Sleep and its regulation in zebrafish

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Abstract
The function of sleep remains a central enigma of modern biology, in spite of the obvious importance of sleep for normal physiology and cognition. The zebrafish has emerged as a promising new model for studying sleep, its changes with age, and the impact of sleep alterations on cognitive function. Recent studies of this diurnal vertebrate have provided new insights into the dual role of the pineal hormone melatonin and its receptors, regulating sleep in diurnal vertebrates through both homeostatic and circadian mechanisms. Research in zebrafish has also revealed interactions between melatonin and the hypocretin/orexin system, another important sleep-wake modulator. Future investigations should benefit from the conservation in zebrafish of mechanisms that regulate normal sleep, our extensive knowledge of their molecular biology, the availability of multiple transgenic and mutant phenotypes, and the feasibility of applying sensitive in vivo imaging techniques to record sleep-related neuronal activity in these optically transparent subjects. The established sensitivity of zebrafish to many pharmacological hypnotics should also contribute to the development of new, safe and effective sleep medications.

Keywords: circadian; homeostatic; hypocretin; melatonin; sleep; zebrafish.

Introduction
Sleep is a universal behavioral and physiological phenomenon in vertebrates and is likely to share basic functions and mechanisms with similar periodic rest behavior in non-vertebrates. In-depth scientific investigation into the problem of sleep in mammals, including humans, taught us a lot about the sleep process and what is happening with different physiological systems during sleep. Many ideas, hypotheses and theories have been put forward to explain the ubiquitous phenomenon of sleep. The negative impact of altered sleep on mood, cognitive functions and overall health is all too evident from both clinical observations and experimental data on the effects of sleep deprivation (Van Cauter et al., 2008; Walker, 2008). Despite this, as of now, no unifying theory explains the overall phenomenon of sleep and its principal physiological functions, common to all organisms that display sleep behavior.

There is a wide range of sleep patterns and responses to sleep deprivation among animals. Some species, including humans, have a consolidated sleep period, occurring at night or during the day. Others have a highly fragmented process with only several minutes of sleep at a time. Similarly, whereas most animals stay quiet during sleep and have characteristic sleep postures, others have to continue moving. This is typically the case when the needs of normal respiration must be served by passing water through gills, e.g., in sharks, or by frequently breaking the water surface to breath in air, as marine mammals do. Thus, the manifestations of sleep and, perhaps, even some of its secondary functions necessarily reflect diverse adaptations of a species to its environmental niche and specific physiological characteristics of the organism. However, the primary function(s) of sleep is likely to be well conserved. It is thus crucial to broaden the search for common sleep function and the mechanisms involved by using diverse animal models and applying novel methodological approaches.

Why do we find the zebrafish (Danio rerio) to be a suitable animal model for studying sleep? In spite of the phylogenetic remoteness of zebrafish from the principal interest of translational research, the human, and their adaptation to an aquatic environment, there are many benefits that zebrafish can bring to sleep research. Some of these are as a result of the extensive use of this small teleost in the fields of developmental biology and genetics, resulting in a fair amount of accumulated information about its molecular biology, the generation of diverse mutant and transgenic phenotypes, and the development of many molecular probes and techniques. Additional advantages arise from the visual transparency of young animals and even adult zebrafish of some strains, allowing for the use of efficient imaging techniques to study the functioning central nervous system in behaving animals. The diurnal nature of zebrafish sleep, similar to that in humans but in contrast to the nocturnal rhythms of rats or mice commonly used in sleep research, is a major advantage for studying circadian regulation of the sleep process.

There is also a more practical matter that favors the zebrafish model. Sleep is an integrative process, involving multiple, if not all, physiological systems and organs. As a result, it has to be studied in a whole organism. Typically, this calls for the use of a large number of animals, which is more feasible in highly prolific fish, when compared to mammals. Indeed, one female
zebrafish can generate hundreds of embryos in one week and, under laboratory conditions, the majority of them survive to maturity. Moreover, their fast development results in fast generation of tiny zebrafish larvae. Being several days old and several millimeters long, they are miniature fish with complex behaviors. By 6 days post-fertilization (dpf), zebrafish are actively swimming, responding to diverse stimuli, hunting for prey and displaying distinct periods of rest.

When we started working with zebrafish, all these potential advantages of the model were outweighed by an apparent disadvantage: at that time, nobody had characterized sleep in zebrafish. Thus, to start with, it was important to determine whether rest behavior in young and mature zebrafish exhibited essential similarities with sleep.

