Origins

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Published in:
Biological Timekeeping

DOI:
10.1007/978-81-322-3688-7_1

Publication date:
2017

Chapter 1
Origins: A Brief Account of the Ancestry of Circadian Biology

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Abstract Who were the investigators and what was the path that enabled the launch of modern mechanistic research on circadian biology in the 1970s? Here we trace the origins of ideas from antiquity to the experimental study of the daily movements of leaves; on to the twentieth-century realization that circadian rhythms are widespread, endogenous, and innate; and finally to the appreciation that such rhythms could be utilized by organisms for the measurement of time. The conceptualization of the internal "clock" metaphor was key to the wave of mathematical, neurobiological, and molecular genetic advances that has transformed the field over the last 50 years.

1.1 Introduction

> We are like dwarfs sitting on the shoulders of giants. We see more, and things that are more distant, than they did, not because our sight is superior or because we are taller than they, but because they raise us up, and by their great stature add to ours. (John of Salisbury (c. 1120–1180) *Metalogicon*, 1159)

The last 50 years have seen a remarkable transformation in our understanding and application of the science of biological timekeeping, especially in the circa 24 h domain. We have moved forward from debating the existence of an endogenous "clock" to identifying a pathological point mutation in the human homolog of a fruit fly gene that regulates behavioral rhythmicity. Of course, such an explosion of knowledge does not take place *de novo* or by chance. Who made the antecedent

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V. Kumar (ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour*,
DOI 10.1007/978-81-322-3688-7_1

observations, experiments, and insights that (paraphrasing Louis Pasteur) prepared the minds of contemporary researchers for discovery?

Here we provide a short account attempting to highlight the scientific path that preceded and launched modern mechanistic research in circadian biology, which, by our subjective estimation, dates from the 1970s; the astounding progress made since then is clearly described in the chapters of this volume. The literature already includes notable accounts of the history of chronobiology and the memoirs and reflections of those who made it, including Erwin Bünning [1], Jürgen Aschoff [2], and Colin Pittendrigh [3]. A complete review of the historical development of chronobiological concepts [4] has been composed by one of the present authors (SD).

1.2 From the Mists of Antiquity

The ancient Greeks distinguished their sense of time with two words. *Chronos* (χρόνος) refers to the passage of chronological time, linearly from the past to the future. *Kairós* (καιρός) represents the suitable (right or opportune) moment in time, especially for taking action; perhaps *timing* is our modern English equivalent. Thus, *kairós* is the meaning of the translated aphorism in the poem *Works and Days* by the Greek rhapsode Hesiod c. 700 BCE, "Observe due measure, for right timing is in all things the most important factor." So, arguably, the differentiation of biological from geophysical time, and the notion of optimal phases under entrained conditions, had already been realized thousands of years ago, presaging modern compilations of the ideal times of day for conducting disparate behavioral activities and physicochemical functions.

Aristotle (384–322 BCE) is credited with the proverb, "It is well to be up before daybreak, for such habits contribute to health, wealth, and wisdom." It is tempting to imagine that this ancient endorsement of the benefits of an early chronotype might have reflected even earlier beliefs that derive from solar worship and the need to greet the rising sun. In any case, this advice has been passed down across the millennia, as recognized in English-speaking countries from the quotations of the American polymath Benjamin Franklin (1706–1790) ("Early to bed and early to rise, makes a man healthy, wealthy, and wise." [5]) and English essayist Samuel Johnson (1709–1784) ("I have, all my life long, been lying till noon; yet I tell all young men, and tell them with great sincerity, that nobody who does not rise early will ever do any good." (1773) [6]). Earlier versions of this admonition have been identified in fifteenth-century English writings [7].

1.3 In the Beginning: The Daily Movement of Leaves

Theophrastus of Eresos (371–287 BCE), widely considered the "father" of botany for his work *Historia Plantarum*, cited the observations made by Androsthenes of Thasos, a trierarch (commander of a trireme), on his voyage from the Indus River to the Persian Gulf following Alexander the Great's Indian campaign (326–324 BCE). On Tylos (present-day Bahrain), Androsthenes described the tamarind tree as "...another tree with many leaves...that closes at night, but opens at sunrise, and by noon is completely unfolded; and at evening – again it closes by degrees and remains shut at night, and the natives say that it goes to sleep." Such daily leaf movements, known by the term *nyctinasty*, were further tabulated by others in additional species over the centuries, but reports of experimentation are not known until the eighteenth century.

Jean-Jacques d'Ortous de Mairan (1678–1771) had already published dissertations on subjects in physics, mechanics, and optics before his now famous experiment was reported to the Royal Academy of Sciences in Paris in 1729. He was very well respected, was elected to various leadership positions in the Academy, and eventually was inducted to membership in learned societies throughout Europe (at the time, he was considered one of the most outstanding scientists of the century, and a crater on the moon, Mairan, was named after him in 1935). A one-page "botanical observation" [8] reported on de Mairan's placement of "sensitive plants" in a dark spot, away from the sun and the open air. The plants seemed to continue opening their leaves in daytime and closing them at night. Thus, while de Mairan deservedly receives credit for moving a plant from sunlight, his chronobiological legacy rests on a paper without any description of how dark the place was or of the timing of the observations. Certainly no claim (or even speculation) was made that the plant opened its leaves in the dark by using an internal clock. On the contrary, de Mairan's conclusion was that the plant "sensed the sun without seeing it," so he presumed an external cause.

