Chapter **12**

Chemical Mutagenesis and Screening for Mouse Mutations with an Altered Rest–Activity Pattern

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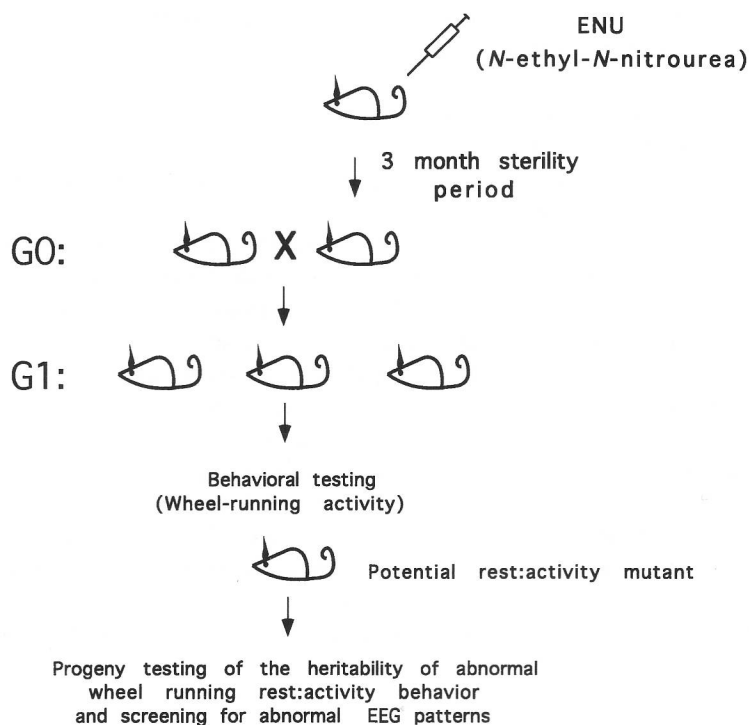
Contents

I.   Introduction

The availability of model organisms harboring mutant variants of genes involved in any biological process is essential for understanding the complexity of these pro-

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cesses at a molecular and biochemical level. A dream of many neurobiologists, including sleep researchers, is to identify and isolate a candidate gene for a controlling element of a neurobiological system or a behavior, based on previous physiological, anatomical, or pharmacological studies. However, the final role and function of a particular gene can only be ascertained by the analysis of the aberrant phenotype in mice with a null mutation of this gene. The mouse is particularly useful as a model in such studies because of the potential of using targeted mutagenesis in embryonic stem cells.[1,2] Although genetic differences have been found in sleep parameters such as total and paradoxical sleep times, circadian rhythms of sleep and the diurnal ratio of total sleep,[3-6] the identification of single-gene mutations affecting sleep is hampered by the invasive techniques necessary to study sleep correlates. Nevertheless, the recent analysis of prion protein-deficient mice would support the argument that a single gene can affect specific sleep parameters.[7] Due to the paucity of candidate genes for key regulators of sleep–wakefulness, we are using random mutagenesis to screen for potential sleep mutants among lines of mice with abnormal rest:activity cycles. This approach involves mutagenesis of the whole genome with a potent chemical mutagen, such as *N*-ethyl-*N*-nitrosourea (ENU),[8,9] and the generation of a large number of progeny (Figure 12.1). Subsequent screening for the presence of behavioral anomalies may reflect the disruption of gene(s) with a general effect on daily activity cycles (for a review see reference 10).

This chapter explores current and potential applications of random chemical mutagenesis to identify novel rest:activity mutations in the mouse. We describe the mutagenesis procedures and behavioral assays and constraints of genetic analysis in the study of rodent behavior. Fully computerized and automated online systems for monitoring wheel-running behavior can be used to simultaneously screen a large number of progeny of mutagenized mice. Analysis of wheel running activity records in light:dark (LD) conditions may provide a prescreen for potential sleep–wake mutants. Based on the statistical analysis of behavioral parameters which can be extracted from the activity records of a large number of tested progeny, several potential mutants with a strikingly different behavioral profile from the norm can be identified. The heritablity of alteration in wheel running rest:activity behavior in these candidates can confirm that the behavioral anomaly is due to a genetic defect. Although monitoring wheel running activity may be sufficient to identify novel mutations affecting circadian behavior,[11,12] potential mutants will have to be examined by electroencephalographic and electromyographic recording and sleep-deprivation assays in order to test whether they affect arousal, wakefulness, or sleep (Figure 12.1). Circadian rhythms of sleep–wakefulness in general covary with wheel running rest:activity cycles. However, concomitant monitoring of EEG recordings and wheel-running has shown periods of "quiet" wakefulness during the wheel running rest phase and fragmented REM and NREM sleep during the active phase of the circadian wheel-running cycle.[13,14] In addition, exercise[15] or access to activity wheels[16,17] can modify sleep patterns in mice, indicating that a prescreen could potentially identify mutations that disrupt the coupling between locomotor activity and sleep homeostasis.