**Do zebrafish sleep?**

Sleep is defined using polysomnographic recordings, including electroencephalograms, in higher vertebrates that have a cerebral cortex. In lower vertebrates and non-vertebrates, sleep is typically defined based on behavioral parameters (Campbell and Tobler, 1984). It thus had to be determined whether rest periods in zebrafish have features similar to sleep. Indeed, we found that, in larval zebrafish, prolonged periods of immobility (up to 10 min bouts, depending on the fish strain and age), occurring predominantly at night, are typically associated with two main postures: either floating with the head down or staying in a horizontal position close to the bottom of the tank (Figure 1). During this time, the fish display an increased arousal threshold to mild stimulation (Zhdanova et al., 2001).

Another typical feature of sleep in many species is lack of eye movements and blinking. Although these behaviors were commonly perceived to be characteristic of sleep, once sleep research progressed from using just behavioral assessments to comparing brain activity during wakefulness and sleep, it was discovered that, at least in mammals and birds, sleep is not a homogeneous state. During one of its distinct stages the eyes actively move, while being closed. For this reason, this sleep stage was called rapid eye movement (REM) sleep. It was the last sleep stage to be discovered and it is so remarkable that the remaining four sleep stages, combined, are now called “non-REM sleep”.

Are there any behavioral signs of different sleep stages in larval fish? Zebrafish do not have eye lids, thus monitoring their eye closure or blinking is not an option. However, their eye movements are very prominent and can be monitored and quantified (Huang and Neuhaus, 2008). We could observe periods of eye movements in larval fish maintaining stable sleep postures. This was sometimes accompanied by changes in respiration rate, another sign of REM sleep in mammals. However, concluding that larval fish have different stages of sleep would be highly premature at this point and requires further in-depth investigation, because such periodic events can represent brief arousals.

In humans and other mammals, the sleep process changes significantly from childhood to maturity, with further modifications observed during aging. Typically, maturity is associated with less total sleep time and the overall daily pattern of sleep becoming less fragmented. Similarly, adult zebrafish show less overall sleep time, with most of it occurring at night. Sleep postures also change with age. Sleeping adult zebrafish float either in a horizontal position or with their head slightly upward, occasionally moving their eyes or fins, or even swimming slowly. Interestingly, there are sleep periods when typical sleep-associated floating ceases. At this time, an animal starts losing buoyancy, gradually sinking to the bottom of the tank. Such behavior can be owing to a loss of tone in major muscle groups or alterations in swim bladder regulation. Arousal threshold during this time is further increased, suggesting deeper sleep. Considering that high arousal threshold, atonia of large anti-gravity muscles and twitching of small distal muscles are characteristic of REM sleep in mammals, we looked for other features of this sleep stage in adult fish during loss of buoyancy. However, those episodes were not associated with apparent eye movements or muscle twitches.

Overall, sleep behavior in both larval and adult zebrafish is very similar to that of other vertebrates studied, being associated with reduced or absent locomotion, specific postures and increased arousal threshold. Although sleep in zebrafish appears to exhibit periodic events that might be consistent with increased depth of sleep or short arousals, it remains to be seen if there are distinct sleep stages in zebrafish.

**Homeostatic regulation of sleep**

The sleep process is known to be regulated by two principal mechanisms, the ‘homeostatic’ and the ‘circadian’. One of the ways to test the presence of the homeostatic component of sleep is to monitor whether sleep deprivation leads to subsequent sleep rebound. Young, larval zebrafish compensate for sleep deprivation by increasing sleep time (Figure 2), indicating that they indeed have a homeostatic component of sleep regulation (Zhdanova et al., 2001). Adult zebrafish, however, show somewhat unexpected complexity in their responses to sleep deprivation. Following several hours of forced wakefulness at nighttime, adult zebrafish display reduced activity levels and increased arousal thresholds (Yokogawa et al.,

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**Figure 1** Typical rest postures in larval zebrafish: floating with head down or staying horizontal, close to the bottom of a chamber. Reproduced with permission from Zhdanova et al. (2001).
Sleep and its regulation in zebrafish 2007) and tend not to perform cognitive tasks as efficiently as control animals that were not sleep deprived (Yu et al., 2006). However, if sleep deprivation lasts longer, for many hours or even days in a light-dark cycle, alterations in fish locomotor activity appear to become less pronounced. In fact, we find many animals becoming hyperactive for some time after sleep deprivation is over and then resuming typical levels of activity, with no sleep rebound observed. This is in striking contrast to the response of mammalian species to prolonged lack of sleep, with a characteristic increase in sleepiness, behavioral alterations and physiological abnormalities mounting over prolonged periods of wakefulness.