De Mairan's paper was not ignored, and within decades the work was pursued by Henri-Louis Duhamel du Monceau (1700–1782) in France, John Hill (1716–1775) in England, and Johann Gottfried Zinn (1727–1759) in Göttingen (then in the Kingdom Hannover, now Germany). De Mairan's basic finding was confirmed, and additional evidence was provided that the perception of daytime did not appear to be due to light leaks or varying temperature [9–11]. A breakthrough came only after the turn of the nineteenth century, by Augustin Pyramus de Candolle (1778–1841), a Swiss botanist. Like de Mairan, he was celebrated in his time, especially for his work on taxonomy [12], a word he coined in 1813; he was the first of four generations of de Candolle botanists, and the journal *Candollea*, published by the Conservatory and Botanical Garden of the City of Geneva, is named after him. De Candolle studied the leaf movements of *Mimosa pudica* in continuous light and found that the periodicity of "sleep and wakefulness" was shorter than 24 h and varied from plant to plant ("in several pots the acceleration amounted to one and a half to two hours per day") [13]. From this deviation from 24 h, de Candolle concluded (p. 861) "that the sleep-wake movements are connected with a disposition for periodic motion that is inherent in the plant." This was the first time, in 1832, that an endogenous cause of the periodicity was postulated.

De Candolle's view was adopted by prominent botanists, notably Julius Sachs (1832–1897) [14], but the first person to write a book about plant movements, the German plant physiologist Wilhelm Pfeffer (1845–1920) – whose interest extended beyond nyctinasty to thermonasty and photonasty and whose opinions carried great weight – was not at all persuaded. He concluded that the movements were "after-oscillations" of a system returning to a stable position after a disturbance ("The experiments reported here show irrefutably that the daily periodic movements can not be considered historically established properties, since they indeed slowly stop in continuous illumination.") [15]. Notably, Charles Darwin (1809–1882) was convinced of the inherited endogenous nature of daily movements. Just 5 years later, he too wrote a book, together with his botanist son Francis, entitled *The Power of Movement in Plants* [16] and even offered a specific idea for selective benefits. He stated (p. 284):

> The fact that the leaves of many plants place themselves at night in widely different positions from what they hold during the day, but with the one point in common, that their upper surfaces avoid facing the zenith, often with the additional fact that they come in close contact with opposite leaves or leaflets, clearly indicates, as it seems to us, that the object gained is the protection of the upper surfaces from being chilled at night by radiation. There is nothing improbable in the upper surface needing protection more than the lower, as the two differ in function and structure. All gardeners know that plants suffer from radiation.

In Leipzig, Pfeffer had moved his attention to other aspects of plant physiology; he did pioneering research on osmosis and acquired a reputation for exacting standards and innovative instrumentation (including photography). He was only drawn back to daily rhythms by the work of the German biologist Richard Semon (1859–1918), who went on to consider the nature of memories, coining the term engram. In 1905, Semon published data on young bean sprouts (*Phaseolus*) that had never seen any light; were then exposed to light-dark cycles of 12, 24, or 48 h; and afterward returned to constant conditions. He expected to see these periodicities reflected in the periods of the ensuing after-oscillations, but these always showed only a circa 24 h period, even when they had never seen one in their own life [17]. Pfeffer then returned to the problem, now applying continuous automatic recording of movements using the kymograph. In 1907, he finally accepted the existence of "autonomous" movements (p. 465) but with a much shorter period than the daily periodicities and their "aftereffects" (p. 472) [18]. Eventually, he found an ingenious way of preserving the rhythm of movements in constant light by darkening the leaf joints in the plants with black cotton. After conducting an extensive series of experiments in this way, he too was forced to conclude that the daily rhythm was sometimes persistent and not driven by an external 24-h force. He was 70 years old when he published his carefully obtained results in 1915 [19] (Fig. 1.1) in a verbose and unclear writing style and in a little-read journal – *Abhandlungen*

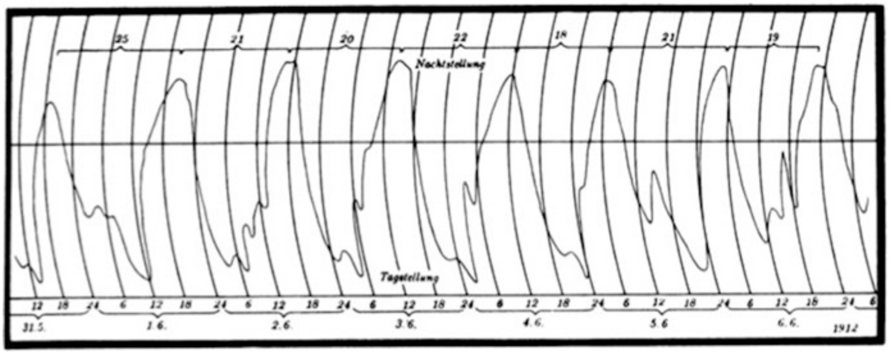

Fig. 1.1 Recording of bean leaf movements in continuous light by Pfeffer [19] (Reproduced with permission [80])

der mathematisch-physikalischen Klasse der Königlich sächsischen Gesellschaft der Wissenschaften – and so its impact at the time was limited.

1.4 Circadian Rhythms: Endogenous and Innate

As the twentieth century progressed, philosophical arguments began to yield to empirical evidence, and the case for an endogenous origin of daily rhythmicity, or at least a component of it, continued to build. Looking back, the experiments of the Dutch botanist Anthonia Kleinhoonte (1887–1960) on jack bean plants (*Canavalia ensiformis*) were especially prescient [20]. In the 1920s, she demonstrated that leaf movements exhibited a circa 24 h rhythm in constant darkness even if the seedlings were first maintained in an 8 h:8 h light-dark cycle and that brief light pulses – as short as one minute in duration – could reset rhythm phase depending on the phase of administration of the light. Plants could be induced to express a range of different phases in darkness, arguing against the view that such rhythms represented a direct response to an unknown exogenous factor [20].

