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NOCTURNAL AND DIURNAL RHYTHMS IN THE UNSTRIPED NILE GRASS RAT, *Arvicanthis niloticus*

By

Julie Anne Blanchong

A THESIS

Submitted to
Michigan State University
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ABSTRACT

NOCTURNAL AND DIURNAL RHYTHMS IN THE UNSTRIPED NILE GRASS RAT, *Arvicanthis niloticus*

By

Julie Anne Blanchong

In a laboratory population of unstriped Nile grass rats, *Arvicanthis niloticus*, individuals with two distinctly different patterns of wheel-running exist, one that is diurnal and the other that is relatively nocturnal. In the first experiment, I found that these patterns are strongly influenced by parentage and by sex. Specifically, offspring of two nocturnal parents were significantly more likely to express a nocturnal pattern of wheel-running than were offspring of diurnal parents, and more females than males were nocturnal. In the second experiment, I found that diurnal and nocturnal wheel-runners were indistinguishable with respect to the timing of post-partum mating, which always occurred in the hours before lights-on. Furthermore, pre-weaning young and their parents all exhibited diurnal patterns of general activity even if they were nocturnal when housed with a wheel. In the third experiment, I discovered that adult female *A. niloticus* with nocturnal patterns of wheel-running were nocturnal with respect to general activity and core body temperature when a running wheel was available, but diurnal when the running wheel was removed. Finally, a field study revealed that *A. niloticus* are almost exclusively diurnal in their natural habitat. Together these results suggest that individuals of this species are fundamentally diurnal, but that access to a running wheel shifts some individuals to a more nocturnal pattern.

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CHAPTER I

INTRODUCTION TO CIRCADIAN RHYTHMS AND *Arvicanthis niloticus*

An animal's environment fluctuates in a rhythmic and relatively predictable manner over the course of the 24-hour day. As a result, animals face temporally varying challenges related to finding food, reproducing and avoiding predators. In order to enhance their chances for survival animals must 'do the right thing at the right time' (Moore-Ede et al., 1982). To accomplish this, animals possess circadian systems responsible for measuring time and synchronizing internal processes with daily cycles in the environment (Moore-Ede et al., 1982). These systems contain physiological pacemakers synchronized with changes in the environment (Moore-Ede et al., 1982). These internal 'clocks' enable animals to anticipate changes in the environment, prepare in advance for periods of activity and promote internal coordination of physiological and behavioral processes (Moore-Ede et al., 1982).

Most animals are specialized to be active at a particular phase of the 24-hour day. An animal's activity is generally organized in one of three broad patterns: diurnal, in which activity is concentrated in the daytime when it is light; nocturnal, in which activity is concentrated during the night when it is dark, or crepuscular where activity is clustered at dawn and dusk (Moore-Ede et al., 1982). Concentration of activity at a particular time of day leads to specializations of other behaviors as well. For example, diurnal animals have sharp vision and tend to rely on social anti-predator strategies while nocturnal animals rely less on their visual systems and often use cryptic strategies for predator avoidance (Daan, 1981). Because so many differences exist between diurnal and nocturnal lifestyles, evolutionary transitions from one of these behavior patterns to the other have been relatively rare.

The first mammals were nocturnal, and this remains the most common pattern of activity in modern mammals (DeCoursey, 1990). In the order Rodentia most families have remained nocturnal. Notable exceptions include most members of the family Sciuridae, some genera of murids and many South American rodents in the families Caviidae, Dasyproctidae, Chinchillidae, Capromyidae and Octodontidae (Nowak and Paradiso, 1983). While most species of rodents can be categorized as diurnal, nocturnal or crepuscular there is some evidence of species in which diurnal and nocturnal individuals coexist. Among cotton rats, *Sigmodon hispidus*, some individuals are diurnal while others are nocturnal in the laboratory and the field (Johnston and Zucker, 1983). In their lab study, Johnston and Zucker (1983), found that half of the juvenile cotton rats showed diurnal patterns of wheel-running while half were nocturnal. As adults, most, but not all, animals showed predominantly nocturnal patterns. However, nocturnal adults sometimes switched to become diurnal either temporarily or permanently. *Octodon degus* are diurnal South American rodents in which morning and evening 'chronotypes' coexist in both the laboratory and the field (Labyak et al., 1997). These different chronotypes exhibit distinct differences in the patterns of their activity and temperature rhythms (Labyak et al., 1997).

A variety of species of voles, mice and lemmings show seasonal variations in their timing of activity in order to avoid the harsh seasonal microclimates (Rowsemitt, 1986; Madison, 1985). Specifically, during the summer when days are longer and temperatures are higher, these animals tend to be more crepuscular, and more active during the cooler parts of the day, whereas in the colder winter when days are shorter they are more diurnal, and express relatively high levels of activity during the middle of the day. For example, montane voles, *Microtus montanus*, from northern latitudes showed crepuscular patterns of activity under long-day conditions in the laboratory and in summer field conditions, while they were more diurnal under short-day lab conditions and winter field conditions (Claypool, 1984). Some species may also switch from one pattern of activity to another in response to the presence of other species. The golden spiny mouse, *Acomys russatus*, native to hot, arid regions, is normally nocturnal, but when it is sympatric with *A. cahrinus* it switches and becomes diurnal (Haim and Rozenfeld, 1993). *A. russatus* also change their activity patterns in response to seasonal changes in photoperiod and ambient temperature, in a manner similar to that described above in some other rodent species (Friedman, 1997).

The unstriped Nile grass rat, *Arvicanthis niloticus*, appears to be another species of rodent in which individuals vary with respect to the distribution of their activity relative to the light-dark cycle. These tropical rodents are widespread in equatorial Africa and are usually the dominant rodent in their ecosystem (Kingdon, 1974). They are stout-bodied rodents five to seven inches in length not including the tail. The coat is sandy colored and peppered with black. Their heads are blunt with a short muzzle and circular ears (Kingdon, 1974). *A. niloticus* are highly opportunistic and almost entirely herbivorous, though analysis of stomach contents sometimes reveals traces of insects (Senzota, 1982). They favor drier grasslands as the major habitat, live in burrows constructed beneath bushes that provide cover, and travel in runways that radiate outward from burrow entrances (Packer, 1983).

Although considerable research has been done on *A. niloticus*, there is a lack of consensus with regard to the pattern of its activity rhythms. Systematic field data have not been reported on this issue, however, while investigating other aspects of this species' ecology, some researchers have concluded that *A. niloticus* are primarily diurnal (Quilici et al., 1969; Delany and Kansiimeruhanga, 1970; Kingdon, 1974; Packer, 1983; Rabiu and Fisher, 1989). However, Senzota (1990) describes them as diurnal with crepuscular tendencies, while others have reported them to be partially nocturnal (Ansell, 1960; Vesey-Fitzgerald, 1966; Rosevear, 1969; Harrison, 1972). Still others assert they are chiefly nocturnal (Schmutterer, 1969; Ghobrail and Hodeib, 1982).

This thesis describes my efforts to systematically characterize the daily rhythm patterns of *A. niloticus* in both a laboratory and a field setting. Chapter 2 describes my efforts to determine if an individual's pattern of activity is influenced by its parentage or by its sex. Chapter 3 describes the timing of mating in diurnal and nocturnal *A. niloticus* as well as the ontogeny of rhythmicity in juveniles of this species. In Chapter 4, patterns of gross motor activity and core body temperature are compared in diurnal and nocturnal individuals. Finally, Chapter 5 describes my efforts to characterize this species' pattern of activity in its natural habitat in Kenya, Africa.

CHAPTER 2

FACTORS INFLUENCING WHEEL-RUNNING PATTERNS IN *A. NILOTICUS*

INTRODUCTION

Almost all animals can be categorized as diurnal, nocturnal or crepuscular. However, there are some rodent species within which both diurnal and nocturnal patterns are exhibited (e.g. cotton rats, *Sigmodon hispidus*, Johnston and Zucker, 1983; degus, *Octodon degus*, Laybak et al., 1997). The unstriped grass rat, *Arvicanthis niloticus*, appears to be another such species.

Within a population brought to the laboratory from the Masai Mara National Reserve (MMNR) in Kenya, most individuals tested in running wheels were diurnal, with elevated levels of activity during the daylight that decreased dramatically shortly after dark. One female, however, showed a distinctly different and nocturnal pattern of wheel-running with high levels of activity several hours after the lights went out. She was mated with two males and 65% (15/23) of her offspring also expressed this nocturnal pattern of wheel-running. Only a small proportion of the offspring of other breeding pairs expressed nocturnal patterns of running (7%, 3/43) .

Though the coexistence of diurnal and nocturnal individuals has been reported in a number of rodent populations, nothing is currently known about the causes of this intraspecific variability. In this experiment, the range of activity patterns seen in a laboratory population of *A. niloticus* is described as well as efforts to determine if a grass rat's wheel-running pattern can be predicted by the activity patterns of its parents, by its sex or by its age.

METHODS

General Methods

In August 1993, 29 *A. niloticus* were captured in Masai Mara National Reserve, Kenya and imported to the USA. These animals were housed at Michigan State University and breeding couples were formed to establish a laboratory colony of animals. All animals were maintained in a 12:12 light-dark cycle and maintained at a constant temperature of 22 degrees Celcius. Animals were housed in 34 x 30 x 16 cm cages containing aspen chip bedding. All animals were provided with water, Harlan Teklad rodent diet 8640, carrots and oats. Mating couples were supplemented with Harlan Teklad high-fiber rabbit diet 7015 once a week and were provided with plastic tube or metal shelters. Juveniles were housed with their parents until 21 days of age at which time they were weaned and placed as a group into a separate cage. At 50 days of age male and female siblings were separated.

To monitor wheel-running, animals were housed individually and provided with a seven cm diameter running wheel to which a magnet was attached. With each revolution of the wheel, the magnet closed a switch attached to the cage. The number of running wheel revolutions was recorded in five minute bins using the Dataquest III Minimitter system (SunRiver, OR).

Experiment 1

I established mating couples of *A. niloticus* with either (1) two diurnal (DD; n= 4), (2) two nocturnal (NN; n= 4) or (3) one diurnal and one nocturnal (DN, n= 6; 3N females, 3D females) animals. Prior to pairing, the rhythms of these breeders were determined by testing them individually in running-wheels for two weeks. I housed offspring of these animals with both parents until weaning (21 days of age), at which time they were placed into individual cages with running wheels (n= 38, 52, and 38 from DD, DN and NN mating couples respectively). Wheel-running was recorded continuously for one month to establish whether patterns were diurnal or nocturnal and to assess the hour after lights-out in which animals became inactive.

These analyses were based on data averaged over the last five days of the first month of testing in running wheels. I defined diurnal animals as those whose activity by the fifth hour after lights-out was less than ten percent of the 24 hour peak. Nocturnal animals were those whose activity during and/or following the fifth hour after lights-out was greater than twenty percent of the daily peak. I defined the hour after lights-out in which an animal ceased to be active as the hour during which activity dropped to less than ten percent of the 24 hour peak.

I continued to monitor a random subset of offspring from each category of mating couple for an additional two months to test for the effects of age on the pattern of activity (n=12, 17 and 19 offspring of DD, DN and NN mating couples, respectively). I classified these animals as diurnal or nocturnal at the end of the third month, again by averaging the hourly activity over the last five days of each testing period. These data were also analyzed to determine the hour after lights-out in which animals stopped wheel-running.

In order to be certain that the differences between diurnal and nocturnal animals were not the result of masking, three nocturnal offspring were placed into constant darkness after three months in a 12:12 light-dark cycle.

Data were analyzed using chi-square tests with Yate's corrections for continuity, analyses of variance (ANOVA) with repeated measures, Pearson correlations and t-tests. Results were considered significant when $p<0.05$.

Experiment 2

I tested whether the influence of fathers on their offsprings' rhythm patterns was mediated by the experience of growing up in the presence of the father. I tested this by comparing the activity patterns of animals that were raised by their diurnal mother with or without their nocturnal father. The parents' wheel-running patterns were determined by testing in running wheels for one month prior to pairing. Four mating couples consisting of a diurnal female and a nocturnal male were established. When a litter was born the father was removed, and the female was left to raise the pups on her own in two of the mating couples. For the other two mating couples, both parents remained with pups until they were weaned. All pups were weaned at twenty-one days of age and immediately placed in individual running wheels where they were kept for one month in order to assess their pattern of activity. After one month these wheel-running patterns were assessed to determine if they were nocturnal or diurnal as in the first experiment. Patterns that developed in animals that were raised with diurnal mothers with or without the nocturnal fathers were compared.