There are several potential explanations for this phenomenon. One possibility is that zebrafish lose the homeostatic regulation of sleep with maturation. Lack of apparent sleep behavior has been attributed to some animals, although these claims are highly debatable (Kavanau, 1998; Cirelli and Tononi, 2008). Older zebrafish might also develop the ability to have brief but frequent microsleep episodes, allowing them to continue their locomotor activity without accumulating substantial sleep deprivation. Yet another possibility is that fish, similar to marine mammals and birds, might have regional distribution of sleep, with a characteristic increase in sleepiness, behavioral alterations and physiological abnormalities mounting over prolonged periods of wakefulness.

Circadian regulation of sleep

The complex machinery of the intrinsic circadian clock includes several core clock genes and proteins serving as transcription factors that are organized in complex feedback loops to maintain close to 24-h oscillations (Reppert and Weaver, 2002). As a result, the clock-controlled genes and their products modulate multiple output processes, including the sleepwake cycle. The majority of studies addressing this issue were conducted in nocturnally active mice, rats and hamsters. However, in spite of the highly conserved core mechanisms of the clock, the circadian regulation of downstream physiological and behavioral processes in diurnal and nocturnal species can differ significantly. For example, daytime activation of the neuronal master clock structure in mammals (the suprachiasmatic nuclei of the hypothalamus, SCN) correlates with daytime activity in humans and sleep in nocturnal species, can switch from unihemispheric sleep while in water, to sleeping simultaneously with both hemispheres while on land. Learning about the neurochemical mechanisms involved in such unique sleep adaptations might help us not only to better understand the overall phenomenon of sleep but, potentially, to design some ways for temporarily increasing periods of human vigilance with minimal side effects. If zebrafish have some similar sleep adaptations, they might become an outstanding animal model to dissect the different mechanisms underlying homeostatic regulation of sleep.

Figure 2  Daily variation in locomotor activity and arousal threshold in larval zebrafish maintained in constant darkness and a compensatory reduction in locomotor activity and increase in arousal threshold following rest deprivation.

(A, B) Zeitgeber time (ZT) and horizontal white/black bars indicate subjective day versus subjective night, according to 12:12 light-dark cycle prior to the beginning of recording, with ZT0 corresponding to lights on time. Each data point represents mean±SEM group locomotor activity for preceding 2 h of recording (pixels per minute). n=60 for each group. The rest deprivation was scheduled either (A) during subjective night (ZT18–24), or (B) during subjective day (ZT0–6). Closed diamonds – rest deprivation group, open squares – control group. (C) Arousal threshold was measured in constant darkness during subjective day (ZT3–5) or subjective night (ZT15–17); n=20 for each group. (D) Changes in daytime arousal threshold (% of basal) starting an hour after daytime or nighttime rest deprivation. White bars – control; striped bars – rest deprivation. n=20 for each group. *p=0.05; **p=0.01. Reproduced with permission from Zhdanova et al. (2001).
species. Similarly, the nighttime production of the principal circadian hormone (melatonin) occurs during the sleep phase in humans and at the peak activity period in rats and mice. Not surprisingly, melatonin promotes sleep in diurnal but not nocturnal species (Zhdanova, 2005). Thus, some aspects of translational research would significantly benefit from studies of intrinsic circadian rhythmicity in species featuring diurnal activity and nocturnal sleep patterns.

Our knowledge of the well-conserved molecular mechanisms of the fish circadian system is based mainly on the original studies conducted in zebrafish (Cahill et al., 1998; Pando and Sassone-Corsi, 2002; Kaneko et al., 2006). Zebrafish have an SCN, but its role in circadian rhythmicity has not been confirmed (Cahill et al., 1998). Instead, the eyes and pineal gland appear to be the principal central clock structures in zebrafish. They carry out autonomic oscillations, photoreception and melatonin production. Starting with early embryogenesis, melatonin production and melatonin receptor expression provide a unifying neuroendocrine circadian signal through specific melatonin receptors (Cahill et al., 1998; Danilova et al., 2004). Hence, as in the case of mammals, melatonin is the major neurohumoral output of the circadian system in zebrafish.