Erwin Bünning (1906–1990), although entering the field as a *bona fide* botanist at the University of Jena in Germany, could be considered to be the first real circadian biologist. He helped to legitimize circa 24 h rhythms by referring to them by a name (endodiurnal; only later was the term circadian introduced by Franz Halberg (1919–2013), in 1959 [21]), and he wrote what was arguably the first accessible "textbook" on the subject (*Die Physiologische Uhr*, 1958). But perhaps most importantly, his research was not restricted to a single organism but spanned both plant and animal kingdoms. In the 1930s, he showed that endodiurnal rhythmicity in *Phaseolus* was clearly and significantly longer than 24 h, that raising the plants in anomalous light-dark or constant conditions did not affect the periodicity of succeeding generations, and that studying hybrids of plants with different periods implicated a genetic basis [22–24]. And then he proceeded to become among the

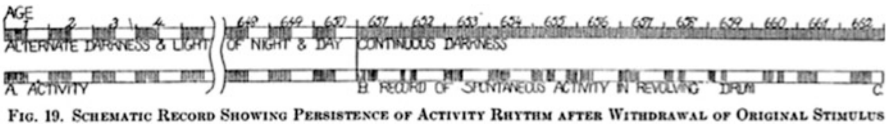

Fig. 1.2 Recording of rat locomotor activity in light-dark cycles and continuous darkness by Richter [27] (Figure 19, reproduced with permission [27])

first (see also the work of Hans Kalmus (1906–1988) in Prague [25]) to undertake studies of another 24 h rhythm, that of adult emergence from pupae (eclosion) in populations of fruit flies (*Drosophila*), reporting its persistence in the progeny of flies maintained in dim continuous light for multiple generations [26].

As mid-century approached, it had become clear that 24 h biological rhythms were a general phenomenon, encompassing a wide range of functions in a variety of organisms (although their endogenous generation had not been universally accepted). Vertebrate animals were no exception. By the end of the 1920s, the American psychobiologist Curt Richter (1894–1989) had already accumulated a wealth of locomotor activity data from rats [27] (Fig. 1.2), including those with access to "revolving drums" (running wheels) [28] invented some years earlier by Colin Stewart at Clark University in Worcester, Massachusetts [29]. For larger animals – including humans – body temperature became the assay of choice and a natural one for a new cadre of investigators entering the field after training in medicine and physiology. Sutherland Simpson (1863–1926), a Scottish MD who later moved his physiological laboratory to Cornell University in New York, performed heroic recordings of the axillary temperature rhythms of rhesus monkeys (*Macaca mulatta*) in light-dark, reversed light-dark, and constant dark conditions [30]. American physiologist Nathaniel Kleitman (1895–1999), considered a founder of modern sleep research, was intrigued by the regulation of the timing of sleep and wakefulness. In 1938, he and his assistant Bruce Richardson isolated themselves in Mammoth Cave, Kentucky, for 32 days, attempting to adjust to a 28 h day (19 h awake with lights on and 9 h in bed with lights off) at a subterranean-constant temperature of 54 °F (12.2 °C). Their body temperature recordings published later (Fig. 1.3a) hint that different periods were expressed by the two men [31]. Incidentally, decades later he and his PhD student Eugene Aserinsky (1921–1998) discovered REM sleep [32], effectively overturning the notion of a quiet, resting brain during sleep.

Jürgen Aschoff (1913–1998) was the youngest son of the famous German cardiac pathologist remembered eponymously by generations of medical students as the Aschoff-Tawara node (now known as the atrioventricular node) and Aschoff bodies (myocardial nodules following rheumatic fever). The younger Aschoff also studied medicine and in 1939 began to investigate the physiology of human thermoregulation; through self-experimentation, he discovered a previously unreported daily rhythm in heat loss under constant conditions without food intake, sleep, or locomotion [33]. He became fascinated by the subject, and – in the course of expanding his knowledge to include ethology and applied mathematics and

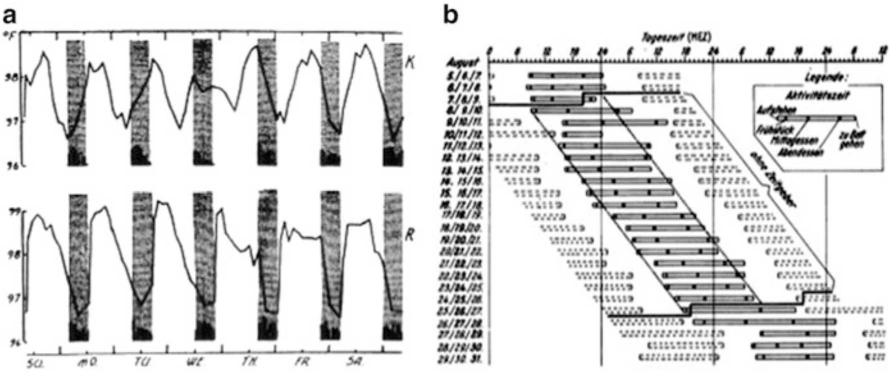

Fig. 1.3 Recordings of human rhythmicity. (**a**) Recording of Kleitman's (K) and Richardson's (R) body temperature rhythms in Mammoth Cave (1938) (Figure 18.4, reproduced with permission [31]). (**b**) Demonstration of the free-running endogenous circadian oscillator in humans by Aschoff and Wever [34] (Reproduced with permission [34])

working with rodents, birds, and humans – he produced a rigorous oeuvre on the properties and entrainment of physiological rhythms. Later, in 1961, he had the chance to use a civil air-raid shelter deep underground below the university's surgery clinic in the center of Munich and studied nine human subjects who lived there, one after the other, each for 2–4 weeks in isolation without any contact with the outside world and without access to any clocks or timing signals. Aschoff demonstrated in all of the subjects continuing rhythms with a period in excess of 24 h (Fig. 1.3b) [34]. After this first demonstration of human circadian rhythms, Aschoff built an underground bunker near his institute in Erling-Andechs (less than 50 km from Munich), specifically designed as a temporal isolation facility in which two subjects could be studied simultaneously. This led to a series of unprecedented experiments on nearly 450 subjects over 25 years that would set the standard for analyses of human circadian rhythmicity [35]. As an energetic, enthusiastic, and dramatic spokesperson for the field, Aschoff succeeded in bringing its emerging insights to international biomedical attention.