RESULTS

Experiment 1

Almost all animals exhibited clear patterns of rhythmic wheel-running that fit characteristic diurnal (Figure 1a) or nocturnal (Figure 1b) patterns. Of 128 animals tested, six could not be categorized as clearly diurnal or nocturnal and were classified as intermediate. Among the remaining 122 animals, nocturnal patterns of activity were expressed by 14% (5/37) of offspring from DD parents, 53% (25/47) from DN parents and 68% (26/38) from NN parents. The ratio of diurnal to nocturnal offspring differed significantly among the three categories of mating couples (x^2_2=26.9, $p<0.001$). Specifically, there were more nocturnal offspring from NN mating couples than from DD mating couples (x^2_1= 20.0, $p<0.001$) and from DN parents than from DD parents (x^2_1= 15.8, $p<0.001$). When male and female offspring were analyzed separately I found a difference among mating couple types with respect to the proportion of both their male offspring (x^2_2= 9.3, $p<0.01$) and their female offspring (x^2_2= 13.6, $p<0.005$) that were nocturnal. In addition, I found that when animals from all mating couples were pooled, females were more likely to express a nocturnal pattern of activity than were males (x^2_1= 12.5, $p<0.001$; Figure 2). Specifically, among offspring of NN parents, more females (89%) than males (50%) were nocturnal (x^2_1= 5.9, $p<0.01$; Figure 2). The sex difference among offspring of DN (x^2_1= 2.5, NS; Figure 2) and DD parents was not significant (x^2_1= 0.3, NS; Figure 2).

In order to examine the influence of parentage and sex more precisely, I compared offspring of different mating couple types with respect to the hour after lights-out in which they ceased to be active (Figure 3). This value differed as a function of the parents' rhythm patterns. Offspring of NN parents continued their activity after lights-out longer than did offspring of DN parents (x^2_8= 17.2, $p<0.05$) or DD parents (x^2_8= 26.3,

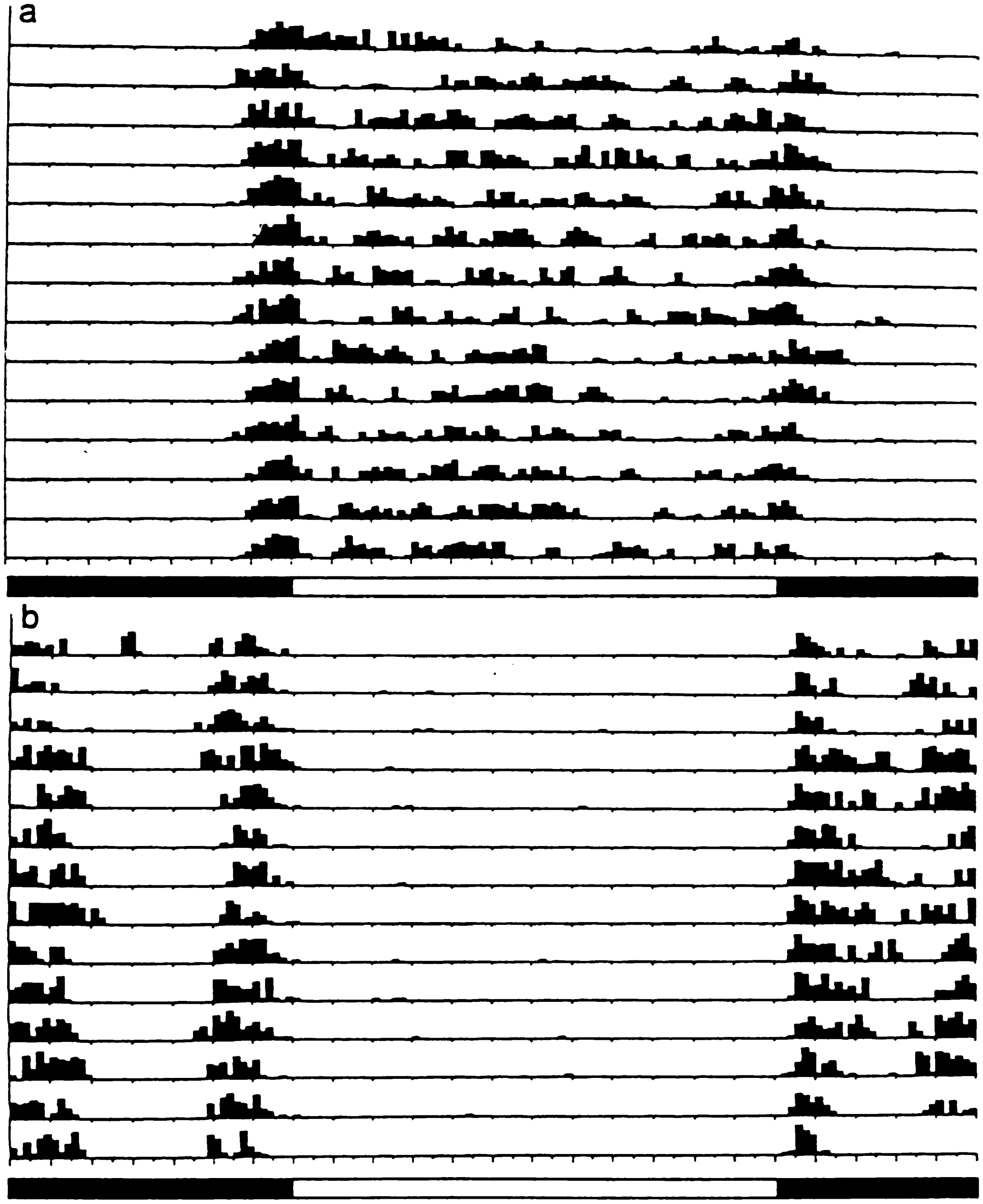

Figure 1. Actograms of typical (A) diurnal and (B) nocturnal wheel-running patterns of *A. niloticus*.

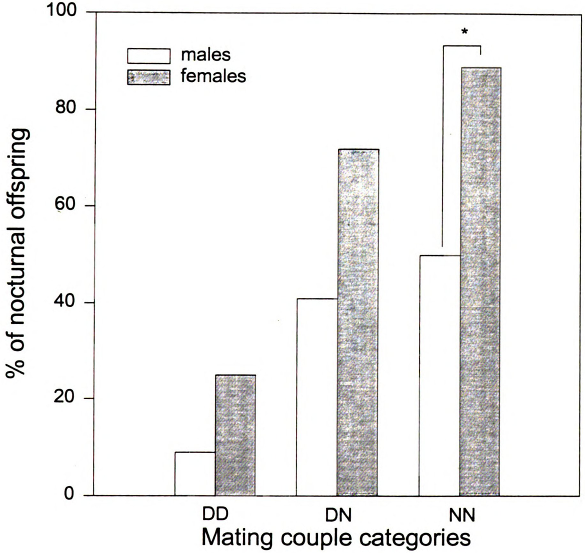

Figure 2. Histogram representing percentages of nocturnal male and female offspring from each category of mating couple.

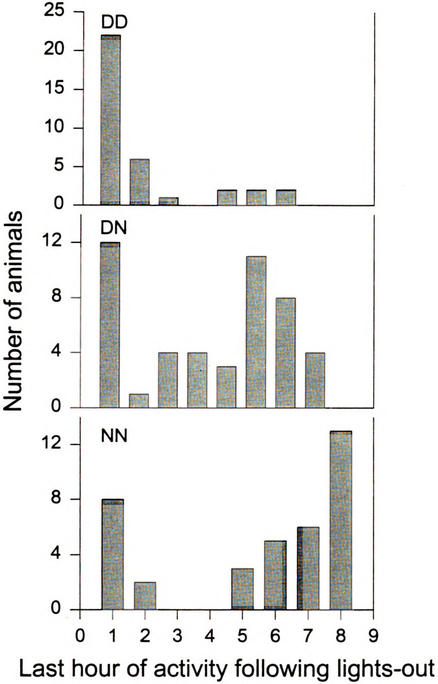

Figure 3. Histogram representing numbers of offspring of each category of mating couple with activity offsets occurring in each of 8 one-hour intervals following lights-out.

$p<0.001$). In addition, offspring of DN parents continued to be active longer after lights-out than offspring of DD parents ($x^2_8 = 27.6$, $p<0.001$). Overall, the distribution of animals that stopped wheel-running at various times after lights-out was distinctly bimodal, with many animals stopping at one hour or six hours or more, while relatively few stopped at intermediate times (Figure 3). When I analyzed the sexes separately, I found that female offspring of the different types of mating couple ceased wheel-running at different times after lights-out (Table 1). Specifically, females of NN parents continued to be active longer than did females from DD parents. I found no significant differences when comparing female offspring of DN parents to those of DD parents or NN parents with respect to the time after lights-out when wheel-running stopped (Table 1). This analysis, in contrast to my more powerful categorical analyses, also failed to detect significant differences between male offspring of the three different categories of mating couples. Overall, males and females differed with respect to the hour after lights-out in which they became inactive (Table 1). Specifically, females continued to be active longer after lights-out than did males.

To determine if activity patterns changed as animals aged, I conducted a repeated measures ANOVA with which I compared the hour at which wheel-running stopped after one and three months in wheels, in animals originally classified as diurnal or nocturnal. The pattern of activity expressed by an individual in month one changed slightly, but significantly by month three ($F_2 = 3.132$, $p=0.049$). This difference is due to 10 out of 38 animals that switched their patterns completely. When the data was analyzed without these animals I found that there was no effect of age on the pattern of activity ($F_2 = 2.362$, $p=0.105$). I also found an interaction between the initial pattern of activity (diurnal or

Table 1. Analysis of Differences Between Groups With Respect to the Hour After Lights-Out That Wheel-Running Stopped.

Animals	Comparison	x^2	df	p value
Females	DD:DN:NN	34.0	16	<0.01
	DD:NN	18.0	8	<0.025
	DD:DN	3.8	8	NS
	DN:NN	13.2	8	NS
Males	DD:DN:NN	22.3	16	NS
All	M:F	22.4	8	<0.005
DD	M:F	7.7	8	NS
DN	M:F	4.1	8	NS
NN	M:F	11.7	8	NS

nocturnal) and the direction of change in the pattern over time (F_2= 19.647, p<0.001). Specifically, animals classified as diurnal in month one remained active longer after lights-out at month three than at month one, while animals initially classified as nocturnal became inactive sooner after lights-out at month three than at month one (Figure 4). Despite these changes, at the end of three months, animals initially classified as diurnal remained significantly different from those initially classified as nocturnal (t_{44}= 4.898, p=0.001, Figure 4).

A one-way ANOVA comparing the total number of daily wheel revolutions by animals that stopped running in each of the first eight hours after lights-out showed that there was a difference in the total amount of wheel-running among animals that stopped activity at different times after the lights went out (F_7= 8.04, p= 0.001). Animals that continued to run for eight hours after lights-out ran approximately 49% more than did animals that ceased running in the first hour after lights-out (Figure 5; t_{57}=5.78, p=0.001). However, the heightened activity among nocturnal individuals was disproportionately concentrated in the dark period of the 12:12 light-dark cycle. This can be seen in the comparison of hourly wheel-running rates of individuals that stopped running one or eight hours after lights out (Figure 6). Furthermore, the number of hours that animals were active after lights-out was actually negatively correlated with the number of hours they were active during the light period (r= 0.594, p= 0.001, Pearson Correlation). Thus, nocturnal animals were not simply more active than diurnal ones, but rather, the fundamental waveform of their rhythm differed. This basic nocturnal waveform was maintained in the three animals that were released into constant darkness (Figure 7).