Consistent with other diurnal species, zebrafish sleep predominantly at night, at the time of peak melatonin production. This daily pattern of sleep and wakefulness in larval and adult zebrafish is preserved after they are transferred from a light-dark cycle to constant conditions. Indeed, monitoring fish locomotion in constant darkness or dim light shows robust circadian variation in zebrafish activity levels, higher during subjective day and lower at subjective night. Under both light-dark cycle and constant darkness, a decline in motor activity during habitual nighttime rest is accompanied by a significant increase in mean arousal threshold (Figure 2C). This proves that the sleep pattern follows the endogenous clock, rather than responding to changes in environmental illumination.

The brain and other tissues can rhythmically express the core clock and clock-controlled genes in zebrafish (Whitmore et al., 1998). We find that in zebrafish brain and eye, the expression of core genes of the positive limbs of the clock – e.g., \( bmal1 \) and \( clock1 \) – is initiated close to the habitual sleep time (Figure 3A,C) (Zhdanova et al., 2008). The expression of the genes of the negative limb of the clock – e.g., \( per1 \) – occurs at the end of the sleep period and continues into the early hours of the daily activity phase (Figure 3B). With age, the amplitude of daily variation in circadian gene expression declines in zebrafish (Zhdanova et al., 2008), as was reported in other species studied (Kondratov, 2007). Constant bright light is well known to significantly alter the circadian clock mechanisms and their behavioral manifestations in many species (Ohta et al., 2005). If zebrafish are kept under constant light conditions for several days, they do not show regular signs of sleep, prolonged inactivity periods or increased arousal thresholds. After being held under these unusual conditions for a week or two, the fish can again display a sleep-like state, which, however, lacks a clear circadian pattern (Yokogawa et al., 2007). Constant light exposure can significantly reduce the amplitude of expression of the clock and clock-controlled

![Figure 3](image-url)
genes (Shang and Zhdanova, 2007), which might, at least in part, be responsible for the sleep alterations in bright light. Regular nighttime administration of melatonin, providing strong circadian modulation, can improve sleep in zebrafish when it is altered by constant light or aging (Zhdanova et al., 2008).

**Neurochemical mechanisms of sleep regulation in zebrafish**

The neuroanatomical structures involved in sleep regulation in mammals are also present in zebrafish, with the corresponding neurotransmitters and their receptors being typically well conserved in this teleost (Eriksson et al., 1998; Panula et al., 2000; Kaslin and Panula, 2001; Clemente et al., 2004; Kaslin et al., 2004; Arenzana et al., 2005; Clemente et al., 2005; Ruuskanen et al., 2005; Faraco et al., 2006). Studies on the hypnotic effects of drugs and physiological agents targeting the GABAergic, melatoninerigeric, histaminergic, hypocretinergic, cholinergic, dopaminergic and adrenergic signaling pathways confirm that these anatomical similarities translate into functional ones (Zhdanova et al., 2001; Ruuskanen et al., 2005; Renier et al., 2007; Zhdanova et al., 2008; Rihel et al., 2010).

**Melatonin**

This principal hormone of the circadian system is known to have a mild hypnotic-like effect in humans when used in physiological or pharmacological doses (Zhdanova, 2005). Its administration also promotes sleep in diurnal primates (Zhdanova et al., 1998, 2002) and birds (Mintz et al., 1998; Aparicio et al., 2006; Paredes et al., 2007). However, the nocturnal species appear to be immune to this effect of melatonin, consistent with the nocturnal production of this hormone corresponding to their active period.

In larval and adult zebrafish, exposure to melatonin promotes sleep, reducing their locomotor activity and elevating their arousal threshold (Figure 4A,B). Independent of the dose used, this effect does not induce anesthesia. Rather, following melatonin administration, both locomotor activity and arousal threshold reach a plateau at higher doses, and the resulting behavior remains close to that observed normally at night. Interestingly, the effects of this dose-dependence of melatonin on sleep is documented in both humans and non-human primates, suggesting that the mechanisms through which this effect is mediated are also likely to be the same in fish and primates.

Moreover, for the first time, studies in zebrafish allowed us to establish that the effects of melatonin on sleep are mediated through melatonin receptors (Zhdanova et al., 2001), because they can be blocked by a specific melatonin receptor antagonist, luzindole, but not by the benzodiazepine receptor antagonist, flumazenil (Figure 5A).