1.5 Paradigm Shift: Rhythms as Clocks

A few individuals, well ahead of their times, anticipated the concept that the internal "clocks" that were generating overt 24 h oscillations could be utilized by organisms for actual time measurement. Julien-Joseph Virey (1775–1846) was chief pharmacist at the Val-de-Grâce hospital in Paris until he was awarded an MD degree in 1814. Having observed the periodic nature of clinical phenomena in the hospital, he had the temerity to speculate (in his doctoral thesis [36]) on the existence of a kind of living clock – "une sorte d'horloge vivante" – *entrainée* (entrained), as he called it for the first time, by the movements of the sun and earth.

In 1936, a 30-year-old Bünning hypothesized how a 24 h clock could be part of a mechanism for measuring changing daylength [37], and in 1939 the American zoologist Maynard Johnson published activity records (cage movements) of deer mice (*Peromyscus leucopus*) in continuous light and concluded that the animals have "…an exceptionally substantial and durable self-winding and self-regulating physiological clock, the mechanism of which remains to be worked out." [38]. It was Johnson's final contribution to circadian biology (see below for a key earlier contribution); he left the Biological Laboratories of Harvard University for the Lake Mattamuskeet Wildlife Refuge in North Carolina.

It was in the 1950s that the conceptualization of rhythms as clocks truly began to take shape. In order to use an internal clock for the measurement of external time (both the time of day and the passage of time), the clock's oscillation must adopt a stable phase relationship to the environment. American biologists J. Woodland ("Woody") Hastings (1927–2014) and E. Beatrice ("Beazy") M. Sweeney (1914–1989), assaying the circadian bioluminescence rhythm of a photosynthetic dinoflagellate (*Gonyaulax polyedra*, now *Lingulodinium polyedrum*), and Patricia DeCoursey (b. 1932), recording the circadian wheel-running rhythm of the flying squirrel (*Glaucomys volans*), rigorously quantified the resetting responses of these rhythms to light pulses presented across the circadian cycle. The resulting "phase-response" curves [39, 40] revealed how the interaction of an endogenous oscillation with a rhythm of light responsiveness could lead to precise and accurate entrainment to the environmental light-dark cycle.

Colin S. Pittendrigh (1918–1996) earned his initial degree in botany at the University of Durham in England, and during World War II, he was posted to Trinidad to tackle malaria control; it was there that he first noted the daily activity rhythms of mosquitoes. He then studied genetics and evolutionary biology for his PhD (1948) at Columbia University in New York, under the famous geneticist Theodosius Dobzhansky (immortalized by his axiom that "nothing in biology makes sense except in the light of evolution" [41]). Pittendrigh remained in the United States, and his influence on circadian biology, and on the formulation of the "clock" metaphor, was pivotal, continuing even after his formal retirement (1984) to homes in Arizona and Montana. While Kalmus had claimed that the period of the *Drosophila* eclosion rhythm depended on temperature [42], Pittendrigh argued that temperature independence (or temperature compensation) of circadian period must be a fundamental feature of a reliable clock – actually, that it must hold true for the spontaneous frequency of any sensory organ, if such organs are to be employed for purposes other than temperature sensing [43]. In a masterful and lucid set of experiments on the timing of eclosion in *Drosophila pseudoobscura* [44], he rigorously analyzed the rhythm and the effects of light and hypoxia, demonstrated the rhythm's near independence of ambient temperature (Fig. 1.4), and provided an explanation for Kalmus' mistaken conclusion (he had measured only the first cycle after a temperature step). Moreover, Pittendrigh laid a foundation, in this and future work on flies and on nocturnal rodents with colleagues, for crucial conceptual advances that the mechanism of the circadian clock is separate (and separable) from the downstream behaviors it regulates and that the clock is used not only to

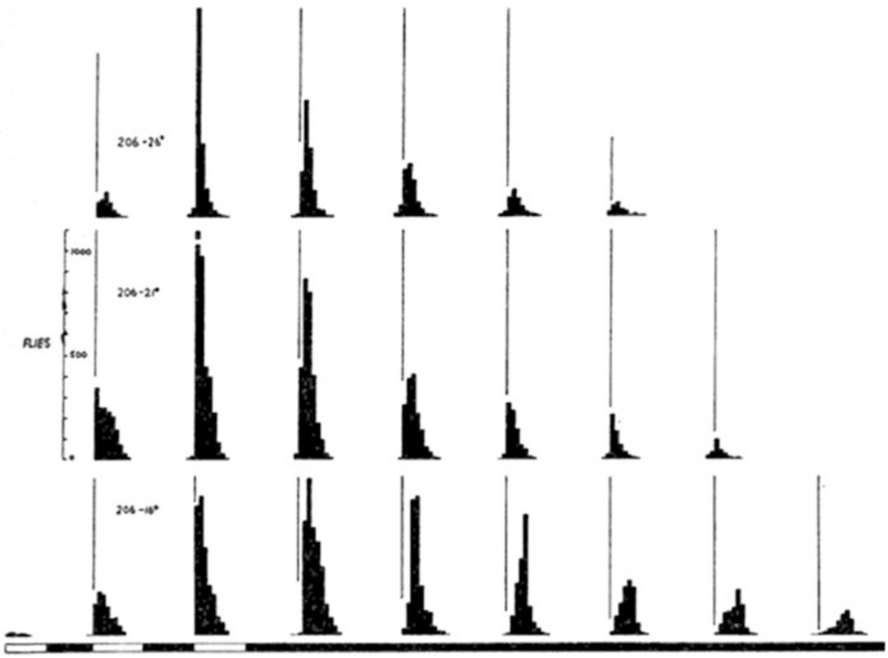

Fig. 1.4 Temperature compensation of the *Drosophila* eclosion rhythm in constant darkness, at 26°, 21°, and 16°C (1954). *Vertical lines* represent 24 h intervals (Reproduced with permission [55])

align the timing of an organism to the environment but also to temporally organize rhythmic subsystems ("slaves") within an organism. Imperious and insightful, at 45 years of age, he was the first circadian biologist to be elected to the US National Academy of Sciences. Notably, for "Pitt" (as he was called by his colleagues), teaching (including to undergraduates) served a critically important role; he was a passionate and electric speaker, pacing the floor and usually drenched in sweat by the end of his lectures.