Experiment 2

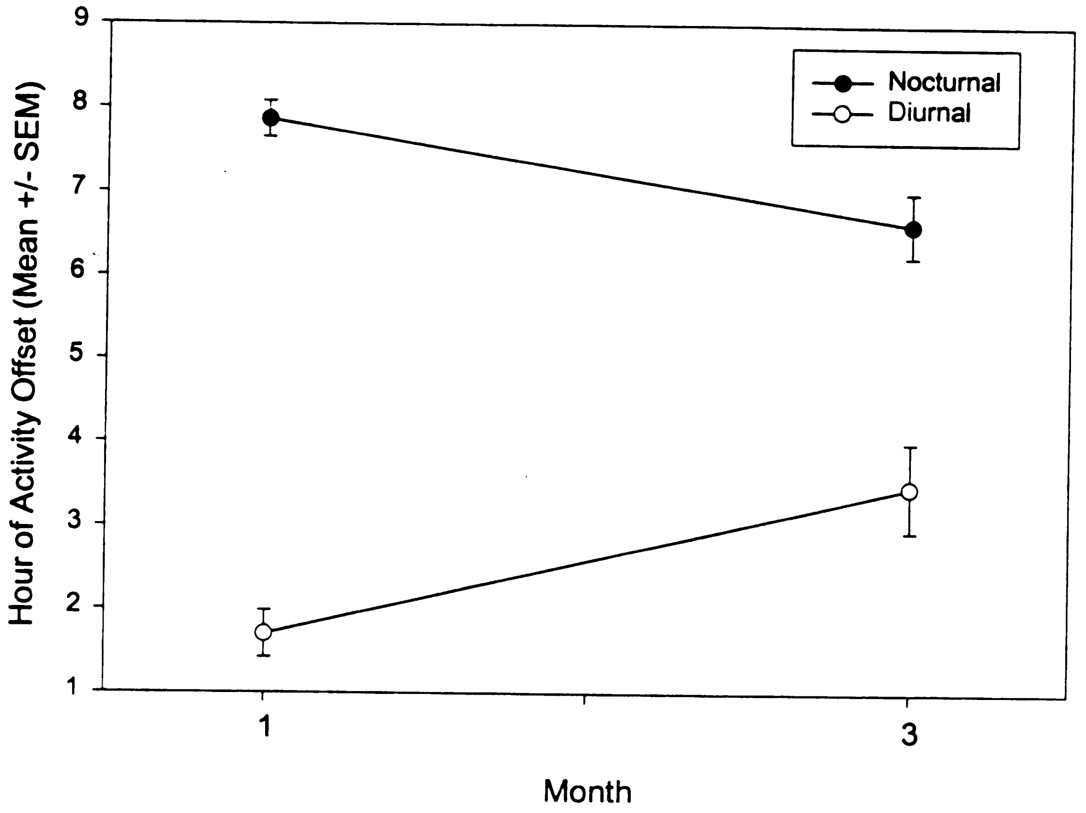

Figure 4. Hour of activity offset and SEM (standard error of the mean) for animals classified as nocturnal or diurnal at the end of their first and third months in running wheels.

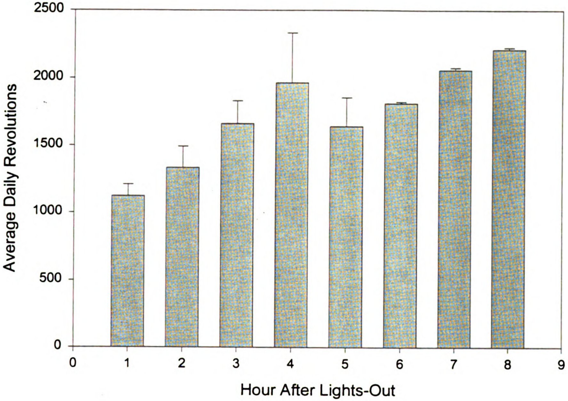

Figure 5. Average number of wheel revolutions (SEM) per day for animals that ceased wheel-running in each of 8 one-hour intervals following lights-out.

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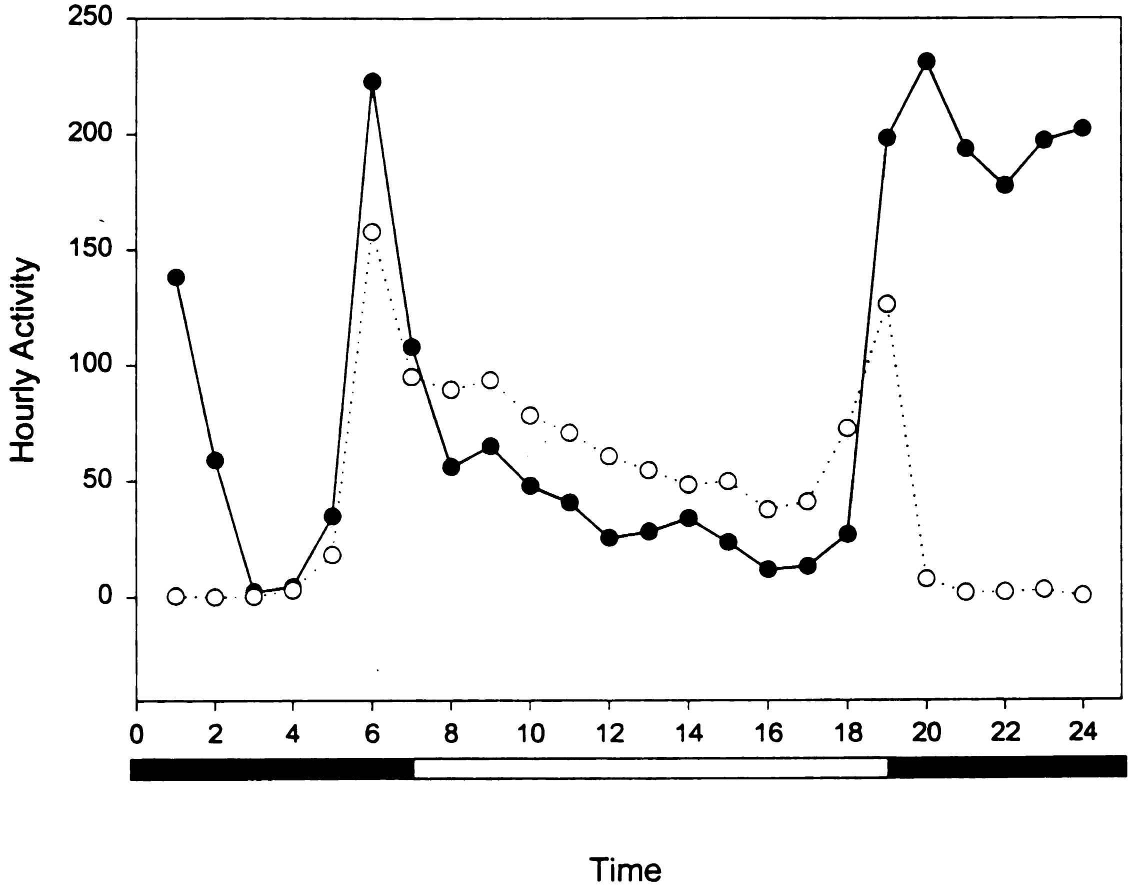

Figure 6. Average number of wheel revolutions per hour of animals that stopped running 1 hour (open circles) or 8 hours (dark circles) after lights-out.

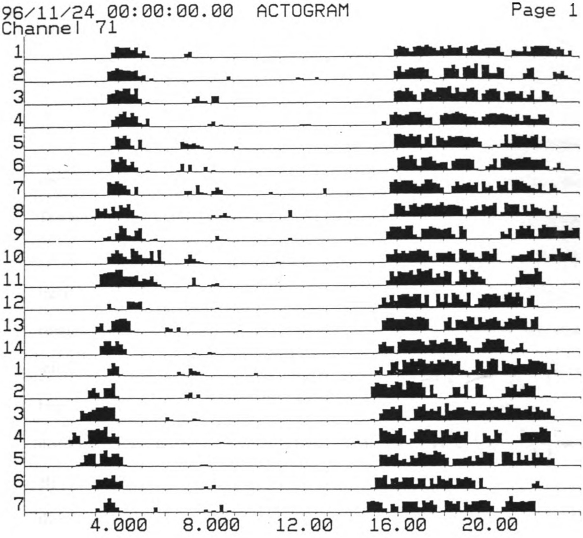

Figure 7. Actogram depicting a nocturnal animal maintained in constant darkness

Three litters (n= 11 offspring) were raised with the father present and three litters (n=10 offspring) were raised without the father present. The proportion of animals in these two groups that were nocturnal was almost identical. Eight out of eleven pups raised with the father present expressed a nocturnal pattern of wheel-running while eight out of ten pups raised without the father were nocturnal.

DISCUSSION

These results suggest that the group-living murid rodent species, *A. niloticus*, contains individuals with two relatively distinct patterns of daily wheel-running activity. The most dramatic difference between these nocturnal and diurnal individuals occurred during the hours after lights-out. Most animals increased wheel-running immediately after lights-out (Figure 6), but then some stopped running within one or two hours while others continued for approximately six to eight hours, and relatively few animals stopped at intermediate times (Figure 3). In contrast to these evening bouts of activity, which were bimodally distributed, activity levels at other times of day were more normally distributed. Animals that ran for a long time at night ran more overall (Figure 5), but the elevated running was disproportionately concentrated in the evening (Figure 6). In fact, there was an inverse correlation between the number of hours that animals ran during the light and dark periods of the day.

One possible interpretation of the differences between nocturnal and diurnal wheel-running patterns involves masking. For example, nocturnal individuals might find the light aversive or frightening, and may therefore wait until dark before becoming active. This interpretation was tested by transferring nocturnal individuals to constant darkness, which did not change the fundamental waveform (Figure 7). That is, the evening bout of wheel-running remained lengthy, and daytime wheel-running remained minimal. Earlier work found that constant darkness similarly did not substantially alter the waveform of the wheel-running rhythm of diurnal *A. niloticus* (Katona and Smale, 1997). Differential masking effects of light are therefore unlikely to account for differences between nocturnal and diurnal wheel-running patterns.

Individual differences in *A. niloticus* wheel-running patterns were strongly influenced by parentage and by sex. Specifically, animals with two nocturnal parents were significantly more likely to be nocturnal than were animals with two diurnal parents, and females were more likely to be nocturnal than were males. Although the data provided by this study cannot definitively rule out possible mechanisms by which mothers and fathers influence the development of rhythm pattern, these data can help to clarify some possibilities. If the pattern of wheel-running was influenced solely by rearing environment it would be expected that the majority of offspring raised only in the presence of one diurnal parent would have shown a diurnal pattern of wheel-running. The pattern of results obtained in experiment two was inconsistent with this prediction. The results of this experiment suggest that the influence of the father on the rhythms of his offspring is not mediated by his presence, but, most likely, by his genes.

Several genetic mechanisms could account for the effective of selective breeding on rhythm patterns in *A. niloticus*. First, a single autosomal gene could account for the influence of parentage on whether an animal is nocturnal or diurnal. If this is the case, however, then that gene does not have complete penetrance. If it did, then either NN or DD mating couples would have two recessive alleles, and 100% of their offspring would exhibit the same pattern as the parents, which was not what I found. However, it remains possible that a single gene responsible for the activity pattern may have incomplete penetrance such that a small subset of the population that is homozygous for this gene would express the alternate phenotype. For example, some NN individuals may be diurnal, or some DD individuals could be nocturnal because of a non-genetic source of variability. Furthermore, the sex difference suggests that if a single allele is involved, then its penetrance would have to be different in males and females. A second possibility is that a single gene with a sex-linked pattern of dominance determines whether an animal is nocturnal or diurnal. Specifically, a nocturnal allele could be dominant in females and recessive in males. This pattern has been documented for the inheritance of some forms of baldness in humans and for the spotting pattern of Ayrshire cows (Dodson, 1956; Griffiths et al., 1993). According to this hypothesis, males and females heterozygous for this gene would exhibit different phenotypes. This hypothesis is consistent with this data. A third possibility consistent with the results is that multiple genes are involved in determining whether an animal exhibits a nocturnal or diurnal pattern of wheel-running.

Populations within which individuals with dramatically different patterns of rhythmicity coexist have been described in a variety of other rodent species, including degus (*Octodon degus*; Labyak et al., 1997), cotton rats (*Sigmondon hispidus*; Johnston and Zucker, 1983), a variety of vole species (Madison, 1985), inbred strains of rats (Wollnik, 1991) and selected lines of mice (Bult et al., 1993). Rhythm “chronotypes” have also been described in degus, a diurnal species in which a relatively rare evening chronotype exhibits delayed nighttime drops in body temperature and wheel-running activity (Labyak et al., 1997). This species is different from *A. niloticus* in that the evening chronotypes are at one end of a relatively normal distribution, rather than one end of a bimodal distribution.

CHAPTER 3

TIMING OF MATING IN DIURNAL AND NOCTURNAL INDIVIDUALS AND DEVELOPMENT OF RHYTHMS IN JUVENILES

INTRODUCTION

The previous experiments have demonstrated that the timing of wheel-running differs dramatically between individual *A. niloticus* and that this difference is influenced by their parentage and by their sex. The coexistence of individuals with distinctly different patterns of wheel-running raises the question of whether other aspects of temporal organization are different in nocturnal compared to diurnal wheel-runners. Nocturnal and diurnal species typically differ with respect to patterns of a host of variables, including hormone secretion, sleep, body temperature, parturition, mating and general activity (Moore-Ede et al., 1982; McElhinny, 1996). In this and the following chapter, I compared nocturnal and diurnal wheel-runners with respect to the timing of reproductive parameters as well as rhythms of body temperature and general activity.