In several model organisms, such as *Drosophila melanogaster, C. elegans,* and zebrafish (references 18, 19, 20, 21 and other papers in *Development,* Vol. 123), large-

ENU
(*N*-ethyl-*N*-nitrourea)

3 month sterility
period

GO:

G1:

Behavioral testing
(Wheel-running activity)

Potential rest:activity mutant

Progeny testing of the heritability of abnormal
wheel running rest:activity behavior
and screening for abnormal EEG patterns

FIGURE 12.1

ENU mutagenesis. Young (8 to 10 weeks) C57BL/6J male mice are injected intraperitoneally with the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU). After a period of sterility (on average 12 to 15 weeks), mutagenized (G0) males are mated with C57BL/6J females. Progeny from this cross (G1), harboring many different induced mutations on one of their parental chromosomes, are screened for potential dominant or semidominant rest:activity mutations. Families of mice that are selected on the basis of aberrant wheel-running activity can then be tested for the heritability of the abnormal behavior and can be used to define the physiological and anatomical basis of the abnormality.

scale mutagenesis experiments for recessive and dominant mutations involve screening thousands of progeny of mutagenized individuals for the desired mutant phenotype in subsequent generations. In the mouse, extensive mutagenesis screens have not been performed due to the expense of mouse maintenance. Therefore, current efforts to recover a large number of recessive mutations are restricted to relatively short stretches of the mouse genome[22-24]; or are aimed at the identification of recessive visible mutations in a known gene.[25] However, a one-generation screen for dominant phenotypes may also provide an efficient way to recover novel mutations. For example, *Clock*, the first mutation which controls circadian behavior in the mouse,[11] and *Wheels*, a developmental mutation which alters circadian activity and responses to light (references 12, 26, and Nolan, Alavizadeh, Lo, and Bucan, unpublished), were identified in screens for mutants with a dominant mode of inheritance.

The frequency of mutations at any given gene induced by ENU is 1.5×10^{-3} per locus, per gamete[27] which, in effect, means that 1 of 750 progeny of mutagenized individuals should harbor a point mutation in any gene of interest. In the

one-generation screen for dominant mutations, populations of progeny for testing are generated by mating mutagenized mice (males) with wild-type females. However, in a screen aimed at the identification of recessive mutations, where the mutant phenotype is detectable only in individuals with two copies of the mutated gene, a complex three-generation breeding scheme has to be used.

The ultimate goal of the mutagenesis screen described in this chapter is to identify single gene mutations with a profound effect on rest:activity behavior. However, in a dominant screen for behavioral mutants even mutations causing minor, but stably inherited, changes will be of great value; subtle changes in heterozygotes may be associated with a more severe phenotype in homozygotes, as shown for the *Clock* mutation.[11] To facilitate genetic analysis of these subtle mutations it is important to maintain them in a homogeneous genetic background; both mutagenized and wild-type progenitors (GO in Figure 12.1) should be of the same inbred strain. Analysis of a behavioral parameter, such as circadian period (τ_{DD}), in a large number of genetically homogeneous progeny of mutagenized mice will give a continuous distribution of τ_{DD} values, where the individual outliers may represent either 1) normal variance in the population, 2) an ENU-induced single gene mutation, or 3) may be caused by multiple mutation events in several genes. Mating of individuals with extreme behavioral phenotypes with wild-type mice will provide the critical test of heritability of the mutant phenotype, as well as its mode of inheritance

