Number of Immunoreactive GnRH-Containing Neurons Is Heritable in a Wild-Derived Population of White-Footed Mice (*Peromyscus leucopus*)

Paul D. Heideman*  
David R. Broussard†  
Jessica A. Tate  
Mauricio Avigdor  
Department of Biology, College of William and Mary, Williamsburg, Virginia 23187  
Accepted 4/13/2007; Electronically Published 7/13/2007

ABSTRACT

The evolution of mammalian brain function depends in part on levels of natural, heritable variation in numbers, location, and function of neurons. However, the nature and amount of natural genetic variation in neural traits and their physiological link to variation in function or evolutionary change are unknown. We estimated the level of within-population heritable variation in the number of gonadotropin-releasing hormone (GnRH) neurons, which play a major role in reproductive regulation, in an unselected outbred population recently derived (<10 generations) from a single natural population of white-footed mice (*Peromyscus leucopus*, Rafinesque). Young adult male mice exhibited an approximately threefold variation in the number of neurons immunoreactive for GnRH in the brain areas surveyed, as detected using SMI-41 antibody with a single-label avidin-biotin complex method. Consistent with earlier findings of selectable variation in GnRH neurons in this population, the level of genetic variation in this neuronal trait within this single population was high, with broadsense heritability using full-sib analysis estimated at 0.72 (P < 0.05). Either weak selection on this trait or environmental variation that results in inconsistent selection on this trait might allow a high level of variation in this population.

Introduction

The evolution of mammalian brain function should depend in part on levels of natural, heritable variation in numbers, location, and function of neurons. Further, natural genetic variation in neuronal traits is presumably responsible for a significant proportion of intraspecific variation in regulatory physiology and behavior in vertebrates. Genetic variation is presumed to underlie a substantial portion of complex trait variation in mammals (Glazier et al. 2002; Hirshhorn and Daly 2005), including neuronal traits in the brain (Bittner and Friedman 2000). Despite the presumed importance of natural neuronal variation, few studies have reported on heritable variation in neuronal traits from natural populations (Hammock and Young 2005; Hammock et al. 2005), though much more data are available from domesticated or laboratory animals (e.g., zebra finch; Airey et al. 2000) and especially laboratory mice (Rhodes et al. 2005). Information on heritable variation in neuronal traits, especially from natural populations, would provide an indication of how genetic variation in neuronal traits might contribute to fitness and the potential for microevolution of the brain.

An approach to link heritable variation in neurons to functional variation in nature has developed from work on genetic and functional variation in the photoneuroendocrine pathway (Heideman et al. 1999b; Majoy and Heideman 2000; Prendergast et al. 2001; Heideman 2004), through which environmental cues regulate seasonal reproduction in rodents (Prendergast et al. 2002; Ebling 2005). The photoneuroendocrine pathway is a complex neural circuit through which seasonal change in photoperiod causes seasonal change in reproduction, energetics, pelage growth, and other aspects of physiology. The signal for light is passed from retinal photoreceptors to the suprachiasmatic nuclei and then through the hypothalamus and via a sympathetic pathway to the pineal gland (Prendergast et al. 2002). The pineal gland releases the hormone melatonin only during the dark phase, providing a physiological signal proportional to the duration of night and day (Bartness et al. 1993; Goldman 2001; Prendergast et al. 2002). The duration of the melatonin signal regulates multiple seasonal responses (Prendergast et al. 2002), including the reproductive system. One of the indirect or direct targets of melatonin is a group of hypothalamic neurons that secrete gonadotropin-releasing hormone (GnRH), a decapeptide that is the master hormonal reg-
Heritability of GnRH Neurons in White-Footed Mice

535

GnRH neurons are a major component of the reproductive system and are critical for reproductive regulation (Wray 2002). Therefore, individual variation in the GnRH neuronal system might result in individual variation in life-history traits, including fertility, maturation rate, and reproductive responses to environmental cues, all of which can affect fitness. Life-history variation is ecologically important within and among species, but almost nothing is known about natural genetic variation in neural traits that might constrain or shape life-history variation (Sinervo and Svensson 1998; Ricklefs and Wikelski 2002; Heideman 2004). Genetic variation in neuronal traits that contribute to life-history strategies is particularly important to assess because such variation may be important for rapid adaptive responses to environmental change and may also contribute to driving ecological phenomena, including population cycling (Nelson 1987; Price and Schluter 1991; Kruuk et al. 2000; Reale et al. 2003).