The time of mating coincides with the time of peak activity in most animals. For example, female rats are most sexually receptive in the first six to nine hours after dusk (Keuhn and Beach, 1963; Hardy, 1972), when they are most active. Previous investigation of the time of mating of diurnal *A. niloticus* indicates that, unlike nocturnal rodents, they mate in the early morning prior to lights-on (McElhinny et al., 1996). In this study, the time of mating was compared between *A. niloticus* that expressed diurnal and nocturnal wheel-running patterns in order to determine if diurnal and nocturnal individuals' mate at different times of day. I also monitored the development of rhythm patterns in pups before they were weaned, and compared these to their wheel-running patterns expressed after they were weaned.

METHODS

Diurnal (n=2) and nocturnal (n=4) mating couples whose activity rhythms had been determined by testing in running wheels for two weeks were paired and placed in aquaria with aspen chip bedding and a plastic tube for shelter. All animals were kept in a 12:12 light-dark cycle with a dim red light to allow for videotaping when the lights were off. Around-the-clock videotaping of mating couples began eighteen days after animals were paired in order to capture the time of parturition (21 days after mating) and post-partum mating. The time of parturition was recorded as the time at which the first pup could be seen. The time of mating was recorded as the first incidence of lordosis by the female in response to male mounting. Five days after parturition, litter size was culled to two and one of the pup's fur was marked with Nyanzol-D hair dye to allow for individual identification of pups on videos. Videotaping continued until litters were weaned 21 days after they were born. Videotapes from days 10, 15 and 20 after parturition were analyzed in ten minute intervals for the entire 24-hour day in order to determine each individual's pattern of activity (adults and juveniles). An animal was considered active if it was off the nest area for at least five minutes of the ten minute interval. At day 21, pups were placed in individual running wheels for one month to determine their pattern of wheel-running. Data were analyzed using t-tests and chi-square tests, and were considered significant when $p<0.05$.

RESULTS

Parturition occurred at many different times throughout the 24-hour day during lights-on and lights-out for both diurnal (4 litters) and nocturnal (8 litters) mating couples. There was no difference between diurnal and nocturnal mating couples in the time of day in which parturition occurred (t_5= 0.68, NS). All post-partum mating occurred in the early hours of the morning. This was true for animals that had exhibited nocturnal as well as diurnal patterns of wheel-running. In contrast to other nocturnal rodents, which mate in the first hours after lights-out, nocturnal *A. niloticus* mated an average of one hour and 36 minutes (range= 4 hrs prior- 1 hr after) before lights-on. Diurnal mating couples mated an average of 3 hours and 5 minutes (range= 5- 1 hr) prior to lights-on. Diurnal and nocturnal animals thus, both mated in the early morning.

When videotapes were analyzed to evaluate juvenile activity patterns at 10, 15 and 20 days of age it was apparent that all animals, including those that were subsequently nocturnal in running wheels, exhibited diurnal activity (Figure 8a,b). Clear rhythms did not exist at day 10 when juveniles rarely left the nest, but were apparent in all animals by day 15. Surprisingly, all parents were diurnal with respect to their patterns of general activity (Figure 8c), even those that had been nocturnal in running wheels. The temporal distribution of activity of all juveniles paralleled that of their parents throughout development (Figure 8). Juveniles exhibited little activity at day 10, and activity increased progressively until weaning (Figure 8a,b). When juveniles were placed in individual running wheels at weaning, 12/13 and 2/6 with nocturnal and diurnal parents, respectively, exhibited nocturnal patterns of wheel-running. More offspring from nocturnal parents exhibited nocturnal patterns that did those with diurnal parents

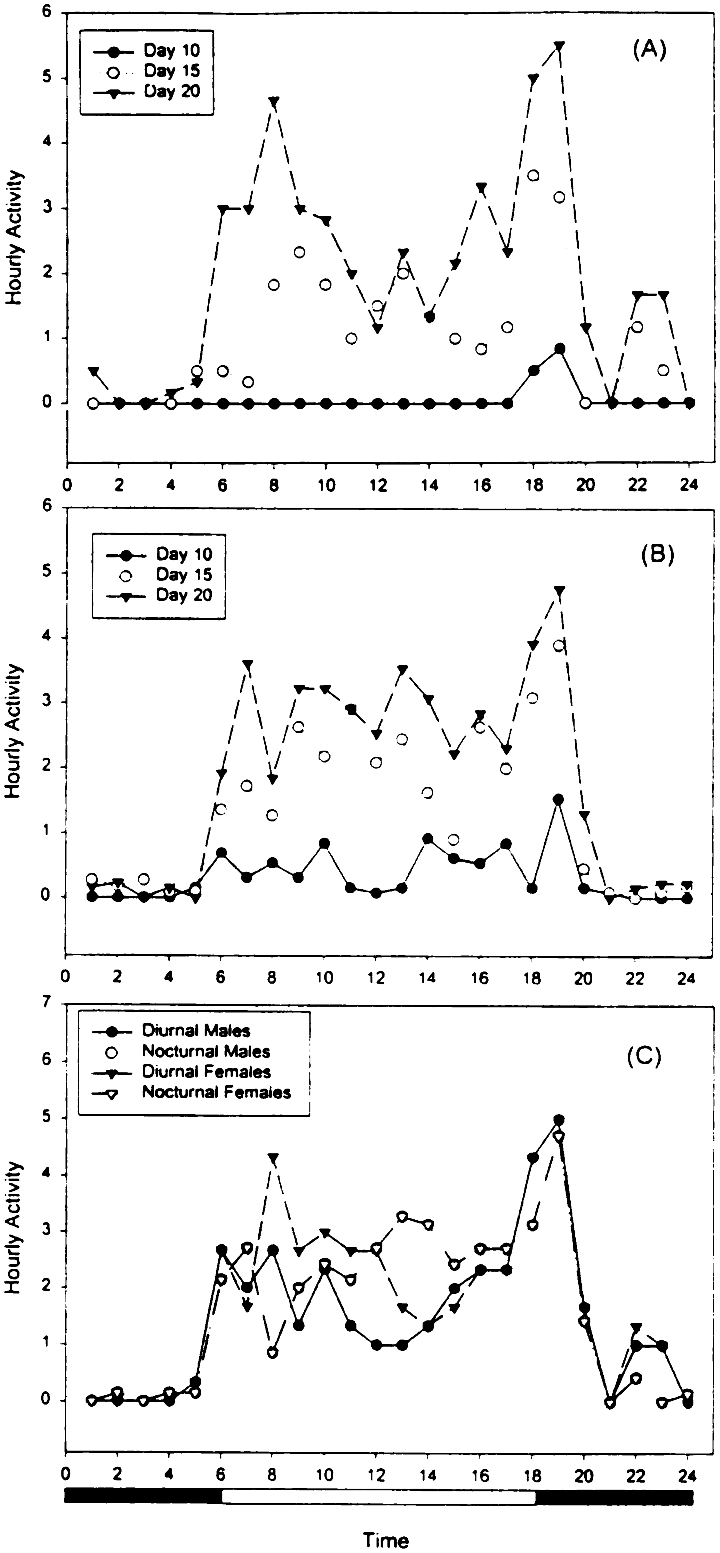

Figure 8. Average activity patterns (# of 10 minute intervals off the nest per hour) of offspring from (A) diurnal and (B) nocturnal parents over time. (C) Average activity of parents at day 20.

(x^2_2= 12.8, $p<0.005$).

DISCUSSION

All *A. niloticus* engaged in post-partum copulation in the early morning regardless of their wheel-running pattern whereas parturition occurred throughout the 24-hour day in both groups. In addition, juveniles in this species were active almost exclusively when their parents were active until the time of weaning. When placed in running wheels at weaning, offspring expressed their own independent patterns of wheel-running that usually, but not always, corresponded to their parents' wheel-running patterns. Very low levels of general activity were apparent in offspring at 10 days of age. Rhythms appeared at day 15 and increased in amplitude at day 20. All animals, adults and their offspring, expressed diurnal activity in this experiment when they did not have a running wheel, even those with nocturnal wheel-running patterns. These data suggest that these nocturnal patterns may not be expressed until: 1. animals are separated from conspecifics, 2. animals are provided with a running wheel or 3. until they are 21 days of age. Alternative number three is contradicted by the fact that nocturnal parents were diurnal when paired without a running wheel present. However, none of these alternatives could be definitively ruled out by this experiment, which was not designed to address this issue. The next experiments examined these issues more explicitly.

CHAPTER 4

GROSS MOTOR ACTIVITY AND BODY TEMPERATURE RHYTHMS IN DIURNAL AND NOCTURNAL INDIVIDUALS

INTRODUCTION

In the previous experiments individual *A. niloticus* showed distinctly different patterns of wheel-running that were strongly influenced by parentage and the sex of the animal. However, all animals mated at the same time of day regardless of the activity pattern they expressed in running wheels. Therefore, while wheel-running can be useful in the study of circadian rhythms, its relationship to other activities is uncertain, and it does not always parallel general activity. A number of researchers have found that patterns of wheel-running do not always resemble the pattern of activity without a wheel. For example, Roper (1976) found that Mongolian gerbils exhibit a nocturnal wheel-running pattern while Lewill (1974) described gerbils as diurnal on the basis of direct observation of their behavior in the absence of a running wheel. In the wild, rabbits are consistently nocturnal, but in the laboratory they have been found to show a wide range of wheel-running patterns (Kennedy et al., 1994). In addition, Wistar-Imamichi rats showed different activity patterns when ambulatory and wheel-running activites were compared (Shinoda et al., 1988; Shinoda and Miura, 1994). In the next experiment, gross motor activity and body temperature rhythms of *A. niloticus* were investigated in order to determine if animals exhibiting diurnal and nocturnal wheel-running patterns differ when a running wheel is no longer present.

METHODS

Adult female *A. niloticus* used in this study were first tested in running wheels for at least one month to determine their pattern of wheel-running. Animals were classified as diurnal (n= 4), nocturnal (n= 5) or intermediate (n= 4). All animals were anesthetized with methoxyfurane (Metofane) and implanted intraperitoneally with paraffin-coated Minimitter transmitters. Both muscle wall and the skin were sutured with dissolvable gut sutures and reinforced with autoclips. Incisions were treated with Nolvasan topical antibiotic and animals were given subcutaneous injections of 1.0 cc Lactated Ringer's solution and 0.2 cc of the analgesic, buprenorphine hydrochloride (Buprenex). Transmitters emitted signals correlated with gross motor activity and body temperature. These signals were picked up by receivers placed beneath the animal's cage and transmitted to a computer equipped with the Dataquest III Minimitter System, (Sun River, OR).

All animals in the study went through the same three phases of treatment. First, immediately following surgery, animals were placed in cages without a running wheel and gross motor activity and body temperature were recorded for three weeks. Second, at the beginning of the fourth week animals received running wheels, and gross motor activity, body temperature and wheel-running were recorded for two weeks. Third, for the final week of the study, the running wheel was removed and gross motor activity and body temperature were recorded as in phase one.

Hourly levels of gross motor activity were averaged over a five day period for each phase of the experiment, and the percentage of activity that occurred in each hour of the 24-hour day was determined for each animal. A repeated measures ANOVA and F-tests were performed to compare the amount of activity in the first five hours after lights-out in the three groups and across the three phases of treatment. Average hourly body temperatures were calculated for each animal from the same five day period of each phase of the experiment.