II.   Methodology

A.   *Mutagenesis Protocol and Breeding Scheme in a Screen for Dominant Mutations*

The procedure for chemical mutagenesis involves administration of the mutagen *N*-ethyl-*N*-nitrosourea (ENU) to 7 to 10-week-old mice with three weekly doses of 100 mg/kg by intraperitoneal injections. A repeated ENU administration protocol has proven to be highly efficient and ensures a greater survival rate for mutagenized animals.[27] Since ENU is mutagenic for mouse spermatogonial stem cells, successfully mutagenized animals will be rendered temporarily sterile. In a typical case, new rounds of spermatogenesis will cease for 10 to 15 weeks, after which surviving mutagenized spermatogonial stem cells repopulate the testis. At four weeks after administration of the mutagen, ENU-treated males are caged individually with two fertile females of the same genetic background (males remain fertile for a few weeks after ENU application). If females become pregnant during this transitional period, it will be an indication that the mutagenesis was unsuccessful and treatment will be repeated. However, restored fertility after a three-to-four month sterile period will likely indicate efficient mutagenesis. Based on the results with mutations identified to date,[28] we found that by collecting and analyzing no more than 50 to 75 generation 1 (G1) progeny from a single mutagenized male, we have a low chance of recovering the same mutation multiple times.

B.   *Behavioral Assays*

Mice, 8 to 14 weeks old, are housed individually in cages equipped with running wheels, with food and water freely available. Cages are maintained in light-tight, ventilated chambers (10 cages to a chamber), with timed lighting. Each animal's wheel-running activity is monitored continuously, via a microswitch located on the outside of each cage. Microswitch closures for 120 cages are automatically recorded and stored on an IBM computer-based data acquisition system (developed by Ralph Mistlberger, Simon Fraser University, British Columbia, Canada). Behavioral analysis is conducted under LD (12 hours light:12 hours dark) conditions for 7 days to assess the ability of mice to synchronize or entrain to the LD cycle, followed by 3 weeks of monitoring in constant dark (DD) conditions to screen for "clock gene" mutations (i.e., mutations which either shorten or lengthen the endogenous circadian period).

C.   *Data Analysis of Wheel-Running Activity by the* Circadia *Program*

A Macintosh-based interactive program, *Circadia*, is used to rapidly visualize, analyze, and quantitate the rhythmicity and activity of mutant mice. Circadian rhythms can be characterized by three quantitative parameters: period, phase, and amplitude.[29] Changes in these parameters may reveal an altered central circadian clock, altered coupling between ambient light cycles and the clock (e.g., due to changes in visual system sensitivity), or altered coupling between the clock and the physiological and behavioral processes it regulates (e.g., sleep and activity cycles). *Circadia* employs standard plotting styles and time-series algorithms that have been extensively used and validated by the circadian rhythm community.

For nocturnal mice, entrained and free-running periods should be assessed under both an ambient light-dark (LD) cycle and under constant-dark conditions (DD) (Figure 12.2 A). Wheel running activity can be visualized through ten different plotting styles with *Circadia*, from a spreadsheet view of individual data points to a strip-chart format. *Circadia* can calculate periodicity from as little as 7 to 10 consecutive days of data using several algorithms: χ^2 periodogram, which estimates period by minimizing variance when averaging data across cycles,[30] autocorrelation,[31] linear fitting to activity onsets or offsets, cosinor analysis (estimating the coefficients of a sinusoidal function by least squares fit to the entire data set).[32] These routines can be used to screen for period changes, including significant deviations of the period from 24 h under LD, significant lengthening or shortening of the period under DD, changes in ultradian or infradian rhythms, and arhythmicity.