Pittendrigh himself acknowledged the great influence of the brilliant German ornithologist and naturalist Gustav Kramer (1910–1959) on his conception of an innate, functional circadian clock. Kramer elucidated how migrating European starlings (*Sturnus vulgaris*) could stay on course by using the sun during their seasonal migration. Through ingenious experiments using circular cages and mirrors that artificially changed the apparent position of the sun in the sky, Kramer tracked the orientation of the birds' migratory restlessness and revealed the existence of a time-compensated sun compass [45], viz., navigation by the direction of the sun, taking into account its movement over the course of the day. In later experiments, a circadian mechanism was implicated by phase shifting the birds' clocks and then exposing them to the natural sun, leading to predictable deviations in their flight direction [46]. Kramer's accidental death while hunting rock pigeons on a Calabrian mountainside – just as he and Aschoff were poised to collaborate – was a tragic loss for the field and for ecology, as memorialized in the obituary [47] by Konrad Lorenz, 1973 Nobel Laureate. Of note, Karl von Frisch, the other 1973 Laureate (along with Nikolaas Tinbergen), concluded, simultaneously with Kramer, that honeybees also possess a time-compensated sun compass [48] as an orientation mechanism for their remarkable time-of-day-dependent choice of feeding location (referred to as "time memory") [49].

The 1960 edition of the annual symposium of the Cold Spring Harbor Biological Laboratory, on Long Island, New York, attracted 150 scientists; it was entitled *Biological Clocks* [50] and chaired by Pittendrigh. It has since assumed a vaunted place in the history of chronobiology. Here Bünning, Aschoff, and Pittendrigh presented a conceptual and experimental framework for the new interdisciplinary field, using the precise language and quantitative methods of oscillator theory: Bünning on photoperiodic time measurement and the heritability of circadian period; Pittendrigh on temperature compensation, transients and aftereffects, nonparametric entrainment, and the distinction between clock and driven rhythms; and Aschoff on zeitgebers, parametric entrainment, and exogenous and endogenous rhythm components. It was also the setting for a memorable exchange [43] between Pittendrigh and Frank A. Brown, Jr. (1908–1983), the persistent champion of the exogenous origin of overt rhythmicity.

> Brown: "…there is no logically defensible proof that the clocks underlying circadian rhythms possess a timing system, a self-sustaining oscillation…such proof has been precluded by the fact that one can never establish through negative evidence alone that nothing on the outside provides essential timing signals….in insisting upon a self-timed, or fully autonomous autonomous, living clock, there always lurks the possibility that we are pursuing a ghost."
>
> Pittendrigh: "…the question of the ghost is simple – either it is an aspect of living organization, or an unknown geophysical variable. My taste in ghosts suggests the latter but, as scientist, I must agree that Dr. Brown may prove right; and as scientist he will doubtless agree he may prove wrong."

For all practical purposes, the search for the exogenous ghost ended some years later, when circadian rhythmicity was found to be sustained in a laboratory setup a few hundred meters from the South Pole with turntables rotating once per 24 h against – and hence fully canceling – the earth's own rotation (in hamsters [*Mesocricetus auratus*], fruit flies [*Drosophila pseudoobscura*], bean plants [*Phaseolus vulgaris*], and fungi [*Neurospora crassa*]) [51]. Additional proof was obtained in the 1980s in *Neurospora* placed in geocentric orbit aboard Spacelab 1, up to 157 miles above earth and circling the globe with a period of 89.5 min [52].

1.6 Ushering in the Modern Era: Mathematics, Maps, and Molecules

As attention turned to the clock itself in the 1960s and 1970s, mechanistic questions recruited a fresh wave of mathematical biologists, neuroscientists, and molecular geneticists newly attracted to the problem: What kind of oscillator is it? Is it physically located in a discrete part of the body? How does it actually work at a tissue, cellular, or molecular level?

American theoretical biologist Arthur Winfree (1942–2002) was a graduate student of Pittendrigh at Princeton University, where he applied his undergraduate background in engineering physics to model the clock as a stable, attractive limit cycle and predict how perturbations affect its behavior. The topology of such a multidimensional nonlinear system led him to reason how light pulses, depending upon their phase of administration and intensity, could generate "strong" and "weak" resetting or even propel the oscillator to an undefined phase ("singularity") (as though transported to the North Pole, where all lines of longitude converge and there is no time of day); he then proceeded to successfully test his predictions by resetting and desynchronizing the *Drosophila* eclosion rhythm with critical photic stimuli [53]. Winfree also considered the dynamics of populations of weakly coupled limit cycle oscillators, realizing that their behavior could be quite different from that of one oscillator alone, and he surmised, as we now know, that intercellular synchronization might determine as much about clock dynamics as does its autonomous cellular mechanism [54].