**Hypocretins/orexins**

The neuropeptides hypocretins (HCRT1 and HCRT2, also known as orexins A and B) were linked to the human sleep...
disorder narcolepsy, characterized by excessive daytime sleepiness, a fragmented sleep-wake cycle, and a sudden loss of muscle tone during waking, called cataplexy (Zeitzer et al., 2006). Deficiency in these peptides or their G-protein-coupled receptors alters alertness and sleep in humans and mammalian models. When HCRT1 is injected into the cerebrospinal fluid, locomotor activity in mammals is typically increased and sleep inhibited.

There is a high level of conservation between the hypocretin system in teleosts, including zebrafish, and mammals. Similar to mammals, zebrafish express HCRT in the neurons of the posterior lateral hypothalamus (Kaslin et al., 2004; Faraco et al., 2006). These are mostly glutamatergic and, although firing predominantly during daytime wakefulness, also maintain some level of activity at night (Appelbaum et al., 2009). As in mammals, the HCRT neurons in zebrafish project to monoaminergic and cholinergic nuclei (Kaslin et al., 2004; Prober et al., 2006; Yokogawa et al., 2007). HCRT receptors are found in telencephalon, hypothalamus, posterior tuberculum, pineal and hindbrain (Faraco et al., 2006; Prober et al., 2006; Yokogawa et al., 2007; Appelbaum et al., 2009). However, in contrast to the major hypocretinergic projections to the wake-promoting histaminergic system present in mammals, HCRT receptors have not been found on histaminergic cells in zebrafish brain (Prober et al., 2006; Yokogawa et al., 2007). This might explain why the absence of these receptors in mutant zebrafish (hcrtR^{-/-}) does not lead to pronounced defects in daytime activity (Yokogawa et al., 2007). Moreover, intracerebroventricular injection of HCRT1 (but not HCRT2) leads to mild reductions in zebrafish activity, in contrast to robust activation following such injections in mammals (Zeitzer et al., 2006). That being said, hcrtR^{-/-} is associated with sleep fragmentation, reminiscent of insomnia in narcoleptic patients (Yokogawa et al., 2007), whereas over-expression of HCRT can increase zebrafish activity during wakefulness (Prober et al., 2006). Further studies in zebrafish and other teleosts should clarify the role of HCRT in fish sleep physiology.

Interestingly, there are both anatomical and functional links between the melatonin and hypocretin systems and these affect regulation of sleep in zebrafish (Appelbaum et al., 2009). Hypocretin receptors are present in the pineal gland and their activation can augment the production of melatonin at night (Figure 6). Moreover, fish lacking hypocretin receptors are hypersensitive to the sleep-promoting effects of melatonin, but not to the effects of other hypnotics (Figure 7). This recent finding further illustrates the many new opportunities for deciphering sleep-related mechanisms that the zebrafish model can provide.

**γ-Aminobutyric acid (GABA)**

The inhibitory neurotransmitter GABA plays an important role in the physiological regulation of sleep. Its receptors mediate an increase in intracellular chloride levels, leading to neuronal hyperpolarization, and are the target of the vast majority of sedative hypnotics (barbiturates, benzodiazepines or nonbenzodiazepine agonists of benzodiazepine receptors), selective targeting of the GABAergic pathway can facilitate sleep onset and extend sleep duration, but it also appears to be responsible for the side effects of available hypnotic substances (e.g., amnesia, ataxia, morning-after sedation or abuse potential). In large doses, these drugs induce general anesthesia and can cause death.

Zebrafish have well-developed GABAergic neurotransmission (Doldan et al., 1999; Higashijima et al., 2004) and are highly sensitive to GABAergic hypnotics (Zhdanova et al., 2001; Renier et al., 2007). The dose-dependence of the behavioral effects of these drugs is somewhat similar to that in mammals. At low doses of drug, the activity levels decline and arousal thresholds rise, followed by inactivity and lack of spontaneous or stimulated arousal in response to higher doses, consistent with general anesthesia (Figures 4 and 5). Finally, if zebrafish are exposed to these drugs for prolonged periods, they do not survive. The latter effect is more pronounced in adult zebrafish than in larvae. This might be explained by...
the apparent reduction in respiratory movements in adult fish treated with barbiturates or benzodiazepines. In larvae, cutaneous respiration can compensate for this effect. Indeed, when larvae are washed after hours of benzodiazepine-induced anesthesia, many of them survive and thereafter show normal behavior.