The phase relationships of circadian rhythms are calculated with reference either to external cycles (i.e., the time of activity onset and offset relative to the time of lights-on or lights-off) or by the phase of internal rhythms relative to a reference phase marker; for example, the timing of daytime activity bouts (internal rhythms) relative to the onset of subjective night (reference phase). *Circadia*'s autocorrelation and cosinor analyses can explicitly calculate phase parameters for circadian rhythms,
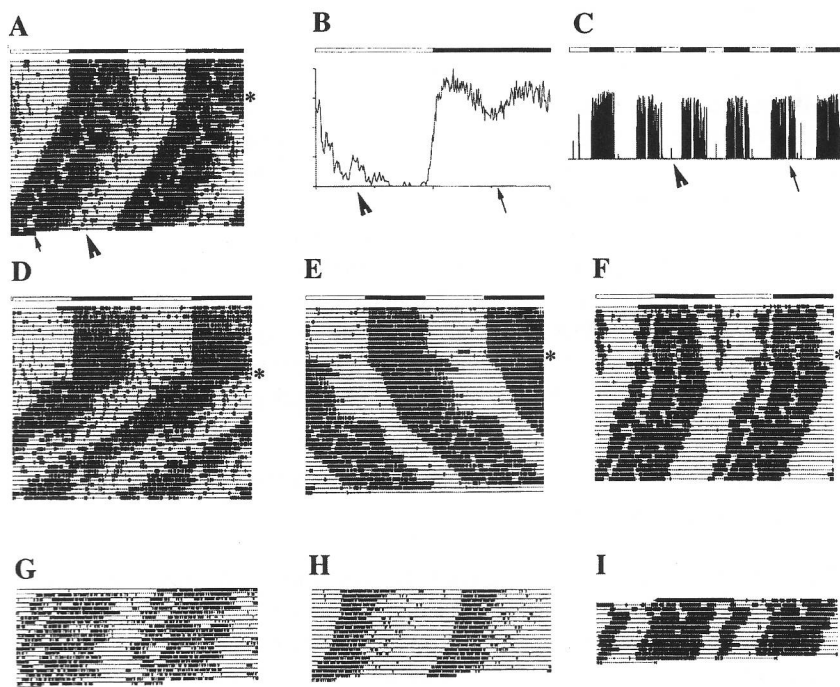
FIGURE 12.2

Wheel-running activity patterns in a typical C57BL/6J mouse and in mice with abnormal rest:activity parameters. Typical activity records for a C57BL/6J mouse A-C. Expression of wheel-running data in all three formats may be useful in the identification of potential rest:activity mutants: **A)** Double-plotted actogram raster plot of wheel-running (indicated by deflections from baseline) over 40 consecutive days under LD (days 0 to 10) and DD conditions. Dense horizontal light and dark bars represent periods of light and dark in LD, and the asterisk represents the day of transition from LD to DD. Variations in the amount, bout-distribution, periodicity, and phase of wheel-running activity are immediately obvious by visual inspection. Note the shortening of circadian period under DD, the greater precision of activity onset compared to activity offset, the brief period of inactivity in the middle of subjective night (arrow), and the short burst of activity in early subjective day (arrowhead). The ability to generate raster plots of the activity patterns of large numbers of mutants rapidly may be sufficient to identify qualitatively obvious mutants, but the statistical analysis of endogenous variation and the detection of significant changes requires additional quantitative computational analysis. **B)** The mean activity waveform (circadian time vs. mean revolutions/5 min bin) of the last 30 days under DD of the activity record in (A) averaged at the free-running period. The intersection of x and y axes represents circadian time 0 (CT0). The mean waveform is the first step in quantitating activity patterns, followed by determining period by χ^2 periodogram and rest:activity ratios by bout analysis. Arrows as in (A). **C)** The same activity record as (A) plotted in strip-chart format, as clock-time vs. revolutions/5 min bins. Although circadian parameters are less obvious, the strip chart reveals the consistent amplitude of wheel-running (~300 revolutions/5 min) during almost all bouts of activity, as well as periods of inactivity during subjective night and bouts of activity in subjective day. Arrows as in (A). **D)** Double-plotted actogram of a representative animal with a short circadian period (22.61 h) in LD and DD conditions. The asterisk represents the time of transition from LD to DD. Activity onsets are similar to (A) in LD conditions, but note the masking effect seen upon entry into DD, high levels and fragmentation of activity in subjective day, and the earlier daily onset of activity in DD relative to (A). **E)** Double-plotted actogram of a representative animal with a circadian

but are computationally intensive. Reliable phase estimates may be rapidly calculated in *Circadia* by the automatic detection of activity bout onsets; the timing of activity onsets or offsets may then be directly compared to the phase of the light cycle. Generating mean daily activity waveforms averaged and plotted at the entrained or free-running period also makes changes in circadian phase immediately obvious (Figure 12.2 B).