Avigdor et al. (2005) recently reported that two selection lines of wild-derived white-footed mice selected for and against gonadal maturation in short winter photoperiods, respectively, differed by 50% in the number of immunoreactive GnRH (IR-GnRH) neurons. The selection line that maintained reproductive condition in short photoperiods had significantly more IR-GnRH neurons compared with the selection line that was reproductively suppressed by a short photoperiod. The suppression of gonad size by a short photoperiod has significant heritability in this population (Heideman et al. 1999a), but it is not known how much genetic variation exists in the number of IR-GnRH neurons or how gonad size is related to IR-GnRH neurons in unselected mice. Here we provide what is, to our knowledge, the first direct measure of heritability of neuron counts from a natural source vertebrate population.

Material and Methods

Animals

Individual Peromyscus leucopus were obtained from a laboratory colony at the Population and Endocrinology Laboratory of the College of William and Mary. The 48 wild founders of the population were captured at 37°16′N in the vicinity of Williamsburg, Virginia. Wild-caught animals were paired in a long-day photoperiod (LP; 16L : 8D), yielding a parental generation (Nelson 1987; Price and Schluter 1991; Kruuk et al. 2000; Reale et al. 2003).

Immunocytochemistry

Immunocytochemistry followed methods described previously (Avigdor et al. 2005) and is briefly described below. Expression of mature GnRH immunoreactivity in the brain was detected using a single-labeled avidin-biotin-peroxidase-complex method. A total of eight independent immunocytochemistry runs were carried out in this experiment, with siblings always separated into different runs to prevent similarities in GnRH complement that arise from shared processing rather than shared genome. This is a conservative approach, which may have increased full-sib differences (potentially decreasing the estimated heritability) compared with a random design. Brain slices were rinsed in a saline buffer and treated with 1% sodium borohydride (Sigma Chemical) for 30 min. Tissues were rinsed again and incubated overnight with SMI-41 monoclonal antibody (Sternberger Monoclonals, Lutherville, MA) at a dilution of 1 : 20,000 in saline buffer. SMI-41 is a mouse monoclonal IgG1 antibody with high affinity and high specificity to GnRH (Kd = 3.0 × 10−10 M; Knapp and Sternberger 1984). SMI-41 is incapable of significantly binding the Gly10-2,4-dinitroanilide GnRH derivative, indicating that an intact C-terminal glycine amide, present at the terminus of mature GnRH but not in pro-GnRH, is necessary for reaction with the antibody. Furthermore, low reactivity for GnRH in frozen coronal sections (30 μm) were cut on a freezing sliding microtome and stored in an antifreeze solution at −20°C until immunocytochemistry.

Perfusions and Sectioning

Fixation and processing of tissues followed methods described previously (Avigdor et al. 2005) and are briefly described below. All perfusions were conducted on mice aged 70–100 d between 1400 and 2200 hours. Mice were weighed, euthanized with an overdose of Isoflurane (Abbott Laboratories), and allowed to enter respiratory arrest before perfusion. Mice were perfused through the left ventricle with 0.1 M phosphate-buffered saline (PBS) at pH 7.4 followed by perfusion with cold 4% paraformaldehyde (Fisher Scientific, Fair Lawn, NJ) and saturated picric acid (Sigma Chemical, St. Louis, MO) in PBS. Brains were postfixed overnight in Zamboni’s fixative in the cold. Brains were cryoprotected before sectioning in a solution of 30% sucrose in PBS. Following perfusion, the weight of the paired testis and paired seminal vesicles was assessed. Frozen coronal sections (30 μm) were cut on a freezing sliding microtome and stored in an antifreeze solution at −20°C until immunocytochemistry.