As early as 1957, Pittendrigh and Victor Bruce (1920–2009) constructed a block diagram of a canonical timekeeping system including input, pacemaker, and output [55] (Fig. 1.5a), a schematic that evolved over 20 years to become Arnold Eskin's iconic blueprint [56] (Fig. 1.5b). This was an era that exploited new techniques and circumscribed lesions in an effort to map various brain functions to distinct sites (e.g., the lateral hypothalamus as a feeding "center," the ventromedial hypothalamus as a satiety "center," various pleasure "centers," and so on). But how could a circadian

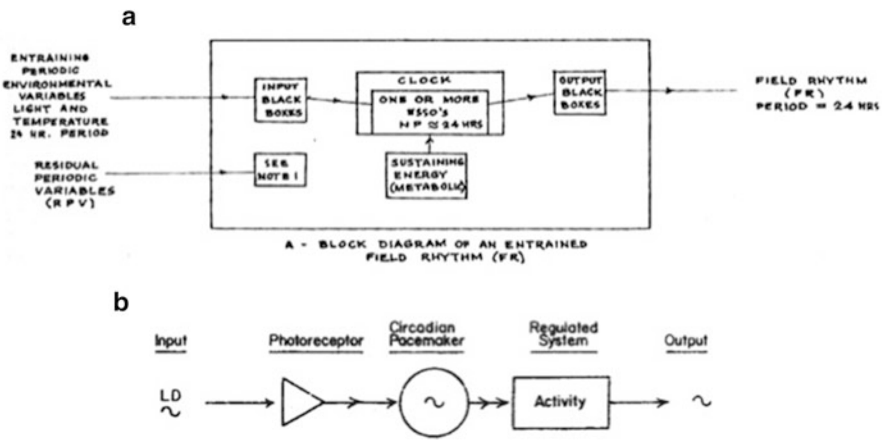

Fig. 1.5 (**a**) Pittendrigh and Bruce's diagram of an entrained circadian system [55], with a clock composed of one or more endogenous self-sustaining oscillators (ESSOs). "Note 1" posits that all periodically fluctuating variables other than light and temperature are not coupled to the oscillator (Reproduced with permission [55]). (**b**) Eskin's original diagram [56] (Reproduced with permission [56])

"center" be distinguished from its downstream effector ("hand of the clock") if a lesion of either would lead to the same arrhythmic phenotype? An answer to this problem was provided in 1970 by James Truman (b. 1945) and Lynn Riddiford (b. 1936) with a daring transplantation experiment that elucidated the timing of ecdysis (a phase of pupal emergence) in two species of silk moths [57]. In a 17 h:7 h light-dark cycle, *Hyalophora cecropia* and *Antheraea pernyi* were shown to emerge in the morning and evening, respectively, a phase difference that was lost in brainless moths. However, when *cecropia* brains were transplanted to the abdomens of brainless *pernyi* and vice versa, the *timing* of ecdysis was restored – with a phase predicted by the donor brain, not the recipient body – while the species-specific behavioral *pattern* of ecdysis remained characteristic of the host. Thus, the clock was in the brain, communicating with the actual behavioral generator (elsewhere) via a neuroendocrine mechanism. Further transplantation studies followed, and the circadian clock regulating locomotor rhythmicity was localized to the brain in *Drosophila* [58], the optic lobes in cockroach (*Leucophaea maderae*) [59], and the pineal gland in house sparrow (*Passer domesticus*) [60]. In 1972, lesions of the suprachiasmatic nucleus (SCN) in rat (*Rattus norvegicus*) [61, 62] implicated this hypothalamic nucleus as the site of the clock in mammals; the decisive transplantation experiments came much later, interchanging fetal SCN tissues between wild-type and short-period mutant hamsters (*Mesocricetus auratus*) [63].

Despite the rise of the "master clock" or "pacemaker" metaphor, it was already clear in the 1960s that the circadian system includes a number of interacting body clocks. In particular, American physiologist G. Edgar Folk, Jr. (b. 1914) cultured hamster adrenal glands and measured oxygen consumption and steroid secretion over a few days [64]. Evidence was presented for a circadian metabolic rhythm in culture, with its period relatively temperature independent, and its phase dictated by the light-dark cycle before animal sacrifice. In 1965, Folk also reported a circadian rhythm of heart rate in isolated rat hearts, proposing that "...the circadian rhythms of resting heart rate are controlled by a "clock" located within the heart cell" [65]. It was another four decades before the further study of such mutually interactive "peripheral" clocks could be undertaken in earnest.

It was in 1959 that Pittendrigh and associates reported a circadian rhythm of asexual spore formation (conidiation) in the filamentous fungus *Neurospora crassa* [66]; this had been the model organism exploited by Norman Horowitz to demonstrate the "one gene-one enzyme" hypothesis of George Beadle and Edward Tatum (1958 Nobel Laureates). Despite Pittendrigh's vehement indifference to searching for "clock genes" – after all, inheritance of clock properties must be polygenic – his graduate student Jerry Feldman (b. 1942), upon completing his PhD in 1967, headed to the California Institute of Technology in Pasadena for a postdoctoral fellowship with Horowitz. The eventual result of his chemical mutagenesis screen with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine was the discovery of a set of single gene mutants that all mapped to the same genetic locus, which he named *frequency* (*frq*) [67]. Remarkably, he isolated mutations at this locus that could either shorten or lengthen the free-running period, with a gene-dosage effect on period length. A friend of Feldman's when he was at Cal Tech, Ronald J. Konopka (1947–2015), the first graduate student of Seymour Benzer (1921–2007) there, set up an ethyl methanesulfonate (EMS) mutagenesis screen in 1968 and generated three *period* (*per*) mutants of *Drosophila melanogaster*, all affecting the same functional gene on the X chromosome, exhibiting either a short (19 h) period, a long (28 h) period, or arrhythmicity, in rhythms of both population eclosion and individual locomotion [68]. Like the skepticism that had previously greeted the evidence for an endogenous clock, there were some who did not readily embrace the fact that single gene alterations could dramatically affect a behavior as complex as the clock's internal mechanism. As recounted by Benzer, Max Delbrück, 1969 Nobel Laureate, was dumbfounded:

> . . .[Konopka] had shown that you could make mutations that changed the biological clock in Drosophila. And he was telling this to Max, and Max said, "No, that's impossible." And then I said, "But Max, he's already done it." And Max said, "No, that's impossible." That was not a completely unusual kind of event. [69]

While 200 years had to pass to realize the significance of de Mairan's botanical observation, it took less than 20 to capitalize – spectacularly – on Feldman's *frq* and Konopka's *per* mutants.