The amplitude of a circadian rhythm is estimated by the number of wheel-revolutions per bin at different phases of the circadian cycle, and by the total duration and amount of wheel-running activity. *Circadia* can directly quantitate wheel-running within user-specified blocks of the daily cycle. For example, the number of revolutions can be calculated for a large number of mice for each quarter of the circadian cycle, to determine if there are significant changes in late subjective night or subjective day activity, or overall rest:activity ratios. *Circadia* can perform automatic bout analysis to detect ultradian rhythms in activity timing that may be modulated by the circadian system. The initial screening may reveal alterations not in circadian rhythmicity *per se,* but in locomotor activity that would require noncircadian analyses (e.g., sleep analysis or open-field activity). Dissecting the mutational effects on locomotion and circadian rhythms is complicated by the feedback effect of activity on the circadian clock.[16]

D.   *Selection of Potential Rest Activity Mutants*

To identify potential mutants, wheel-running activity of progeny of ENU-treated mice is examined in LD and DD conditions. Almost all G1 progeny exhibit robust activity, typical of most inbred mouse strains.[33,34] Based on the previously reported values of 23.7 ± 0.17 hours for the τ_{DD} of circadian activity in C57BL/6J mice,[11,12,34] any mouse

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FIGURE 12.2 (Continued)

period greater than 24 h. Asterisk as in (D). Activity onsets are similar to (A) in LD conditions with a later daily onset of activity in DD relative to (A). No masking effect is evident and activity in subjective day is lower than in (A). Note also the periods of inactivity in subjective night. F) Double-plotted actogram of a representatvie animal with an advanced phase of wheel-running activity. Asterisk as in (D). Note in LD, wheel-running activity starts approximately 3 to 4 h prior to lights-off and the unusual activity onset at lights-on. The circadian period for this animal is comparable to (A) although there appear to be two activity onsets. Wheel-running activity is relatively robust, although there are brief periods of inactivity in subjective night. Subjective day activity is low. G) Double-plotted actogram of a representative animal with a long wheel-running activity phase (α, 18.6 h) in DD. Note that although the time between activity onsets and offsets is greater than in (A), the amplitude of wheel-running activity bouts is somewhat lower with fragmentation of wheel-running activity throughout the subjective night period. Circadian period is within the normal range for C57BL/6J mice. H) Double-plotted actogram of a representative animal with a short α (9.5 h) in DD. Circadian period is within the normal range for C57BL/6J mice. Activity in subjective night is robust, with several short bouts of activity in subjective day. I) Double-plotted actogram of a mouse in DD showing an unusual burst of activity in the subjective day period. Circadian period and activity levels in subjective night are within the normal range for C57BL/6J mice. Although it is usual to see bouts of wheel-running activity in mice at the beginning of subjective day, the activity seen here is in the middle of subjective day and is more robust than in the typical case.

with a τ_{DD} shorter than 23.10 or longer than 24.30 hours is selected for further analysis. Activity records for two mice that display significant shortening and lengthening of circadian period are illustrated in Figure 12.2 D and E. A visual observation of the onset of activity in relation to the offset of light in LD conditions allows selection of potential mutants with an early activity phase, where onset of activity precedes the offset of lights (Figure 12.2 F). Based on our data, some nongenetic factors, such as age or light intensity, may influence the precision of activity onsets.

Locomotor activity records of progeny of mutagenized mice can be analyzed to determine the variation in the length of the activity phase (α). Figure 12.2 G and H illustrate the activity records of two mice with extreme long (18 hours) or short (9 hours) activity phases (α), while circadian periods for both mice are within the normal range. Activity record Figure 12.2 I shows an individual characterized by an additional block of activity during the subjective day (under DD). Although activity at the beginning of subjective day is often noted in C57BL/6J mice, bouts of activity for this mouse occur with extreme regularity, are robust, and occur in the middle of subjective day. The circadian period for this mouse lies within the normal range.