RESULTS

During phase 3, transmitters in two of the nocturnal animals stopped functioning, and data from these two animals were, therefore, excluded from statistical analysis. However, their patterns of general activity and body temperature during phase 1 and phase 2 resembled those of the other nocturnal animals. In the absence of a running wheel (phases 1,3) all animals exhibited diurnal patterns of gross motor activity, including those whose original wheel-running patterns had been nocturnal (Figure 9). When animals were given access to running wheels (phase 2) all animals originally classified as nocturnal showed nocturnal patterns of general activity while animals originally classified as diurnal remained diurnal (Figure 10). The percent of general activity that occurred in the first five hours after lights out was influenced by the phase of treatment (F_2= 14.360, p= 0.001), and by an interaction between the phase of treatment and the initial classification of animals as diurnal, nocturnal or intermediate (F_4= 17.018, p= 0.001). Pairwise comparisons were calculated using F-tests. For animals originally classified as nocturnal, the percentage of total activity that occurred in the first five hours after lights-out changed from phase 1 (wheel absent) to phase 2 (wheel present) ($F_{1,2}$= 126.41, $p<0.001$), and from phase 2 to phase 3 (wheel absent) ($F_{1,2}$= 145.08, $p<0.001$). Specifically, during phase 2, when wheels were present, all animals that were originally classified as nocturnal shifted their patterns of activity so that they became more nocturnal (Figure 11). Animals

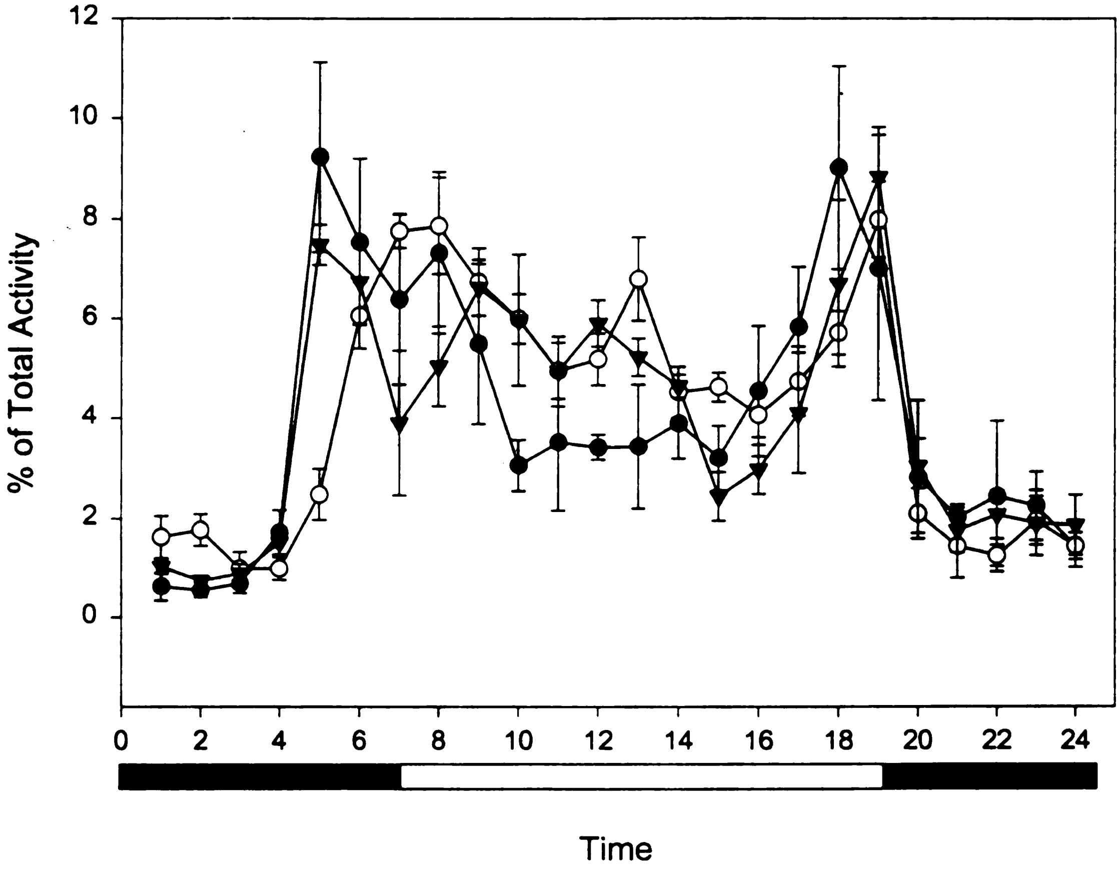

Figure 9. Average daily patterns of general activity and SEM for diurnal (open circles), intermediate (triangles) and nocturnal (dark circles) animals without a running wheel (phase 1).

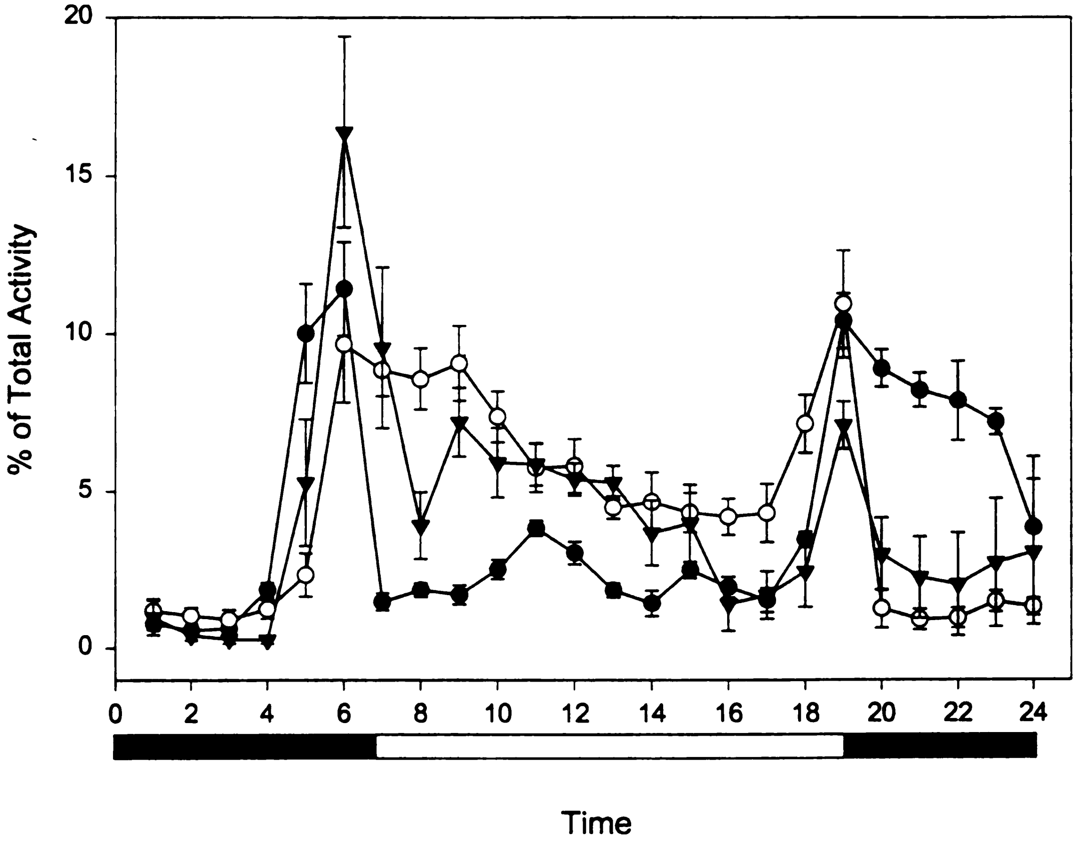

Figure 10. Average daily patterns of general activity and SEM for diurnal (open circles), intermediate (triangles) and nocturnal (dark circles) animals with a running wheel (phase 2).

classified as diurnal or intermediate did not change across phases (Figure 12). In addition, nocturnal animals differed from both diurnal and intermediate animals during phase 2 (wheel present) with respect to the percentage of activity that occurred in the first five hours after lights-out (F_2= 571.88, $p<0.005$; F_2= 411.28, $p<0.005$ respectively). There were no other differences between groups within any phases of the experiment.

The body temperature rhythms of all animals were diurnal without running wheels (Figure 13). When a running wheel was made available (phase 2), the body temperatures of animals categorized as nocturnal remained elevated for several hours after lights-out, as did their activity (Figure 14). The temporal pattern of diurnal animals' body temperature peaks did not change across the phases of the experiment. The body temperature rhythms paralleled patterns of gross motor activity in all animals at all phases of treatment. Body temperatures were elevated for all animals when a running wheel was present. This elevation occurred during the day in animals in the diurnal category, and at night in animals in the nocturnal category (Figure 14). Without a running wheel, animals in the diurnal category appeared to have a higher overall body temperature than did animals classified as nocturnal (Figure 13).

DISCUSSION

As described above, wheel-running patterns do not always parallel patterns of general activity (Kennedy et al., 1994; Lewill, 1974; Mather, 1981; Roper, 1976; Sherwin, 1998; Shinoda et al., 1988; Shinoda and Miura, 1994). This also appears to be true for *A. niloticus*. Regardless of initial wheel-running patterns, all animals implanted with abdominal transmitters showed diurnal patterns of gross motor activity and body

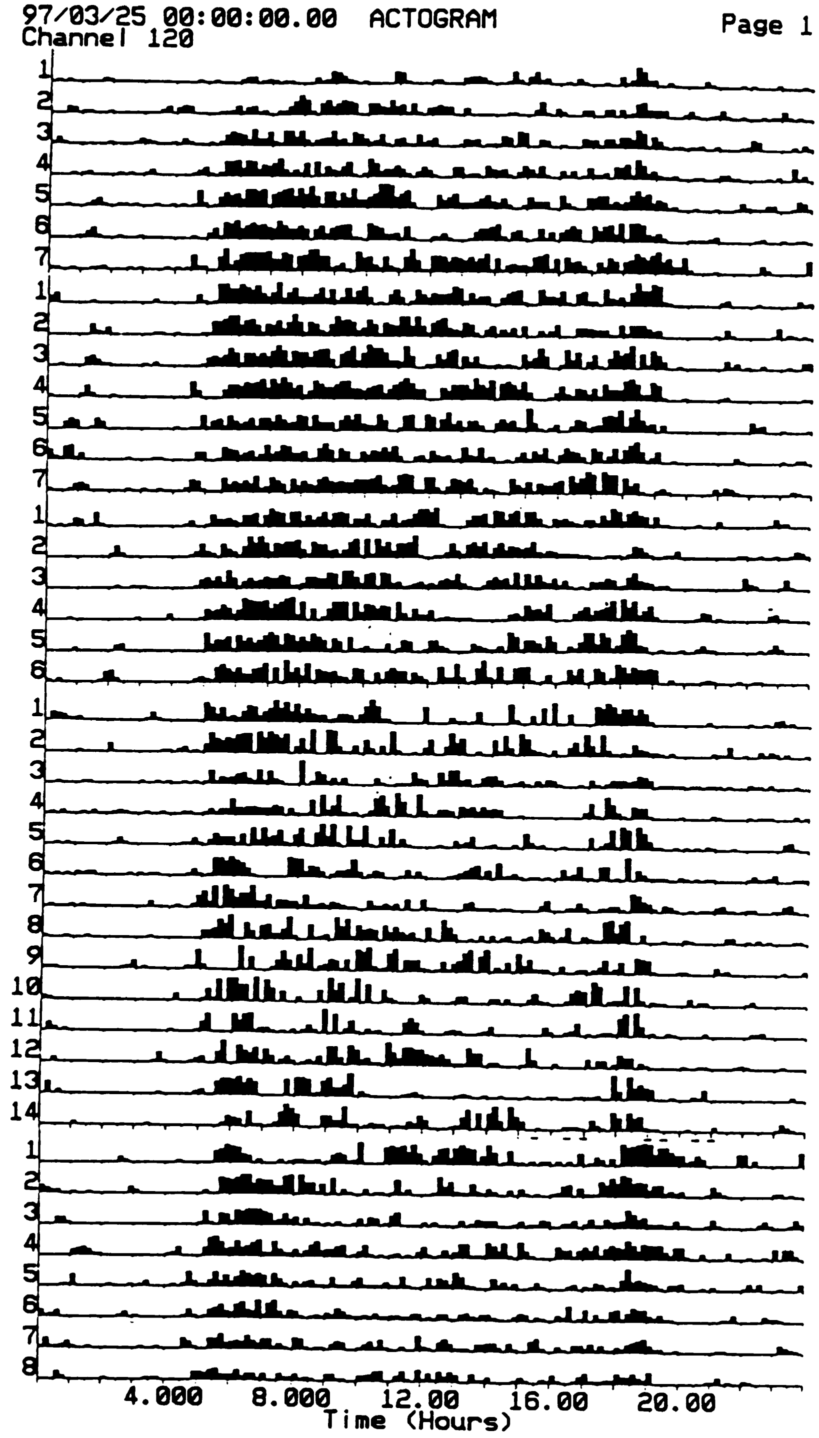

Figure 11. Representative actogram of general activity during phases 1,2 and 3 for a diurnal animal (lights-on 0700, lights-out 1900).

