



Understanding the New Bar Coded Cage Card System

The Animal Resource Program (ARP) is converting to a computer generated cage card system that uses bar codes, similar to those seen on many products sold in retail stores today, to collect and analyze animal census data. Animal care charges in all ARP facilities will be automatically tabulated using this system. The bar coding system will provide for more accurate population counts and charging of per diem costs to investigators. However, the system requires input from both ARP personnel and investigative staff to work correctly.

The ARP office will generate bar coded cage card(s) for placement on each cage. A bar code will be generated for each animal in a cage. Cages containing three or fewer animals will have one to three cage cards with one bar code printed on each card. Cages containing four or more animals will have one cage card with multiple bar codes printed on the card.

ARP personnel regularly scan the bar coded cage cards in the animal facilities with a computerized reader. Do not turn these cards over in order to make notations—the bar codes need to be easily accessible to ARP staff. Also, marking on or near the barcode will delay the scanning process and may lead to an inaccurate census.

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animal use questions*

What's wrong with these mice?



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Let Sleeping Zebrafish Lie: A New Model for Sleep Studies

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Although the function of sleep is hotly debated, one thing is clear—we, and most other animals, cannot do without it. In a new study, Yokogawa et al. describe how zebrafish sleep, finding both striking similarities to mammalian sleep and its regulation and intriguing differences.

How can you tell when a zebrafish is asleep? According to Yokogawa et al., it stops swimming (for at least six seconds), stays immobile at the bottom or on the surface, and becomes less sensitive to external stimuli, such as a mild electric shock. This raised threshold for stimulation is also a feature of mammalian sleep (as is prolonged inactivity). Zebrafish, like humans, are markedly diurnal—they sleep more during the night than the day. By using electrical stimulation to prevent the fish from sleeping, the authors found another similarity between mammalian and zebrafish sleep. Just like mammals, the sleep-deprived zebrafish showed a rebound effect: after being deprived of sleep for a time, they slept more, showing that their sleep is homeostatically regulated.

But when it came to the effects of light on zebrafish sleep, the authors discovered a marked difference from mammalian sleep. When kept in constant light conditions, zebrafish barely slept at all. Light could suppress sleep in zebrafish almost completely, even if they had been sleep deprived. Surprisingly, the suppression of sleep with light did not produce a rebound effect. When zebrafish that had been kept in constant light for three days were returned to darkness, they slept normally, showing no compensatory increase in sleep. The lack of a compensatory rebound effect may more closely resemble sleep in certain birds, such as pigeons, than in mammals.

The authors speculate that this strong effect might result from the fact that all zebrafish cells are directly sensitive to light and that light suppresses the production of melatonin, a sleep-promoting hormone that is particularly effective in zebrafish. Light might act through various pathways to suppress sleep, and this could combine with a lack of melatonin to cause the striking effects of light on sleep in zebrafish and to overcome weaker circadian (cyclical) or homeostatic influences on sleep. By contrast, in mammals, circadian rhythms and homeostatic drives have a much stronger effect than light does.

As vertebrates, zebrafish have a nervous system that is similar in overall architecture to our own. Zebrafish brains also contain hypocretin (also known as orexin), one of the most important sleep-regulating molecules in the mammalian brain. In humans, the sleep disorder narcolepsy is caused by the death of neurons that produce hypocretin, and in dogs, a mutation in one of the hypocretin receptors can cause narcolepsy. Patients with narcolepsy suffer from insomnia at night, but are excessively sleepy during the day. They have a tendency to enter rapid eye movement (REM) sleep—the sleep state during which we dream—abnormally suddenly, and they tend to show some of the features of REM sleep, such as paralysis, when they are awake. This indicates that in mammals, hypocretin promotes wakefulness and regulates sleep.

Hypocretin is found in parts of the zebrafish brain, and its distribution is broadly similar to that seen in mammals. It would be reasonable to expect that mutant zebrafish lacking the hypocretin receptor would thus also show symptoms of narcolepsy. Unexpectedly, however, such mutant zebrafish showed a slight overall increase in activity, with shorter bouts of sleep at night than normal zebrafish, but no increase in sleep during the day, which is one of the primary features of narcolepsy. It may be that light's powerful suppressive effect on daytime sleep in zebrafish might have prevented the increased daytime sleepiness that is found in mammalian narcolepsy.

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Let Sleeping Zebrafish Lie, continued from page 2.

When the authors injected hypocretin directly into the brains of normal zebrafish, the fish slept more; as expected, fish lacking the hypocretin receptor showed no effect. In zebrafish, therefore, hypocretin seems to promote sleep—apparently the opposite of its effect in mammals.