Determining the basic circadian parameters of period, phase, and amplitude is only the beginning of characterizing a circadian system. Mutants which display hereditary alterations of greater than 2.5 SD in these parameters are subjected to more extensive, higher-resolution probing of the circadian clock. Variations in phase or levels of nocturnal and diurnal activity are behavioral variables which can be detected by monitoring robust wheel-running activity, and are relevant for the selection of candidate sleep mutants. Once potential mutants have been identified, more specialized real-time automated sleep scoring can be accomplished with available microcomputer systems.[35]

E.   *Initial Genetic Analysis of Selected Mutants*

The next step in the characterization of selected candidates for mouse mutants with altered rest:activity behavior is a series of genetic crosses to test the following: 1) whether the mutant phenotype is transmitted to the next generation; 2) whether the mutant phenotype is due to a single gene mutation; 3) the mode of inheritance and phenotype of homozygotes; and 4) the influence of genetic background on the mutant phenotype.

The heritability of the abnormal behavior is the critical step in the characterization of potential mutants and is tested by mating the potential mutant to a wild-type mouse of the same genetic background to eliminate the influence of other genetic elements that can mask the effect of the induced mutation. In addition, each potential mutant is bred to a different inbred strain that displays similar behavioral characteristics as the original mutagenized strain. This cross will provide F1 progeny for a backcross, which will be necessary for genetic localization of the novel locus associated with the behavioral anomaly. In both crosses, the ratio of mice with altered behavior compared with the total number of progeny examined will indicate whether the inheritance follows Mendelian ratios for single-gene inheritance of a dominant trait (i.e., 50% mutant phenotype: 50% wild type based on a dominant gene). The

precise ratio of mutant to normal progeny will depend on the penetrance of the mutant phenotype and/or on the relative difference between the mean value for the behavioral parameter in the particular inbred strain and that for the mutant progeny.

Although we screen progeny of mutagenized mice for a particular phenotype — altered rest:activity cycles — it is important to keep in mind that an individual sperm from a mutagenized male contains many mutational events, some of them neutral, some causing other visible phenotypes, and some that cause lethality in homozygotes. With each round of mating to a wild-type mouse (outbreeding), recombination between the mutagenized and normal genome will take place, and the desired mutant phenotype will be selected for, whereas other mutations will be eliminated. In 5 to 6 generations the genome of the mouse line selected for altered rhythms will be cleared of all but tightly linked mutations, and therefore suitable for the interbreeding that will indicate the phenotype of homozygote animals. The information concerning the phenotype in homozygotes may provide a useful insight into the nature of the genetic component that alters rest:activity behavior and other behavioral traits.

The test of the influence of genetic background on the mutant phenotype is a useful one for several reasons. First, it will indicate which mouse inbred lines are favorable for genetic mapping experiments that will be used for the chromosomal localization of the mutant locus. In addition, different expressivity/penetrance of the mutant phenotype in different genetic backgrounds can be used to identify and isolate modifiers (suppressors and enhancers) of the analyzed phenotype.

III. Discussion and Limitations of the Method

The aim of the mutagenesis screen illustrated in this chapter is to identify novel mouse mutations and corresponding disrupted genes which may serve as entry points for the construction of a molecular pathway(s) involved in governing sleep–wakefulness in mammals. The main assumption of our approach is that recordings and analyses of wheel-running rest:activity patterns allow for "behavioral high-throughput screening." The analysis of these rest:activity patterns is two-pronged: the identification of altered circadian oscillator function as revealed by circadian wheel-running parameters and the identification of altered activity parameters that may point to potential sleep mutants. We argue that further behavioral analysis, including electrophysiological recordings (by electroencephalogram and electromyogram) before and after sleep deprivation, may reveal activity mutants that harbor inherited alterations of sleep parameters.

The main limitation of a random mutagenesis screen for behavioral mutants in the mouse is the laborious nature of the large-scale screen and the capacity of the behavioral testing facility necessary to screen and analyze large numbers of mutagenized lines. This aspect restricts current efforts to one-generation screens, which will uncover only mutations with a dominant mode of inheritance. In general, dominant inheritance is rarely associated with loss-of-function mutations which, as stated before, are the most informative class of mutations in the genetic dissection

of a molecular or neuronal pathway. Moreover, dominant mutations are not as frequent as recessive mutations. Therefore, only a three-generation screen and breeding to homozygosity of these randomly induced mutations will offer the opportunity to systematically isolate essential regulatory genes in a particular process such as sleep.

