (2022).
174. Shakespeare, W. & Carabine, D. K. *King Lear*. (Wordsworth Editions, 1994).
175. I heard a Fly buzz—when I died. *Wikipedia* (2021).
176. With a little help from our (insect) friends. *NobelPrize.org* https://www.nobelprize.org/drosophila/ (2020).
177. Gu, S., Wang, F., Patel, N. P., Bourgeois, J. A. & Huang, J. H. A Model for Basic Emotions Using Observations of Behavior in Drosophila. *Frontiers in Psychology* **10**, (2019).
178. Active sampling and decision making in Drosophila chemotaxis | Nature Communications. https://www.nature.com/articles/ncomms1455.

179. Batchelor, A. V. & Wilson, R. I. Sound localization behavior in Drosophila melanogaster depends on inter-antenna vibration amplitude comparisons. *J Exp Biol* **222**, jeb191213 (2019).