Individual Peromyscus leucopus were obtained from a laboratory colony at the Population and Endocrinology Laboratory of the College of William and Mary. The 48 wild founders of the population were captured at 37°16′N in the vicinity of Williamsburg, Virginia. Wild-caught animals were paired in a long-day photoperiod (LP; 16L : 8D), yielding a parental generation (Heideman et al. 1999a, b; Kruuk et al. 2000; Reale et al. 2003). The unselected line was maintained as an outbred line with at least 20 and generally more than 40 breeding pairs per generation in order to minimize genetic drift and the loss of natural variation. The 31 full-sibling pairs of mice used in this experiment belonged to the F3 to F9 generations of the unselected control line. Mice were born in LP and transferred to a short-day photoperiod (SP; 8L : 16D) within 3 d of birth. Male offspring were weaned at 21–23 d of age and singly housed in polyethylene cages with wire tops and pine shavings until 70 ± 3 d of age. Mice were then lightly anesthetized with Isoflurane (Abbott Laboratories, North Chicago, IL) in order to obtain body weight and testis measurements (Heideman et al. 1999a). Mice were provided food and water ad lib. at all times. Procedures were approved by the Institutional Animal Care and Use Committee as projects 9837 and 0429.
Figure 1. Photomicrographs of the ventromedial preoptic area, immediately dorsal to the optic chiasm, taken from the anterior-most section that includes the third ventricle (reference area is plate 18; Paxinos and Watson 1986) from two representative individuals with (a) high neuron count (offspring of dam 29 from Fig. 2a) and (b) low neuron count (offspring of dam 2 from Fig. 2a), respectively. Arrows indicate immunoreactive GnRH neuronal cell bodies. A high density of GnRH-positive fibers creates the appearance of solid dark staining near the third ventricle (3V) in both photomicrographs but was resolvable under the microscope into a three-dimensional network of fibers in which neurons were distinguishable. oc = optic chiasm. Scale bar = 100 μm.

alpha-amino group of the N-terminal glycine interferes with the binding (Knapp and Sternberger 1984). Because SMI-41 reacts only to the five amino acids adjacent to the C-terminus of the GnRH peptide and the amidation site, only mature hormone is likely to be recognized by SMI-41 antiserum (Knapp and Sternberger 1984; Tai et al. 1997). After incubation with the primary antibody, sections were rinsed and processed using avidin-biotin-peroxidase (Vector Laboratories Elite ABC-Peroxidase kit) using diaminobenzidine (0.2 mg/mL), NiSO₄ (24 mg/mL), and H₂O₂ to produce an insoluble colored reaction product at binding sites of the primary antibody. The color reaction was allowed to proceed for approximately 12 min. Sections were mounted on gelatin-coated slides and air dried, dehydrated in xylene, and coverslipped with Permount (Fisher Scientific).

Neuron Assessment

The location and number of mature GnRH-secreting neurons were assessed in counts by eye by M. Avigdor, with independent test counts of approximately one-third of sections by J. A. Tate and of a small additional subsample by P. D. Heideman, all of which gave similar numbers. Counts were carried out blind with respect to sibling using a compound light microscope. The data presented here are from counts by M. Avigdor. In order to assess whether stereological techniques (Wreford 1995) might be necessary for our counts, we tested for potential biases in counting due to neuron size. If IR-GnRH neurons were more similar in size within families than among all individuals, and if size of IR-GnRH neurons affected our counts, then estimates of heritability in number of IR-GnRH neurons might be due in part to heritable size variation of IR-GnRH neurons. For these tests, we measured the greatest width and greatest length of the three IR-GnRH neurons nearest the midline of the first section containing the medial preoptic area (plate 18 in Paxinos and Watson 1986); in mice where there were fewer than three neurons in this section, we measured neurons in the next stained section. The effect of family was significant for neuron width (nested ANOVA using JMP 5 software; \( F = 2.02, P = 0.04 \) but not length (\( F = 1.07, P = 0.43 \)). However, there was no significant correlation between number of IR-GnRH neurons and width (\( R^2 = 0.01, P = 0.43 \)) or length (\( R^2 = 0.03, P = 0.16 \)) of IR-GnRH neurons. Therefore, we did not apply stereological techniques to adjust counts for variation in IR-GnRH neuron size.