**Histamine**

Antagonists of histamine receptors have sedative effects in mammals via direct antagonism of H1 receptors and additional antiadrenergic or antimuscarinic activity (Montoro et al., 2006). Similarly, administration of histamine H1 antagonists, such as diphenhydramine or mepyramine, produce dose-dependent effects in larval zebrafish, ranging from mild sedation to general anesthesia (Renier et al., 2007).

**Neuronal structures involved in zebrafish sleep**

Imaging techniques have dramatically changed research approaches and medical practice. The sleep field is no exception, with magnetic resonance imaging and positron emission tomography contributing to our understanding of structures involved in sleep regulation or sleep alterations. Although neurophysiological experiments in adult and especially in larval zebrafish pose a challenge, the optical transparency of larval fish allows visual observation of their neuronal activity. Larval fish can survive through cutaneous respiration and thus they can be embedded in water-saturated agar to restrain them, allowing for imaging of the same cells being maintained for prolonged periods of time. Because electrically active vertebrate neurons usually exhibit large fluctuations in intracellular calcium levels, fluorescent calcium indicators...
represent an excellent tool for monitoring neuronal activity. Such indicators are readily transported both retrogradely and anterogradely by neurons and can be used to fill populations of motoneurons and interneurons in different brain regions. As a result, confocal or two-photon imaging methods allow the study of neuronal activity with single-cell resolution in intact zebrafish (Fetcho and O’Malley, 1995; O’Malley et al., 2003).

These experimental approaches have begun to yield new insights into the brain structures and individual neurons involved in sleep processes. Our studies in larval zebrafish suggest that multiple brain areas are involved in both arousal and sleep-related states. Typically, the latency and threshold of neuronal calcium responses are increased during nighttime sleep, and this correlates with similarly changed behavioral responses recorded in parallel.

The well-known involvement of the brainstem in sleepwake mechanisms, including motoneuron inhibition during sleep, makes this brain region interesting for analyzing sleep-like behavior in zebrafish. The specific neuronal clusters and individual neurons of the brainstem nuclei can be screened for possible involvement in zebrafish sleep regulation following caudal injections of fluorescent indicators. Using confocal calcium imaging in labeled cells and concurrent recording of locomotor activity, we have screened multiple reticulospinal neurons and their responses to melatonin treatment at concentrations that induce sleep-like states in zebrafish larvae. In the majority of these neurons, melatonin did not affect calcium responses to sensory stimuli (tapping or light). However, the activity of several neurons of the nucleus of medial longitudinal fasciculus, demonstrated a robust response to light stimulation and was inhibited by melatonin treatment (Zhdanova, 2006).

Whereas the effects of melatonin appear to be more localized, GABAergic hypnotic drugs inhibit neuronal activity in the majority of brain areas. This difference could be due to the wide presence of GABA receptors, mediating chloride-dependent hyperpolarization, in contrast to the more discretely localized areas of melatonin receptor expression. Detailed characterization of the neuronal correlates of sleep-related effects of pharmacological and physiological agents is currently underway.

Genetically encoded calcium indicators (Higashijima et al., 2003) offer an even more promising approach to monitoring neuronal activity in sleep and wakefulness. The development of transparent adult zebrafish (White et al., 2008) should further enhance our ability to analyze localized sleep-related processes in adults. Furthermore, by generating mutants with alterations in the sleep-wake cycle or sleep homeostasis (Prober et al., 2006; Yokogawa et al., 2007), and crossing them with transgenic fish carrying genetically encoded calcium indicators, it might be possible to identify the structures defining specific sleep phenotypes and sleep disorders.

Age-related changes in sleep

Sleep is significantly altered by aging in humans and other species (Ancoli-Israel, 2009). The zebrafish has recently become the focus of attention as a promising model for studying aging (Gerhard, 2003; Herrera and Jagadeeswaran, 2004; Kishi, 2004; Zhdanova et al., 2008; Kishi et al., 2009). Under laboratory conditions, zebrafish mature within 6 months and survive for up to 6 years. They undergo gradual senescence and start showing age-dependent changes in multiple physiological and cognitive parameters at around 2 years of age (Yu et al., 2006; Tsai et al., 2007). The distinct interindividual variability in these changes suggests that zebrafish, similar to mammals, can have successful and unsuccessful aging processes.