1.7 A Paean to Actograms

No historical account of circadian biology should fail to mention the actogram, a graphical display method that is essentially unique to the field. Figure 1.6 illustrates how plotting a time series in such a format serves to accentuate rhythm phase and period. To our knowledge, the first actograms were published – although not named as such – by Curt Richter in 1922 [27] (his Figs. 17 and 18), but it was in 1926 that they assumed their familiar form, as part of the PhD thesis of Maynard Johnson

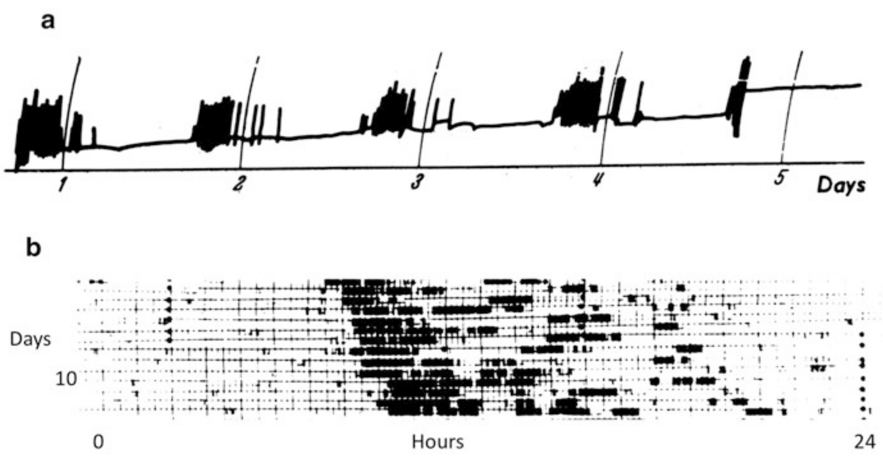

Fig. 1.6 Recordings of locomotor activity rhythms in two different hamsters in constant light (**a**) or constant darkness (**b**), with the latter graphed in actogram format ((**a**) Reproduced with permission [81]; (**b**) modified from [82])

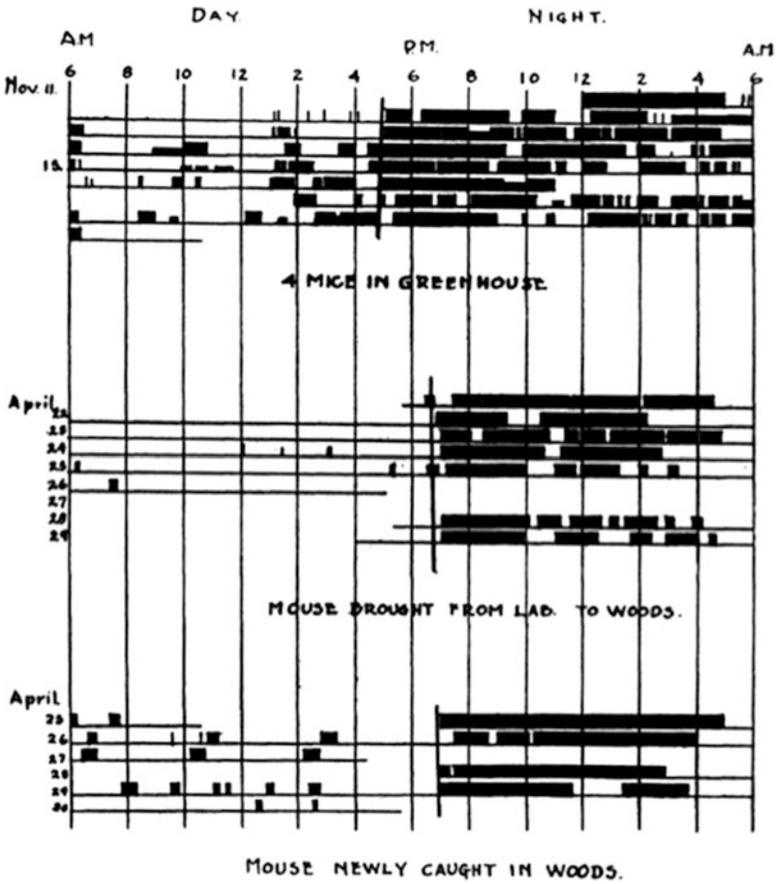

Fig. 1.7 Recordings of rhythmic mouse cage movements in natural light-dark cycles by Johnson [70] (Reproduced with permission [70])

(referenced earlier), graphing rhythms of cage movement in deer mice (*Peromyscus leucopus* and *P. maniculatus*) (Fig. 1.7) [70]. Although his paper provided extensive detail on his recording apparatus, nothing was mentioned about his choice of stacking each day's activity vertically from top to bottom. The origin of the term for such an activity plot is unclear; perhaps it arose from the Prussian psychologist J.S. Szymanski's *Aktograph* apparatus for recording animal activity, with the inscribed trace on a smoked drum called an *Aktogramm* in a 1918 publication [71] (although multiple days were not arranged vertically). In any case, while many actograms appeared in the 1960 Cold Spring Harbor Symposium *Biological Clocks*, none of them was referred to by that name, and as late as the 1981 Handbook of Behavioral Neurobiology volume on *Biological Rhythms* [72], J. T. Enright wrote "actogram" in quotations, John Brady referred instead to actographs, and others used the rather nonspecific term "raster plots."