This difference is probably explained by a differential distribution of hypocretin and its receptor in the zebrafish brain. Although in mammals, the hypocretin-producing neurons send strong projections to “monoaminergic” neurons (neurons that use monoamines such as dopamine and serotonin as their neurotransmitters), this is not the case in zebrafish. Instead, the hypocretin-producing neurons seem to project mainly to inhibitory neurons that use the neurotransmitter GABA. In particular, hypocretin-producing neurons target a group of GABA-producing cells in the anterior hypothalamus (a part of the brain that is important for controlling a variety of homeostatic functions such as appetite and, in mammals, body temperature). The authors suggest that these neurons could be important for modulating sleep in zebrafish.

One theory that might account for the converse effects of hypocretin in mammals and zebrafish is that it might have a dual role in mammals, promoting sleep at night and wakefulness during the day. This would fit with the two main symptoms of narcolepsy, insomnia at night and excessive sleepiness during the day. The authors suggest that zebrafish might share the sleep-promoting neural circuitry of mammals, but not the wake-promoting circuits. Clearly, there is much to discover about the regulation of sleep and its evolution in vertebrates. Zebrafish now join “higher” vertebrates as useful models for studying the physiology, neurobiology, and in particular the genetics of sleep regulation.



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Investigator responsibilities: Cage card deactivation

If an animal is permanently removed from a facility the bar code for that animal must be deactivated so the animal is no longer counted during scanning. Deactivating bar coded cage cards is a simple process that is done by the investigator and/or their staff.

1. For multiple bar coded cage cards, simply cross off a bar code completely so that it is unreadable (a sharpie does this very well) for each animal removed from that cage.
2. For single bar coded cage cards or cards with multiple bar codes all crossed off, remove the bar coded cage card from the empty cage.
3. On the front of the card, write the date in which the cage is no longer in use and initial.
4. Drop the cage card in the cage card deactivation envelopes found in each of our facilities.
5. Cards should be turned in daily. ARP staff will routinely pick up cards and turn them in for deactivation.

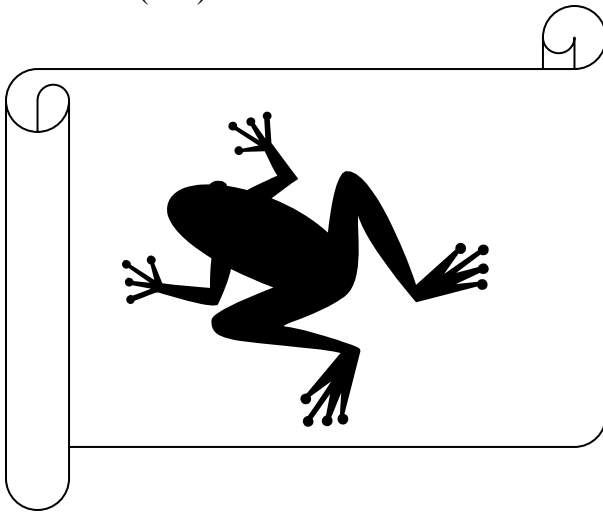
Per diem charges are calculated based on the number of active bar codes listed under an investigator’s name. Deactivation of cage cards is required to discontinue per diem charging for each animal.

If you have questions about the bar coding system or deactivating cage cards please contact the ARP office at 865-1495.

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The Animal Resource Program (ARP) is committed to providing PSU research personnel with high quality animal care services and facilities, to facilitate and improve animal research, and to ensure the health, well-being and humane treatment of all animals at PSU. ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal, agricultural and wildlife technology and medicine. ARP veterinarians have specialized training and are available to assist with animal model development, experimental design, budget projections and grant preparation. Participation in collaborative research projects is welcomed.

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Answer to “What’s wrong with these mice?”

These mice display signs of “barbering”; hair and whisker loss as a result of excessive grooming behavior. Barbering is seen in group housed mice and other rodents. The areas of hair loss are usually well-demarcated with healthy, normal skin underneath. Typically, one mouse in the group shows no hair loss and is the “barber”.

Barbering is seen in both male and female mouse groups in which case the barber is typically the dominant individual in the group. Male/female pairs may also show barbering with the female generally barbering the male. Suckling mice may barber the ventral abdomen of their lactating dam. Barbering severity tends to increase with increased social stress, such as overcrowding.

Mouse strains vary in the incidence and pattern of barbering, although hair loss is typically greatest around the head and neck. Barbering appears to be inversely related to aggression as males in behaviorally aggressive strains display decreased barbering activity. Several known mouse mutants have been shown to display aberrant barbering phenotypes along with altered social interaction and aggressiveness. This has led to the suggestion that barbering may serve as a behavioral mechanism to maintain social harmony in stable groups.

All of this suggests that barbering is a complex behavioral trait that could be a useful marker of neurological and/or behavioral alterations.

Reference: Kalueff AV, A Minasyan, T Keisala, ZH Shah, P Tuohimaa (2006) “Hair barbering in mice: Implications for neurobehavioral research”. *Behavioural Processes* **71**: 8-15.