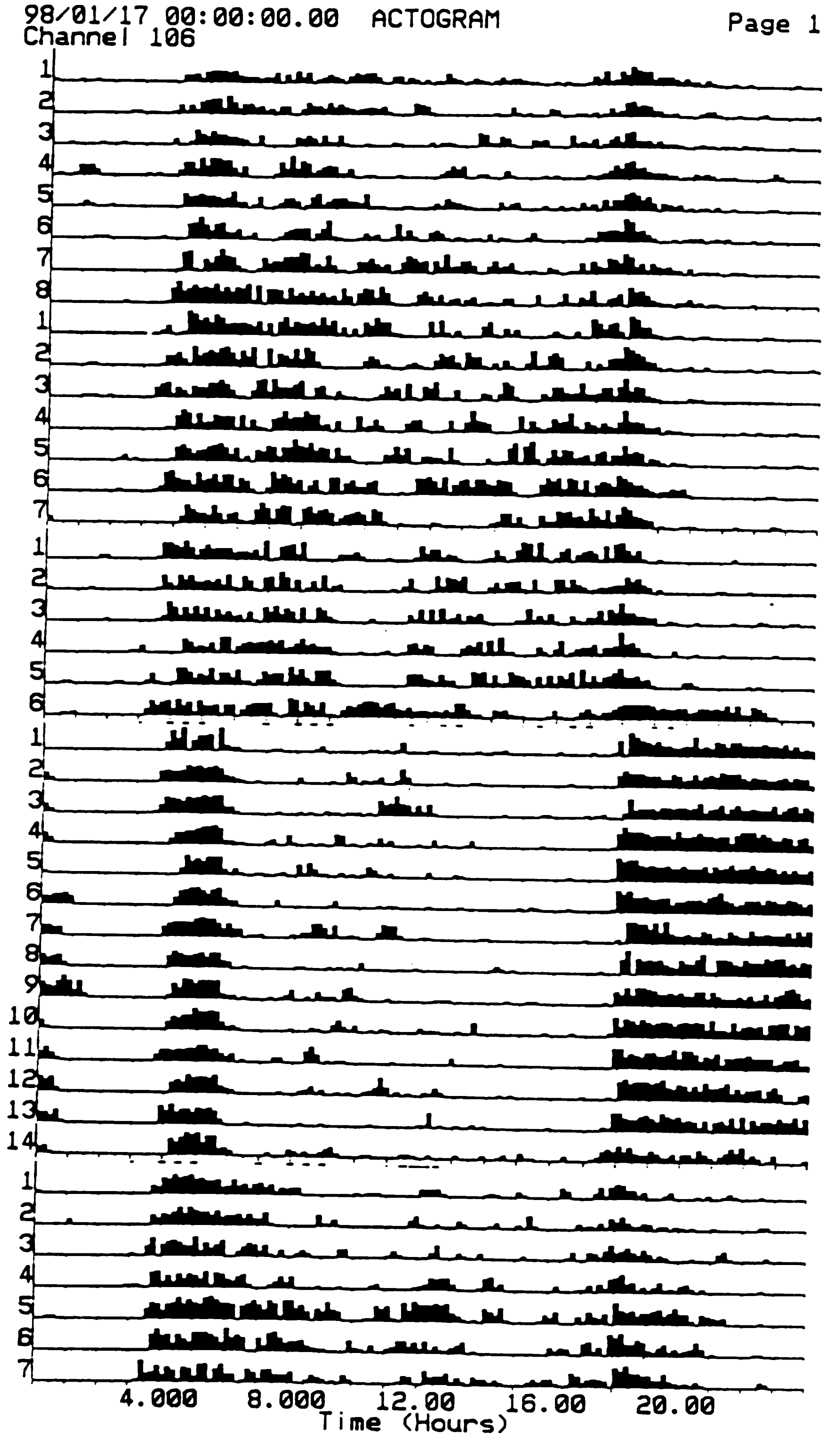

Figure 12. Representative actogram of general activity during phases 1,2 and 3 for a nocturnal animal (lights-on 0700, lights-out 1900).

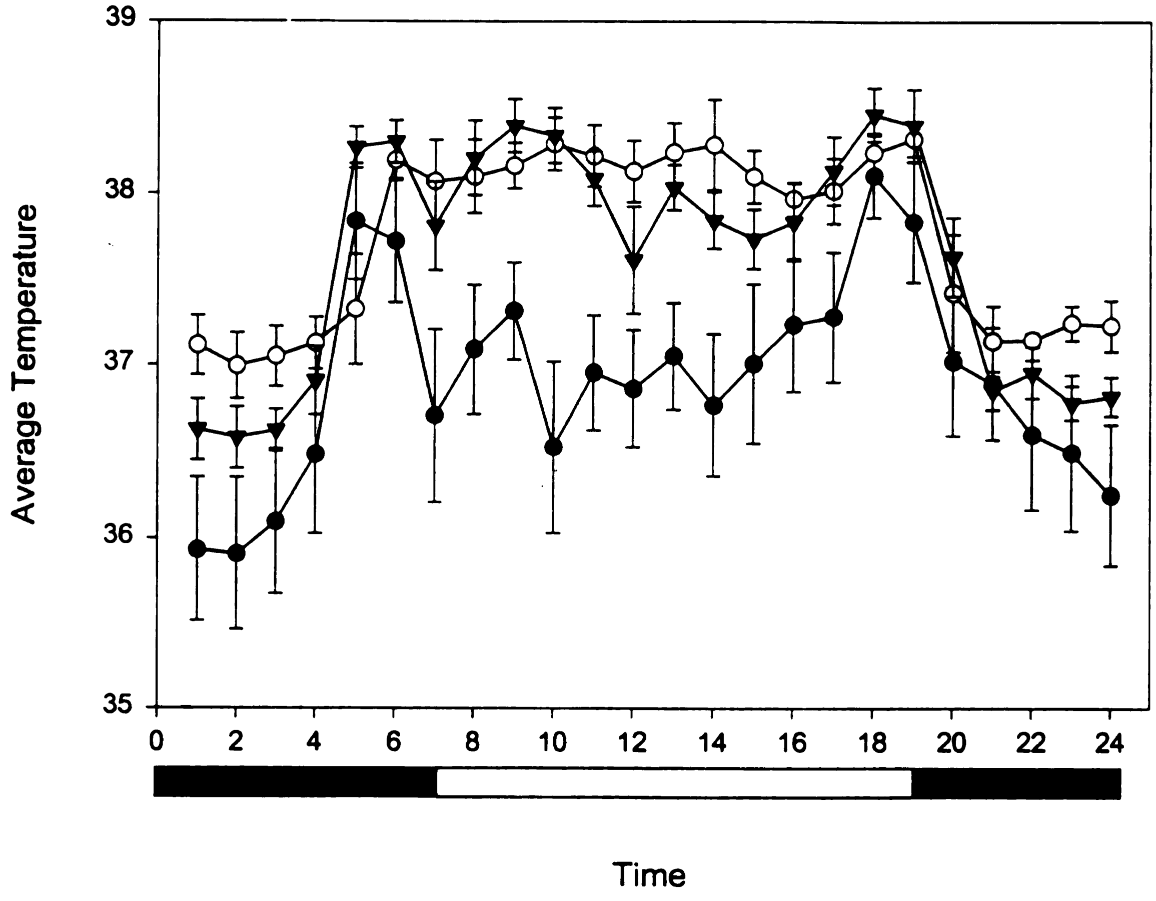

Figure 13. Average daily patterns of core body temperature and SEM for diurnal (open circles), intermediate (triangles) and nocturnal (dark circles) animals without a running wheel (phase 1).

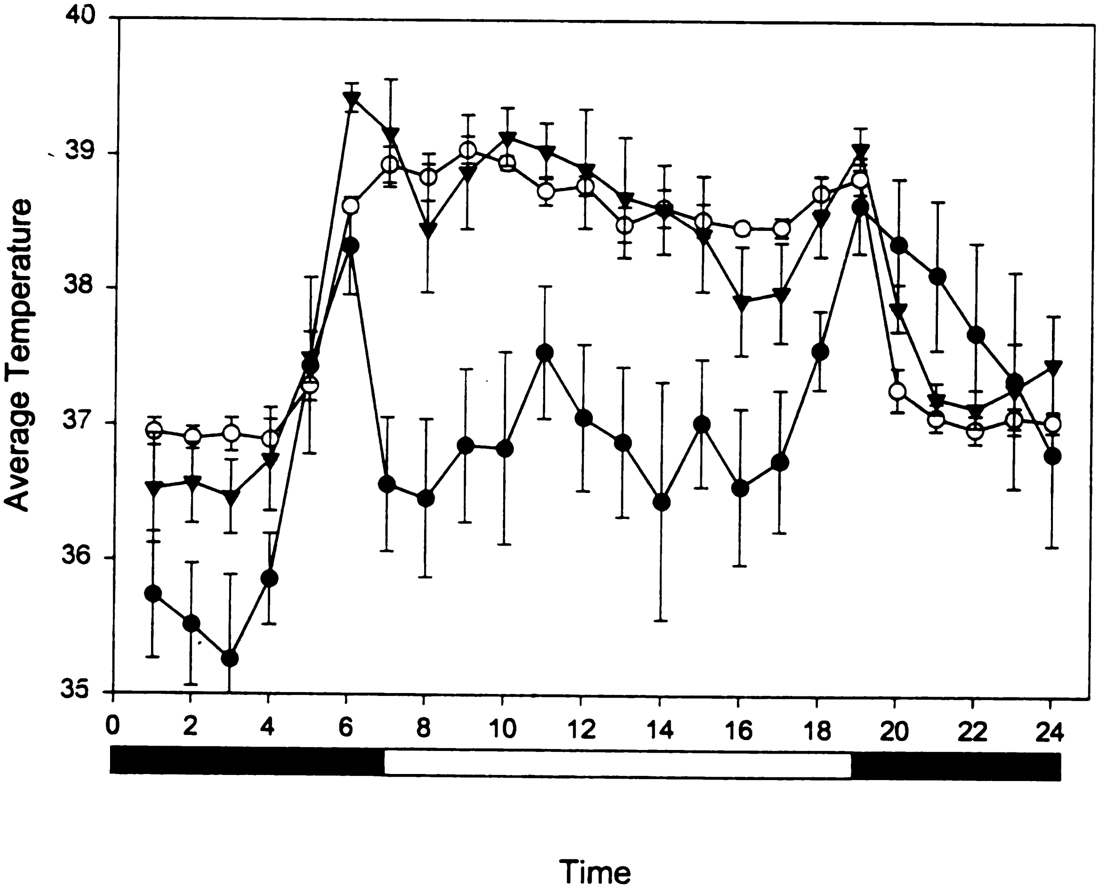

Figure 14. Average daily patterns of core body temperature and SEM for diurnal (open circles), intermediate (triangles) and nocturnal (dark circles) animals with a running wheel (phase 2).

temperature when running wheels were removed. Specifically, animals with diurnal patterns of wheel-running exhibited diurnal patterns of gross motor activity throughout the three phases of the study, while animals with nocturnal wheel-running patterns were diurnal without a running wheel (phase 1,3), but returned to a nocturnal pattern of general activity when a wheel was present (phase 2).

In all species examined, body temperature rhythms are generated by an endogenous circadian mechanism (Moore-Ede et al., 1982). My experiment took place in a light-dark cycle so I cannot say definitively if the body temperature rhythms of *A. niloticus* are endogenous or not. However, previous studies on other species have consistently demonstrated that the circadian rhythm in body temperature and locomotor activity are closely related (Renfinetti and Menaker, 1992). Peaks in body temperature always coincided with peaks in activity in all animals in this review. Body temperature rhythms were assessed in *A. niloticus* with and without running wheels, and compared between diurnal and nocturnal animals. In this study, I found that all *A. niloticus* express diurnal patterns of gross motor activity and body temperature in the absence of a running wheel. I also found that when housed without running wheels, animals originally classified as diurnal had relatively higher average body temperatures than did those classified as nocturnal (Figure 13). This is the only difference that has been found between these two groups of individuals in the absence of a running wheel.

CHAPTER 5

ACTIVITY PATTERNS OF *A. niloticus* IN THEIR NATURAL HABITAT

INTRODUCTION

Arvicanthis niloticus is an herbivorous murid rodent inhabiting dry savanna, woodland and grassland habitats in tropical Africa (Rosevear, 1969; Kingdon, 1974). Males and females have been observed to live in groups in equal numbers (Delany and Munro, 1985; Senzota, 1983) and associations have been described as random with respect to age and sex (Senzota, 1990). *A. niloticus* reside in underground burrows they construct at the bases of bushes, trees, rocks, banks, trash piles and termitaria (Delany and Neal, 1966; Packer, 1983; Senzota, 1983) and maintain runways that radiate outward from burrow entrances (Vesey-Fitzgerald, 1966; Senzota, 1990).

As described in the previous experiments, I have studied patterns of wheel-running, gross motor activity, and body temperature in members of a captive colony of *A. niloticus* descended from animals trapped in East Africa in 1993. In these laboratory animals, individuals express distinctly different diurnal or nocturnal patterns of wheel-running. The pattern expressed by an individual depends on the pattern expressed by its parents and on its sex. However, gross motor activity and the peak in body temperature are diurnal in all animals housed without running wheels, including those that are nocturnal with a wheel. This raises the question of whether any *A. niloticus* are nocturnal in nature, where running wheels are not available. To determine if diurnal and nocturnal *A. niloticus* actually coexist in a natural setting, and to systematically characterize their rhythms in the wild, I conducted a field study in the region from which the founders of this colony were collected, the Masai Mara National Reserve (MMNR), Kenya.