All brain structures and nuclei in this article are referred to using abbreviations and nomenclature consistent with those given by Paxinos and Watson (1986). Brain areas were first estimated relative to major landmarks using a stereotaxic coordinate atlas for the rat brain (Paxinos and Watson 1986). Those regions where IR-GnRH cells were scored using the brain atlas for the rat were later compared with those identified in a stereotaxic coordinate atlas for the deer mouse *Peromyscus maniculatus* (Eleftheriou and Zolovick 1965). Because the deer mouse atlas lacked detail in much of the hypothalamus, the rat brain atlas was used to estimate boundaries of many of the brain areas, and terminology from this latter source is used here. *Peromyscus leucopus* have much larger eyes and optic nerves than the similarly sized laboratory mouse, and in our judgment, the hypothalamic structures and landmarks are more similar to those of the rat than those of the lab mouse. However, because brain areas in the rat may differ from brain areas in
Figure 2. a, Counts of GnRH neurons (total cell bodies counted from four brain sections) for each pair of siblings plotted along the Y-axis, from smallest average for a sibling pair to largest average for a sibling pair. Dam identification numbers were assigned for this figure, from smallest to largest average GnRH neuron number of offspring; dam order does not correspond to that in Figure 1b. b, Paired testes masses for each pair of siblings plotted along the Y-axis, from smallest average for a sibling pair to largest average for a sibling pair. Dam identification letters for this figure were assigned in order from smallest average mass of the testes of offspring to largest average mass; dam order does not correspond to that in Figure 1a.

*P. leucopus*, areas to which we assigned the same name may not be homologous or functionally equivalent.

Previous results (Avigdor et al. 2005) indicated that four specific coronal brain sections were good candidates for assessing heritability of IR-GnRH neurons. These include the brain section containing the most posterior parts of the vertical limb of the diagonal band of Broca (plate 17 in Paxinos and Watson 1986), the first two sections containing the medial preoptic area (plates 18 and 19 in Paxinos and Watson 1986), and the most caudal section containing the medial preoptic area but no parts of the lateroanterior hypothalamic nucleus or anterior hypothalamus (plate 22 in Paxinos and Watson 1986). These four sections contained a large fraction of the total GnRH neuronal complement of *P. leucopus* and include regions of the brain where differences in IR-GnRH neurons were typical of the overall differences between lines (Avigdor et al. 2005).

**Statistical Analysis**

Data were analyzed using Statview SE + Graphics software and SuperANOVA software (Abacus Concepts, Berkeley, CA) running on a Macintosh computer. To test for any potential differences in procedure reactivity across immunocytochemistry runs, we performed a one-way ANOVA on neuron number with runs as the factor. Means among runs were very similar, and no significant differences across runs were detected ($P > 0.20$). To determine within-group and between-group variances for body weight, testis weight, seminal vesicle weight, and number of GnRH neurons, we carried out a one-way ANOVA for each variable using dam as a factor. Broadsense heritabilities were calculated using the following formula (Becker 1984):

$$h^2 = \frac{2\sigma_i^2}{\sigma_i^2 + \sigma_w^2},$$

where $\sigma_i^2$ is an estimate of between-group variance and $\sigma_w^2$ is an estimate of within-group variance derived from ANOVA tables. Confidence intervals (CI) for heritabilities were calculated using the following formula (Becker 1984):

$$2(1 - K_{0.025}) \leq h^2 \leq 2(1 - K_{0.025}),$$

where

$$K_i = \frac{k MS_w F_i}{MS_i + MS_w (k - 1) F_i}.$$

Lowercase $k$ is the number of individuals per sibship (family), $MS_w$ is the mean square within sibships, $MS_i$ is the mean square between sibships, $\alpha = 0.05$, $F_i$ is $F_{0.025} = 2.07$, and 0.95 is the level of confidence (Becker 1984). Correlations between family averages of testis weight and seminal vesicle weight, body weight and testis weight, body weight and seminal vesicle weight, body weight and total number of neurons, and testis weight and total number of neurons were calculated using simple regressions, with $P < 0.05$ as the level of significance. Heritability estimates using full siblings include more sources of variation than heritability estimates by other methods, such as half-sibling or parent-offspring regression. Parental effects and dominance interactions are potential sources of variation that cannot be partitioned out of a full-sibling estimate of heritability. Therefore, heritability estimates with full siblings can only set an upper limit to narrow-sense heritability. We chose to use full siblings for this study for practical reasons, on the basis of the difficulty
in obtaining breeding pairs for the destructive sampling necessary to conduct neuron counts.

