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6. AUTHOR(S)
DR MAJA BUCAN

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pennsylvania School of Medicine
Dept of Psychiatry
One Clinical Research Building
415 Boulevard
Philadelphia PA 19104-6140

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
110 Duncan Ave Room B115
Bolling AFB DC 20332-8050

Dr Genevieve M. Haddad

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We have developed a routine procedure for random mutagenesis in the mouse and have demonstrated the feasibility of rapidly screening for aberrant behavioral parameters. We believe that this classical genetic approach, as well as the screening of progeny of mutagenized mice for altered sequence and/or expression pattern prior to phenotypic analysis, will play an important role in the elucidation of the functional content of the mammalian genome.

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Final Report

AFOSF 94NL009A: Genetic Approach to Mammalian Circadian Rhythyms

Dr. Maja Bucan, P. I.

The availability of model organisms harboring mutant variants of genes involved in any biological process is essential for understanding the complexity of these processes at a molecular and biochemical level. We used random mutagenesis to screen for dominant mutations in circadian rhythymicity by examining G1 progeny from ENU-mutagenized mice for abberant wheel running activity. Based on a statistical analysis of behavioral parameters which can be extracted from the activity records of a large number of tested progeny, several mutants with a strikingly different behavioral profile were identified.

In the course of our studies we performed four experiments using 8-10 week old mice for mutagenesis:

1) BALB/c males injected with 1X150 mg/kg *N*-ethyl-*N*-nitrosourea (ENU) mated with C57BL/6J females
2) C57BL/6J males injected with 1X150 mg/kg ENU mated with BALB/c females
3) C57BL/6J males injected with 1X150 mg/kg ENU mated with C57BL/6J females
4) C57BL/6J males injected with 3X100 mg/kg ENU mated with C57BL/6J females

At four weeks after administration of the mutagen, ENU treated males were caged individually with two fertile females of the same genetic background. We found that by collecting and analyzing no more than 50 - 75 generation 1 (G1) progeny from a single mutagenized male, we had a low chance of recovering the same mutation multiple times. Comparisons of single and multiple dosage regimens in C57BL/6J males indicates that the two procedures have similar affect long term survival (greater than 6 months after the final injection). Mutagenized males (1X150 mg/kg) were mated to either C57BL/6J or BALB/cJ females and produced comparable litter sizes. The repeated dose (3X100 mg/kg) results in

slightly smaller litter sizes (Table 1). Deaths prior to or at weaning were comparable in progeny of males treated with either 1X 150 mg/kg or 3X 100 mg/kg ENU.

The most striking result in this pilot screen comes from the difference in numbers of detected anomalies and recovered mutations in the various treatment groups and crosses. These results of our screen suggest that repeated doses of ENU in C57BL/6J mice may be most effective at the generation of visible and behavioral anomalies. Visible defects observed in this screen included dominant spotting, curly or kinked tails, runting, eye anomalies and craniofacial defects. The incidence of detectable visible anomalies was higher in isogenic crosses than in F1 hybrids where mutagenized C57BL/6J males were crossed to BALB/cJ females.

To screen for mutations in circadian rhythms, 8-14 weeks old G1 mice were housed individually in cages equipped with running wheels, with food and water freely available. Cages wre maintained in light-tight, ventilated chambers (10 cages to a chamber), with timed lighting. Each animal's wheel-running activity was monitored continuously, via a microswitch located on the outside of each cage. Behavioral analysis was 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). A Macintosh-based interactive program, *Circadia* , was used to rapidly visualize, analyze, and quantitate the rhythmicity and activity of mutant mice. 3 quantitative parameters—period, phase, and amplitude — were used to characterize the circadian rhythm of G1 mice.

To date we have obtained the distribution of values of circadian periods for 1000 progeny of mutagenized males in three experiments. Values determined in these experiments vary depending on the genetic background of mutagenized mice and their breeding mate. Almost all G1 progeny exhibited robust activity, typical of most inbred mouse strains. Based on the previously reported values of 23.7 $\pm$0.17 hours for the τ_{DD} of circadian activity in C57BL/6J mice, any mouse with a τ_{DD} shorter than 23.10 or longer than 24.30 hours was selected for further analysis. The most detailed analysis has been performed

on the *Wheels* mutation. Although this mutation was originally identified based on its circadian period, further analysis showed that *Whl/+* mice also exhibit hyperactive circling and an inner ear anomaly, while *Whl/Whl* die *in utero* at E10.5 and exhibit abnormal anterior brain structures (Pickard et al., 1995; Nolan et al., 1995; Nolan, Alavizadeh, Cohen, Lo, and Bucan, in preparation). More recently, we identified two mutations with a short circadian period, *Esterline* and *Early bird*. Originally, the τ_{DD} value for these two mutations was only 3 standard deviations (SD) shorter than the mean (22.69 and 23.0 respectively); however after their mating to C57BL/6J mice, a significant number of their progeny displayed a circadian period that was more than an hour shorter than the mean for the C57BL/6J genetic background. Genetic mapping and analysis of the recessive phenotype of these mutations is in progress. In addition, we have identified a mapped a mutation which we call *Restless*. Animals with this mutation exhibit increased activity during the subjective day, suggesting that this gene may be involved in the regulation of sleep.