METHODS

From June 15- August 22, 1998, 20 colonies of *A. niloticus* were systematically sampled at MMNR for two periods of eight consecutive days approximately one month apart. During each of these two sampling periods, traps were set between 0700h-1900h or 1900h-0700h. Sherman traps baited with a teaspoon of powdered oats were placed at the entrances to animal burrows and in runways the animals had constructed. Two kinds of traps were used. The first was a standard 9 x 3 x 3.5 inch Sherman trap. The second was a Sherman trap with a timer mounted on the side of the trap that was attached to a button on the floor of the trap that was triggered by the opening and closing of the trap door (Barry et al., 1989). When the trap was set the time of day was recorded, the timer was set at 00:00 and the trap door was opened which depressed the timer button and started the timer's counter. When an animal entered the trap the door closed which released the button and stopped the timer. The timer, therefore, recorded the interval between when the trap was set and when the animal was captured. The time of day that the animal was trapped was determined to the nearest minute by adding the elapsed time to the time at which the trap was set.

Day and night trapping methods were different in two respects. First, traps were checked repeatedly during day trapping periods and only at the end of the period for night trapping. Traps were not checked at night because MMNR regulations precluded walking after dark when many large and dangerous predators (e.g. lions) were most active and difficult to see. During the day, however, traps were checked every hour between 1100h and 1600h in order to avoid animal mortality due to extreme heat. When animals were found between these hours they were identified and released, and the trap was removed. The second difference between day and night sampling protocols involved the deployment of non-timer traps during the day, but not at night. During the day, timer-traps (x= 8.5/ site) were set closest to burrow openings and a small number of non-timer traps (x= 3.6/ site) were placed around the periphery. These additional traps were needed during the day to ensure that traps would not be saturated before the sampling period ended. This was not an issue at night because so few animals entered the traps (see results). Furthermore, at night, when traps could not be checked, only timer-traps could provide information on the time the animal entered the trap. To account for this difference in trap deployment, analyses were done on timer-trap data exclusively as well as on the full data set. The distributions of activity that resulted from these two analyses were then compared.

Upon capture, the time the animal was captured was recorded. The first time an animal was caught it was anesthetized with the inhalant Metofane in order to keep it from struggling and inflicting harm to itself during marking. The animal was sexed, weighed, its physical condition noted (size of testes for males; pregnancy and lactation for females) and its ear marked with an ear-punch. Animals were then released at the site of capture. Animals were considered to be adults if they weighed >40 grams.

Data were analyzed using chi-square tests, and were considered significant when $p<0.05$.

RESULTS

One hundred seven *A. niloticus* were trapped at 20 different sites a total of 372 times. This number includes pilot data obtained while locating animal burrows. Seventyseven individuals were systematically captured 287 times using both timer and non-timer traps following the protocols described in the methods. The time of all of these trappings could be assigned to one of six four-hour intervals (2300h-0300h, 0300h-0700h, 0700h-1100h, 1100h-1500h, 1500h-1900h, 1900h-2300h). These data were used to determine the time of day at which animals were most likely to be captured overall, as well as to compare males with females, and adults with juveniles with respect to the time of day at which they were most likely to be captured. One hundred fifty-one captures of 56 individuals involved traps with timers in which the exact time of capture was recorded (mean number of timer-trap captures per animal= 2.66, SEM= 0.28). These 151 captures were used to analyze the distribution of activity at a finer scale as well as to compare timer-trap data to data that included non-timer trappings during the daytime.

During the field study it became light at approximately 0630h and dark at approximately 1915h. Almost all 287 *A. niloticus* trappings occurred during these daylight hours (Figure 15). All individuals trapped appeared to be diurnal. Only five out of 77 animals (two adult females, one adult male and two juvenile males) were captured after dark or before sunrise. Each of these five individuals were only caught once at these times, and all were also captured during the daytime (34 times for all five animals). Using data from both timer and non-timer traps, a comparison of the proportion of traps containing animals during the light versus the dark showed that animals were significantly more likely to be captured during the day than at night (x^2_2=150.6, $p<0.001$). When the 24-hour day was divided into four-hour intervals there was a significant difference between intervals with respect to the probability a trap would contain a grass rat (x^2_5=195.6, $p<0.001$) (Figure 15). Males and females did not differ with respect to the

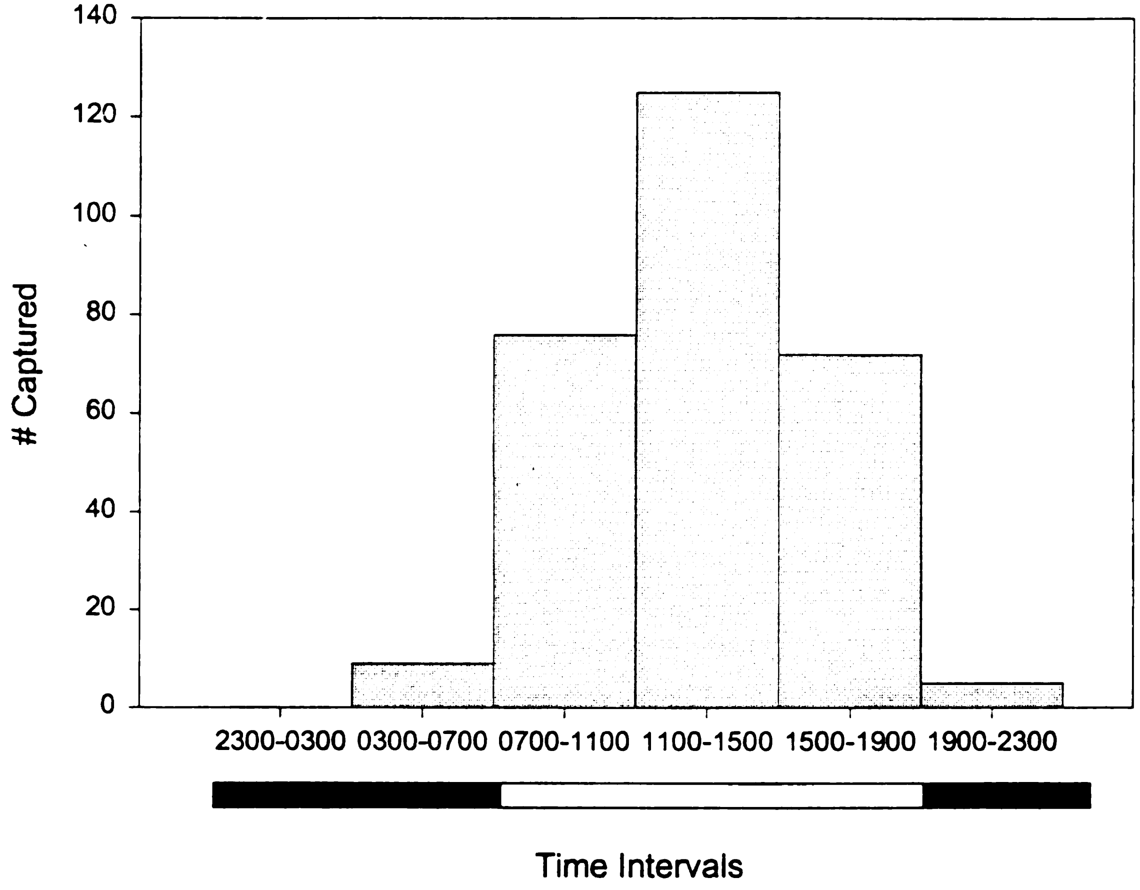

Figure 15. Total number of *A. niloticus* captured in timer and non-timer traps in each of six 4-hour intervals throughout the 24-hour day (sun rises 0630, sets 1915).

four-hour interval in which they were most often captured (x^2_5=0.67, NS) (Figure 16). There was also no difference between adults and juveniles in the four-hour interval in which they were most likely to be trapped (x^2_5=2.43, NS) (Figure 17). The fundamental pattern observed when only timer-trap data was examined (Figure 18) was the same as the pattern obtained from the combination of timer and non-timer trap data (Figure 15).

Occasionally, other species including shrews and various species of mice were captured at sites in which *A. niloticus* were also captured. However, *A. niloticus* were captured more frequently (287 trappings) than were members of all other species (49 trappings). In addition, all trappings of other species in *A. niloticus* study sites occurred during the night (Figure 18).

Although this study was not designed to elucidate social structure or population dynamics, some observations were consistent with the notion that *A. niloticus* live in social groups containing at least one adult male and female and their offspring. These animals were found living in underground burrows constructed under thorny bushes. Group size and makeup appeared to depend on the proximity of habitable bushes. At six sites, bushes were distinctly isolated from other bushes harboring *A. niloticus.* Animals trapped at these sites generally included a single adult male and female pair and juveniles presumed to be their offspring (Table 2). When these sites were sampled again approximately one month later the original animals were usually still present and often a second group of offspring was present as well, suggesting that the population was expanding at some sites. This was most clearly seen at bushes that were isolated from other bushes, and a clear assessment could be made as to which animals were living there (Table 3). At six trapping sites where bushes were in close proximity more animals were

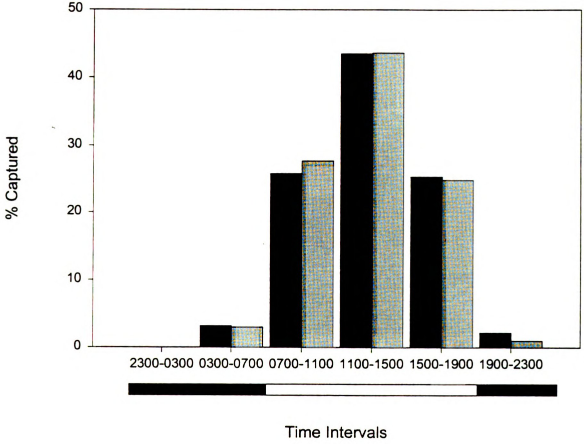

Figure 16. Percent of male (black bars) and female (gray bars) *A. niloticus* caught in timer and non-timer traps in each of six 4-hour intervals throughout the 24-hour day (sun rises 0630, sets 1915).

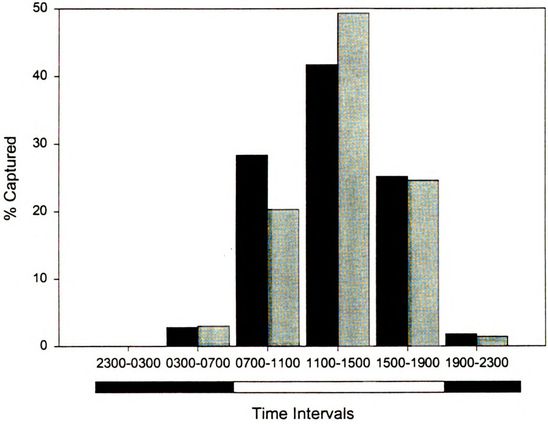

Figure 17. Percent of adult (black bars) and juvenile (gray bars) *A. niloticus* captured in timer and non-timer traps in each of six 4-hour intervals throughout the 24-hour day (sun rises 0630, sets 1915).

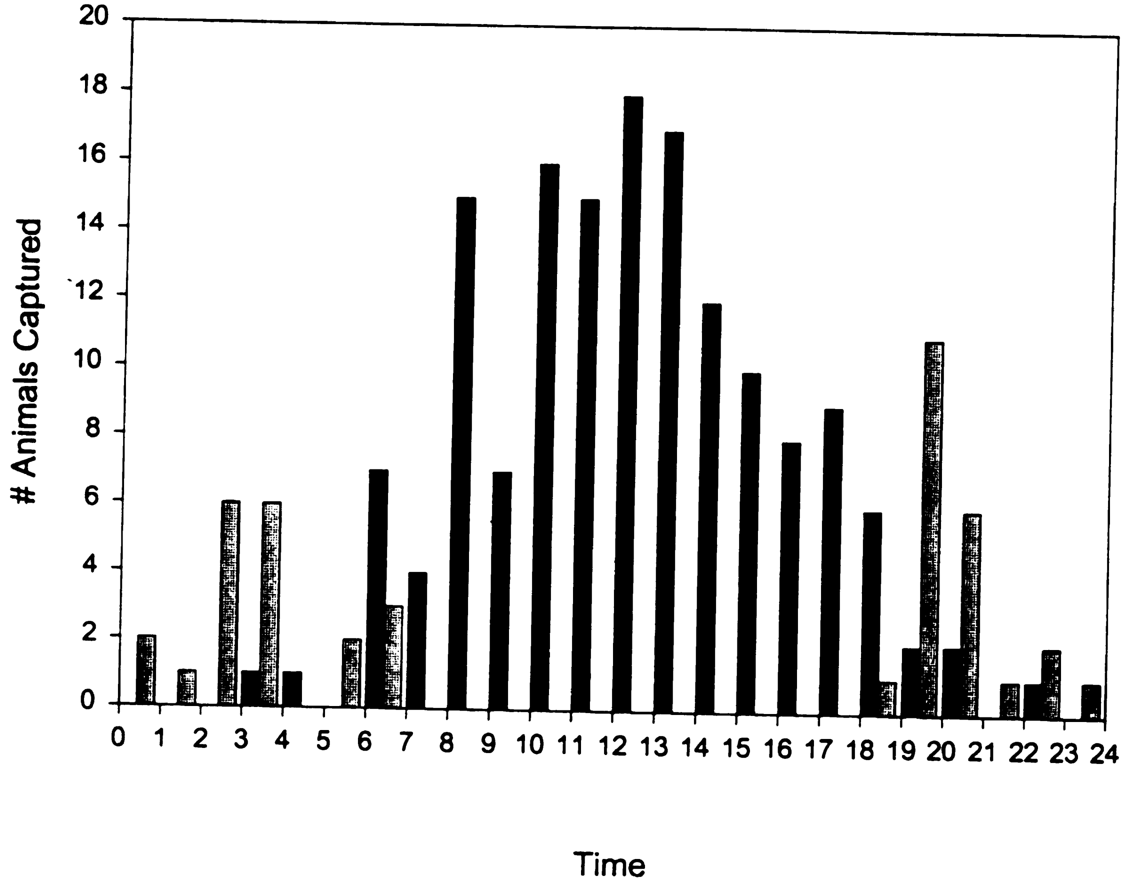

Figure 18. Number of *A. niloticus* (gray bars) and other rodent species (black bars) captured in timer-traps in each hour of the 24-hour day (sun rises 0630, sets 1915).