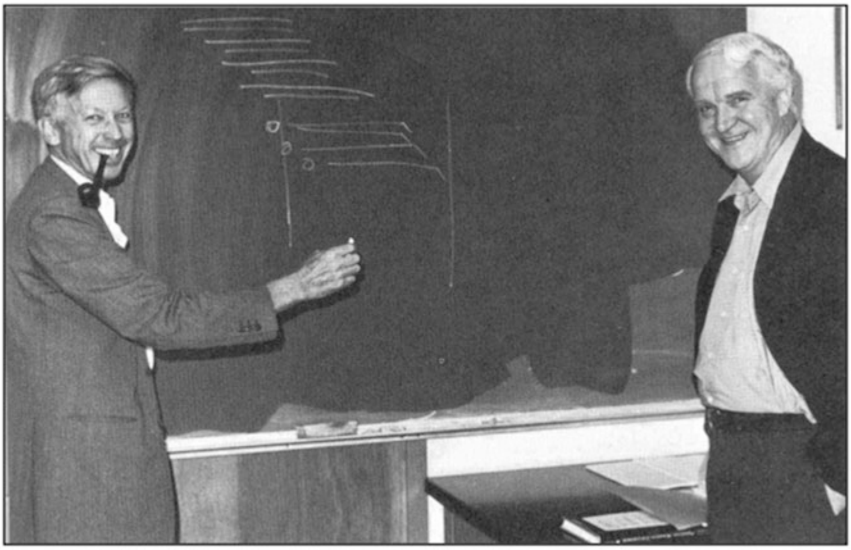

Fig. 1.8 Jürgen Aschoff (*left*) and Colin Pittendrigh in 1970. Their diagrammed actogram clearly shows phase and period but no amplitude (Reproduced with permission [83])

As a means to analyze the burgeoning studies on rodent rhythmicity [73], actograms meshed well with the field's emphasis on clock phase and period (Fig. 1.8) and with the technical limitations of most of the recording equipment of the time, especially pen and ink chart recorders (e.g., Esterline-Angus) that could not provide reliable measures of rhythm amplitude (Fig. 1.6b) [74]. There were attempts to incorporate amplitude in actographic displays [75] (Fig. 1.9), but these failed to achieve widespread acceptance, and rigorous analyses of rhythm amplitude had to await the computer.

1.8 Coda

Our brief essay cannot do justice to all who have contributed to the success of modern circadian biology; the giants whom we have highlighted stood on and by the shoulders of many others. And here we have not reviewed the historical development of other domains of biological timing, including biomedical rhythmicity, non-24 h functions that depend on the clock, and adaptation to the seasons and the tides. Future historians will have access to fantastic resources, including video histories of lectures (e.g., a 1992 lecture by Pittendrigh at the University of Virginia [76]) and investigator interviews [77, 78].

Our review has taken us to the end of the beginning. Near the end of his life, Pittendrigh realized that the *clock* paradigm, which had proven so useful for the field's development, needed to be re-framed as a temporal *program* of events at

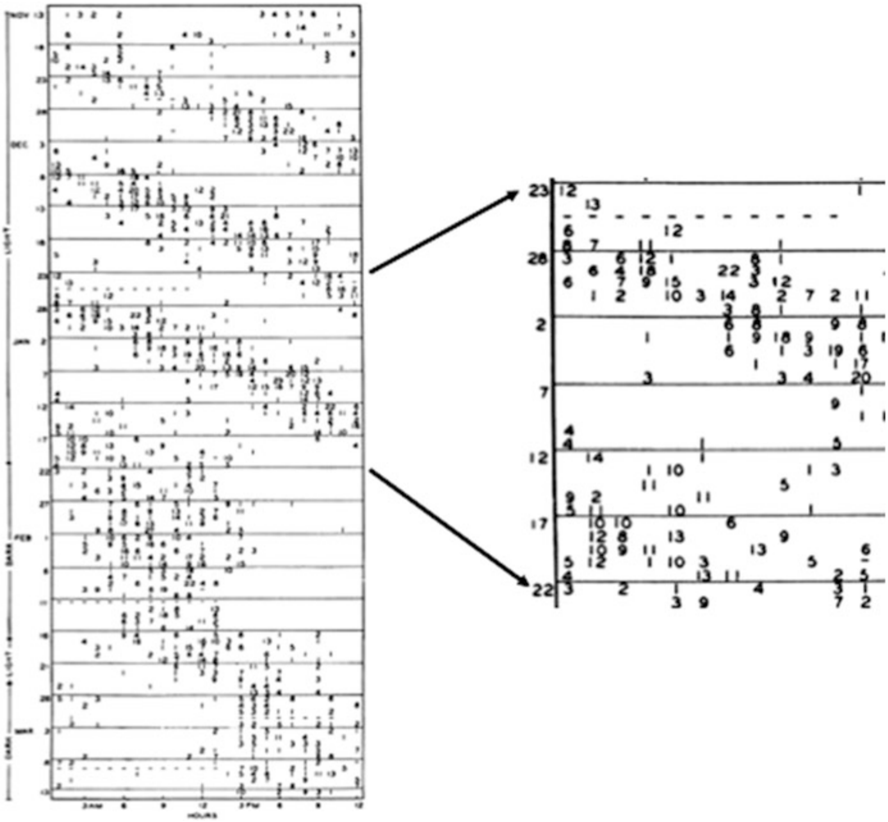

Fig. 1.9 Plot of rat locomotor activity, with amounts in arbitrary units, in constant dim light (Modified from [75])

different times of day, orchestrated by a multiplicity of circadian oscillators ultimately entrained by light [79]. The interdisciplinary investigation of that concept – from molecules, to cells and tissues, and even to communities – is now well underway. The far-reaching importance of circadian organization as a ubiquitous property of living things that evolved on this rotating planet, as well as a prominent phenomenon in human life, behavior, and health, has come to be widely appreciated by both science and society at large.

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