In addition to screening wheel-running behavior, we examined the feasibility of using other simple tests to identify mutations in various aspects of mouse behavior. These included the acoustic startle response (ASR), and the inhibition of ASR by a weak prestimulus (prepulse inhibition). In humans and rodents, sensorimotor gating, or normal filtering of external sensory stimuli, is reflected by the reduced amplitude of startle response when the strong stimulus is preceded by a weaker stimulus. Since patients with schizophrenia, schizotypal personality disorder, obsessive-compulsive disorder and Huntington's disease have impaired PPI, we reasoned that loss of PPI of a normal startle response in mice may be used as a simple screen for a model of attentional impairment -- one component of this complex psychiatric disorder. As a first step in our pilot screen for anomalies in startle-responsiveness and sensorimotor gating we determined the baseline responses for ASR and PPI in three inbred strains of mice, C57BL/6J, C3H/HeJ and BALB/cJ. Tested inbred strains were 4 months old (5 males and 5 females) and tests were performed at the same time each day (between noon and 5 pm). In both ASR and PPI, the three inbred strains displayed consistent and reproducible results. Among 10 C57BL/6J mice, ASR was calculated as 988 ± 104 (mean $\pm$ sem) and PPI was $39.6\% \pm 4.0$. For C3H/HeJ subjects, ASR was 619 ± 168; and PPI $28.9\% \pm 5.6$. The ASR for BALB/cJ was 2058 ± 238; and PPI was $46.5\% \pm 4.5$. The significantly

greater ASR seen in BALB/cJ mice relative to C57BL/6J and C3H/HeJ is consistent with strain differences in ASR previously reporte. PPI was comparable in all three strains.

We also tested the behavior of several inbred strains in the Porsolt Swim test. This test is a simple and rapid experimental procedure widely used in rodents as an efficacious screen for antidepressants. We found substantial inter-strain differences in behavior in the Porsolt Swim test. C57BL/6J mice had an initial active period of 67.8 ± 6.5 seconds (Mean ± sem) and remained immobile for 225.4 ± 14.4 seconds during the final 5 minutes of the test. Similarly, BALB/cJ mice had an initial active period of 63.8 ± 10.0 seconds and a total immobility time of 249.8 ± 11.9 seconds during the final 5 minute period. Remarkably, however, C3H/HeJ mice were more active and/or appeared to be less prone to "behavioral despair". Their average latency was 161.9 ± 18.5 seconds and they were immobile for only 84.1 ± 10.6 seconds in the final 5 minute period. C3H/HeJ mice also adopted a characteristic "vertical" posture in the water and continuously attempted to "climb up" the side of the container in order to escape.

Our preliminary results with these two sets of tests have convinced us that it will be feasible to screen for mtuations using these paradigms, and that it may also be possible to examine the genetic basis for natural variation in these behaviors.

In summary, we have developed a routine procedure for random mutagenesis in the mouse and have demonstrated the feasibility of rapidly screening for aberrant behavioral parameters. We believe that this classical genetic approach, as well as the screening of progeny of mutagenized mice for altered sequence and/or expression pattern prior to phenotypic analysis, will play an important role in the elucidation of the functional content of the mammalian genome.

Papers resulting from sponsored research

Pickard, G. E., Sollars P. J., Rinchik, E. M., Nolan, P. M., and Bucan, M. (1995) Mutagenesis and behavioral screening for altered circadian activity identifies the mouse mutant, *Wheels (Whl), Brain Research*, 705, 255-266.

Nolan, P. M., Sollars, P. J., Bohne, B. A., Ewens, W. J., Pickard, G. E., Bucan, M. (1995) Heterozygosity mapping of partially congenic lines: mapping of a semidominant neurological - mutation, *Wheels (Whl)*, on mouse chromosome 4, *Genetics* 140, 245-254.

Nolan P.M., Houpt, T. and Bucan, M. (in press) Chemical mutagenesis and screening for mouse mutations with altered rest:activity pattern. In: "Molecular Regulation of Concious States" (ed. R. Lydic), CRC press

Nolan, P. M., Kapfhamer, D. and M. Bucan (in press) ENU-Mutagenesis and a screen for dominant behavioral mutations in mice. In: "Functional Genomics in the Mouse" (ed. M. Seldin) Methods: A companion ot Methods in Enzymology: (in press)