Table 2. Average Number of Animals Captured Per Site.

Trapping Site:	Adult Males (SEM)	Adult Females (SEM)	Juvenile Males (SEM)	Juvenile Females (SEM)
Isolated (n=6)	1.2 (0.2)	0.8 (0.2)	1.0 (0.4)	0.7 (0.3)
Interconnected (n=6)	4.2 (0.7)	2.0 (0.8)	1.3 (0.5)	0.8 (0.3)

Table 3. Number of Animals Captured in Period 1 and Period 2 at Isolated Sites.

	Adult Male	Adult Female	Juvenile Male	Juvenile Female
Period 1	6	4	4	1
Period 2: # still present	5	4	3	1
Period 2: # new animals	0	0	2	3

captured (Table 2). At these sites, animals were observed moving from bush to bush, and it was not possible to determine under which specific bush each animal was living. At the remaining eight sites too few animals were captured to conclude anything about their group makeup. Two animals were captured in the same trap on six occasions. The pairs were as follows: adult male and adult female (twice), adult female and unidentified adult, adult male and juvenile male, two juvenile males and two juvenile females. Based on these findings animals appeared to associate in a variety of age and sex combinations.

DISCUSSION

A. niloticus are clearly diurnal in their natural habitat in the Masai Mara National Reserve in Kenya. These animals were captured throughout the daylight hours and very rarely during the night (Figures 15 and 18). Five *A. niloticus* were captured, each on one occasion, in the late evening or early morning hours, and each of these animals was repeatedly captured during the day. One potential problem interpreting these results is that sampling methods for day versus night trapping were somewhat different. This was necessary because at night, a variety of dangerous animals (e.g. lions) are active, making it dangerous to check traps in the dark. During the day, traps had to be checked at hourly intervals because the extreme heat would have killed animals had they been left in traps. In addition, more traps were set during the day than at night to avoid trap saturation which would have biased against sampling times late in the light period. Trap saturation was avoided by the use of a small number of non-timer traps around the periphery of the burrow systems during the day. Timer-traps in daytime and nighttime sampling periods were set in the same places at the entrances to burrows. One way to try to account for these differences in sampling methods was to compare the distribution of activity including and excluding non-timer trap data. There was no difference in the resulting distributions of activity when the data were compared (Figures 15 and 18).

This discrepancy with regard to the activity pattern of this species may be due to the taxonomic confusion for the genus *Arvicanthis*. Different authorities have described from one to five different species throughout the range of this genus (Misonne, 1974; Corbet and Hill, 1980; Honacki et al., 1982). Because of this, studies of these animals in other regions of Africa may not be describing the same species characterized here. Specifically, the papers that describe *A. niloticus* as primarily nocturnal (Schumutterer, 1969; Ghobrail and Hodeib, 1982) both took place in Sudan and might actually be a different species from the animals in southern Kenya that I have found to be diurnal. In order to be confident the lab and the field animals were from the same species, my field study was done at the same sites from which our lab colony was collected. This was the first study done with the specific purpose of describing the activity pattern of this species.

The diurnal pattern of activity in *A. niloticus* is unusual and quite different from the nocturnal pattern expressed by most murid rodents. One possible explanation for *A. niloticus'* diurnal pattern of activity may be predator avoidance. *A. niloticus* are preyed upon by numerous species of birds, reptiles and mammals. The following have all been observed to be their predators: spitting cobra (*Naja nigricolis*), black-backed jackal (*Canis mesomelas*), long-crested hawk eagle (*Elanus caeruleus*), black headed heron (*Ardea melanocephala*) and dwarf mongooses (*Helogale parvula*) (Packer, 1983). These predators have different patterns of activity which may make *A. niloticus* vulnerable to predation throughout most of the day and night. *A. niloticus* may feed during the middle of the day because this is a time of rest for numerous birds of prey and small carnivores

(Kingdon, 1974).

Group living is also uncommon in murid rodents, yet *A. niloticus* living in isolated bushes generally consisted of 'family groups' of an adult male and female pair and juveniles presumed to be their offspring (Table 2). In addition, these pairs were often found together with a second group of offspring when sites were re-sampled approximately one month later (Table 3). This raises the possibility that this species may form pair bonds for at least short periods of time. Many studies of the social organization of various species of voles have led to the hypothesis that the nature of the habitat and population density affect an animal's mating system (Getz, 1978). Prairie voles, *Microtus ochrogaster*, which live in large, continuous habitats in which they compete for resources with other larger herbivores, have been described as monogamous at low population densities and in the laboratory (Thomas and Birney, 1979.) Getz (1978) however, stated that at high population densities this probably would not hold true. *M. californicus*, another species of vole that lives in habitats similar to those of prairie voles also tends toward monogamy (Lidicker, 1979). *M. pennsylvanicus*, however, which live in smaller, more isolated, ephemeral areas are described as polygynous (Tamarin, 1984). *A. niloticus* live in a habitat in which they, like *M. ochrogaster*, are in constant competition with other large herbivores for resources and may also tend toward monogamous behavior, at least when population density is low (personal observations).

This field data provides no evidence of the nocturnal pattern evident in the wheel-running behavior of some *A. niloticus* in captivity (Katona and Smale, 1997; Blanchong et al., unpublished). This apparent discrepancy could be explained in a number of ways. Perhaps the nocturnal wheel-running pattern, which was rare in wild-caught animals

(Katona and Smale, 1997), reflects a temporal variant which is rare in wild-populations. If I had sampled a larger population, or if I had sampled at different seasons or at different phases of a population cycle, I might have detected nocturnal individuals. Alternatively, the nocturnal patterns of wheel-running seen in some captive *A. niloticus* may not reflect patterns of general activity that these animals would exhibit in free-living conditions. Wheel-running has often been used as an easy method of assessing patterns of activity in rodents and it generally provides accurate information as to whether rodents are diurnal or nocturnal (e.g. DeCoursey, 1973; Johnston and Zucker, 1983; Kavanau, 1971; Kenagy, 1978; O'Reily et al., 1986; Rowsemitt,1986). However this may not always be the case (Kennedy et al., 1994; Lewell 1974; Mather, 1981; Roper, 1976; Sherwin, 1998; Shindoa et al., 1987; Shindoa et al., 1988; Shindoa and Miura,1994). The current field data reinforce the view that one should not assume that wheel-running provides accurate information as to whether animals are diurnal or nocturnal in nature.

CONCLUSIONS

In summary, I have demonstrated that individual *A. niloticus* differed with respect to their patterns of wheel-running, and that these differences were influenced by parentage and by sex. However, when a running wheel was not present the pattern of activity for all animals was almost exclusively diurnal. A running wheel caused a shift in the timing of activity of only some individuals, specifically those that expressed nocturnal wheel-running patterns, but diurnal gross motor activity patterns. This may be related to the higher average body temperature found for animals classified as diurnal versus those that were nocturnal in the absence of a running wheel. In addition, I found that all animals observed in a field setting showed diurnal activity. Surprisingly, the pattern of diurnal activity exhibited by *A. niloticus* in the field differed dramatically from the diurnal pattern of activity seen in the laboratory. In the field, animals' activity peaked in the middle of the day while in the lab diurnal animals showed a more crepuscular pattern in which there were two peaks of activity, one shortly after the lights came on and the second shortly before the lights went out.

This combination of results provides a new way of looking at possible mechanisms underlying individual differences in wheel-running patterns in *A. niloticus*. It raises the possibility that some individuals shift to the nocturnal pattern because they run in their wheels more than other individuals. That is, high levels of running may feed back to change the basic waveform of the rhythm. This hypothesis is consistent with the positive correlation between the number of hours an animal runs after lights-out and the overall level of wheel-running (e.g. Figure 5). This correlation, however, is far from perfect, raising the possibility that some individuals may be more sensitive than others to feedback effects of wheel-running on the pattern of rhythmicity. The mechanisms underlying the influence of parentage and sex on rhythm patterns (Chapter 2) could involve effects of these variables on either the levels of wheel-running, or sensitivity to feedback effects of wheel-running.

Feedback effects of wheel-running on circadian rhythms have been documented in a number of nocturnal rodents (Pratt and Goldman, 1986; Mistleberger, 1991; Yamada et al., 1988; Reebs and Mrosovsky, 1989). Most of this work has focused on properties of rhythms under free-running conditions. For example, in hamsters, wheel-running in response to a novel wheel phase shifts the endogenous rhythm in a precise phase dependent manner (Reebs and Mrosovsky, 1989). These shifts only occur in animals that run above threshold levels when presented with the novel wheel (Mrosovsky and Biello, 1994). Such effects were not found in *A. niloticus* when animals kept in constant darkness were enclosed in novel wheels for three hour periods at a variety of circadian times (Smale, unpublished observations). A dramatic effect of wheel-running on the pattern of entrainment was recently reported in *Octodon degus* (Kas and Edgar, 1998), a normally diurnal species (Fulk, 1976). In this species, animals that were diurnal without a wheel became more active at night than during the day when given a running wheel (Kas and Edgar, 1998). The effect of running wheels on the pattern of activity thus appears to be somewhat similar in *A. niloticus* and *O. degus*, two rather distantly related rodents that independently evolved a diurnal lifestyle.

From the point of view of trying to understand animals in their natural habitat, the effect that wheel-running has on the fundamental pattern of activity in nocturnal *A. niloticus* is a puzzle. The switching system that responds to wheel-running in the laboratory could theoretically have evolved to respond to some other stimuli in nature. It could reflect some ancestral nocturnal mechanism that is no longer active in modern *A. niloticus* in their natural habitat, or it could be a byproduct of selection for some other characteristic(s). My field study provides some evidence against the first of these hypotheses. I found no evidence that any modern *A. niloticus* are nocturnal. In fact, in the field, these animals appear to be even more diurnal and less crepuscular than they are in the lab. These animals were rarely trapped before sunrise, their activity progressively increased until it peaked in the middle of the day, and was essentially over by sunset. Although the details of the activity pattern deduced from trapping are unlikely to perfectly reflect natural activity patterns (Hicks et al., 1998), the huge difference between day and night trapping results provides clear evidence that these animals are diurnal (Figure 15). Of special note is the dramatic difference between the number of animals trapped in the 5 hours after traps were set in the morning compared to evening. However, it remains possible that I would have detected nocturnal patterns of activity in some animals had I sampled a larger population, or if I had examined animals at a different season, or when the population density was dramatically higher or lower.

In summary, a nocturnal pattern of wheel-running in captive *A. niloticus* was prevalent in offspring of nocturnal parents, and was more common in females than in males. However, a nocturnal pattern was only expressed in these animals when a running wheel was present. Nocturnal wheel-runners were indistinguishable from diurnal ones when other behaviors and body temperatures were monitored in the absence of a running wheel. In captivity, it therefore appears that running in a wheel feeds back to change the mechanisms responsible for the temporal organization of wheel-running, as well as other behaviors. The role that this system might play in the regulation of rhythms in their natural habitat is unclear. The activity pattern deduced from two months of trapping a free-living population of *A. niloticus* suggests that all individuals are diurnal.

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