Any knowledge of the mode of inheritance of the phenotype of interest in other model organisms may give an important clue for the design of a mutagenesis screen/scheme in the mouse. For example, the existence of an array of semidominant alleles at the *per* locus in *Drosophila* (for a review see references 36 and 37) and the *tau* mutation in the hamster[38] provided a rationale for a dominat screen for mutations that affect circadian behavior in the mouse.[11,12] In the case of sleep anomalies, the study of narcolepsy in dogs, *canarc1*, serves as a useful example of a recessive trait which could be potentially detected in heterozygotes (G1) by pharmacological treatment; Mignot and colleagues showed that activation of cholinergic and deactivation of monoaminergic systems can induce narcoleptic/cataplectic behavior in normally asymptomatic carriers.[39,40] Neuropharmacological testing of novel behavioral mutations recovered in a random mutagenesis screen will be of great value in the initial characterization of these mutants and in the evaluation of their resemblance to corresponding human inherited disorders. Hypothetically, if a novel mouse mutant with sudden sleep attacks displays the same therapeutic response as narcoleptic subjects, this mutant will be a likely candidate for a mouse model of narcolepsy.

In a screen for a dominant mutation, the desired phenotype observed in heterozygotes (G1 progeny) may dramatically differ from the phenotype in mice with two copies of this mutant locus (homozygotes). The true nature of the induced mutation and the corresponding disrupted gene will only be ascertained following its breeding to homozygosity — intermating of two affected individuals and analysis of the mutant phenotype in their offspring. For example, we have recently shown that the *Wheels* mutation, identified in our pilot mutagenesis screen for dominant mutants with anomalies in circadian activity, is associated with embryonic lethality and anomalies in the developing forebrain in homozygotes (Nolan, Alavizadeh, Lo, and Bucan, unpublished). In the case of circadian rhythms, homozygosity at the *per*, *tim*, *tau*, and *Clock* loci results in a more severe effect on circadian rhythmicity and is not associated with lethality.[11,36-38,41] However, in the case of other dominant behavioral mutations in the mouse, it is important to await more studies to evaluate how many of them will be associated with developmental defects or lethality in homozygotes. This is particularly relevant in the case of gene(s) that may regulate such an essential biological process as sleep. The association of developmental anomalies with sleep disturbances and/or sleep apnea has been reported for several human inherited disorders, such as Smith-Magenis[42] and Treacher Collins[43] or Prader Willi syndromes.[44]

Monitoring wheel running activity in rodents provides an efficient and inexpensive screen for abnormal circadian behavior. In our effort to use the same paradigm to screen for potential sleep mutants, we are characterizing mice with abnormal values for activity phase (α) and mice with high levels of activity during the subjective day, as well as those whose rest:activity phases seem fragmented. Although, by

limiting ourselves to a noninvasive prescreen we may miss subtleties in sleep–wake architecture, it is important to keep in mind that our long-term goal will be to positionally clone genes disrupted in the sleep mutations. Positional cloning involves extensive breeding and phenotypic analysis of several hundreds of mice. Therefore, at this stage of sleep genetics, sleep mutants that are "marked" by an additional phenotype and scoreable in the high throughput assay are an important advantage.

The ultimate goal of a screen for mutations with abnormal rest:activity behavior is the identification of a disrupted gene. How does one discover the molecular identity of a gene whose function is not known? The strategy that allows cloning of a gene based on its chromosomal map location is positional cloning.[45] Currently, procedures for positional cloning include genetic mapping of the locus of interest to a 0.5 cM interval (approximately 1 Mb), physical mapping, cloning of large genomic DNA fragments, and finally a search for genes of interest in the cloned region. On average, it takes two to three years to positionally clone the gene disrupted in a novel mutation in the mouse. With comprehensive physical and transcription maps, valuable resources currently being developed by the Human Genome Project, conventional positional cloning may soon be replaced by techniques that allow one to search for point mutations in candidate genes in the immediate vicinity of the mutant locus rather than search for these candidate genes in a larger chromosomal segment.[46] In other words, the availability of transcription maps and expression profiles for genes placed on these maps will allow faster identification of genes, disrupted in novel behavioral mutants in the mouse, as well as identification of their homologs in humans.

Acknowledgments

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