Circadian functions are disrupted in aged zebrafish, as evidenced by reduced daily amplitude of locomotor activity and melatonin production (Zhdanova et al., 2008). Similarly, the amplitude of daily expression of core clock genes is also reduced during zebrafish aging (Figure 3). This might explain why the daily activity rhythms in aged zebrafish become more vulnerable to environmental factors. Although both young and aged animals show more robust daily patterns of locomotion in the light-dark cycle, lack of entraining environmental cues under constant dim light conditions reduces both rest and alertness in aged zebrafish and severely disrupts their circadian pattern of activity.

Such changes in the circadian system can also, at least in part, underlie age-dependent modifications in zebrafish sleep, manifesting as low nighttime sleep duration and high sleep fragmentation (Zhdanova et al., 2008). At the same time, the basal daytime arousal threshold during wakefulness is increased in aged zebrafish, potentially reflecting a lower level of alertness owing to reduced circadian amplitude and/or nighttime sleep deficits. This possibility is further supported by the observation that overnight melatonin treatment promotes sleep and reduces daytime arousal threshold in aged fish. The sensitivity to melatonin typically does not decline with age, consistent with the similar expression levels of melatonin receptors in young and aged zebrafish. In addition, the ability of melatonin to improve cognitive performance in aged zebrafish following overnight administration (Yu et al., 2006) also suggests that sleep-related and circadian abnormalities are part of the reason for cognitive alterations in aged fish.

Conclusion

Sleep remains an enigma of modern biology. This is especially surprising in view of the substantial time animals and humans spend in this distinct physiological state, the major similarities in its behavioral manifestations observed in different species, and the deleterious effects of sleep deprivation on behavioral, autonomic and cognitive functions. Although all this attests to sleep being a basic necessity, the question of whether the function of sleep is single and universal among diverse taxa remains to be determined. To establish a common function would require in-depth investigation of sleep processes in phylogenetically distant organisms adapted to different environments. In this regard, fish are interesting for several reasons. They represent some of the earliest vertebrates on the planet, with abundant variation in species, habitats and adaptations to periodically changing environments. Fish have...
a well-developed brainstem, midbrain and diencephalon, with sensory, motor and integrative central nervous circuits that are highly comparable to those in mammals. However, the forebrain in fish is relatively small and its structure is distinctly different from that of mammals, including the lack of a regular cerebral cortex. Considering that the cortex in mammals contributes to such distinct sleep-related phenomena as slow-wave sleep, the study of sleep processes in fish might help to elucidate the extent to which cortical changes affect the intrinsic mechanisms of sleep in humans.

The results of in-depth evaluation of rest behavior in zebrafish support the idea that prolonged rest in these vertebrates shares essential similarities with mammalian sleep. Changes in cognitive performance following sleep deprivation also imply that sleep deficits affect brain function in fish (Zhdanova et al., 2008), as has been observed in mammals (Stickgold, 2005). There are, however, many questions in fish sleep physiology that require further investigation. One such question concerns the remarkable ability of fish to seemingly circumvent the need for sleep when challenged with constant bright environmental illumination or other stimuli causing sleep deprivation (Yokogawa et al., 2007). There might be special adaptive strategies in play during such challenging conditions (e.g., that are similar to the unihemispheric sleep found in aquatic mammals and birds). This issue needs further elucidation.

Studies of endogenous agents that promote or attenuate sleep in both fish and mammals are of special interest. This is because their neurochemical actions and target structures should help us to decipher the complex network of sleep-regulating mechanisms and to develop drugs to combat human sleep disorders. Melatonin, with its sleep-promoting effect in both humans and zebrafish, is one example of such a physiological agent. Its role in homeostatic and circadian sleep regulation is clearly conserved in diurnal taxa, including fish (Zhdanova, 2005). The role of another important endogenous regulator of sleep, hypocretin (orexin), which promotes wakefulness in humans and other mammals, merits intense investigation in fish, especially owing to the somewhat controversial results obtained so far (Prober et al., 2006; Yokogawa et al., 2007). Further studies on the neurophysiological correlates of sleep in fish should be of great value and will benefit from new techniques involving electrophysiology or the in vivo imaging of changes in intracellular calcium responses and gene expression. Together, these studies should significantly contribute to our understanding of sleep evolution and its physiological functions.

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References