**Results**

The broadsense heritability of GnRH neuron number was 0.72 ($P < 0.05; CI = 0.03$ to $1.00$). The heritability estimate of testis weight was 0.96 ($P < 0.05; CI = 0.32$ to $1.00$). Heritability estimates for seminal vesicle weight ($h^2 = 0.59$; $P > 0.05$; $CI = -0.12$ to $1.00$) and body weight ($h^2 = 0.22$; $P > 0.05$; $CI = -0.49$ to $1.00$) were not significantly different from 0. Across families, the range of GnRH neuron numbers varied approximately threefold (Figs. 1, 2a), and the range for testes mass varied approximately fivefold (Fig. 2b).

Genetic and phenotypic correlations of male reproductive characters were calculated using data averaged for 31 sibling pairs (genetic correlations; Table 1) and for 62 individuals (phenotypic correlations; Table 2), respectively (Lynch and Walsh 1998). Number of GnRH neurons was not significantly correlated with average body weight, testes weight, or seminal vesicle weight in either genetic or phenotypic correlations. Average testis weight and average seminal vesicle weight were significantly positively correlated in both comparisons (Tables 1, 2). Among all other genetic and phenotypic correlations, only the positive phenotypic correlation between body weight and testis weight was statistically significant (Table 2).

**Discussion**

Adult males from an unselected outbred line recently derived from a single natural population of white-footed mice exhibited an approximately threefold variation in the number of immunoreactive cells of a specific category of neurons, those containing GnRH (Fig. 2a). Estimates of the total number of marked GnRH neurons were consistent with those of other rodents similar in brain size (Wray 2002; Avigdor et al. 2005). Differences among individuals might represent either differences in the number of GnRH neurons in the brain or differences in the number of neurons that contain detectable quantities of the mature GnRH peptide. The differences in number of IR-GnRH neurons are unlikely to be due to differences in reproductive state, since IR-GnRH neuron counts in our previous study on this population (Avigdor et al. 2005) were not significantly different between SP and LP, nor was the number of IR-GnRH neurons related to reproductive status or androgen manipulation. Testis mass in SP was significantly heritable, consistent with an earlier measure of narrow-sense heritability for testis mass of mice in this population also raised in winter photoperiods (Heideman et al. 1999a). In this study, neither seminal vesicle mass nor body mass was significantly heritable in SP. Because our sample sizes gave low statistical power for low values of heritability, it is not possible for us to distinguish whether these traits are not heritable in SP in this population or are heritable but with $h^2$ too low to detect in this sample.

Under the controlled conditions of the laboratory, variation in IR-GnRH neuron counts was significantly heritable, with a broadsense heritability estimate of 0.72. This suggests substantial heritable variation in IR-GnRH neuron numbers within this population. If a significant portion of the heritable variation is due to additive genetic variation, there would be potential for microevolutionary change in response to directional selection on phenotypic variation related to the number of IR-GnRH neurons. In a population containing selectable genetic variation, a strong prediction is that selection would produce a significant change in phenotype. That prediction is supported by recent results from Avigdor et al. (2005), in which lines from this population artificially selected for and against gonadal development in SP differed significantly in number of IR-GnRH neurons and in gonadal development in SP. This suggests that number of IR-GnRH neurons may have responded to selection on gonadal development in SP as a correlated trait, though changes due to genetic drift are also a possibility.

Number of IR-GnRH neurons was not significantly correlated in SP with body weight, testes weight, or seminal vesicle weight. Previous studies on vertebrates have sometimes shown direct relationships between numbers of IR-GnRH neurons and male reproductive characters, while others show no significant relationships (e.g., Molenaar et al. 1993; Ebling et al. 1995; Scaggiante et al. 2004). Studies on laboratory mice with a natural GnRH gene knockout have shown that hypothalamic implants with fewer than 20 detectable GnRH neurons can support reproduction, though these mice do not have normal reproductive endocrinology (Silverman et al. 1990). In mice with high counts of IR-GnRH neurons, some of these neurons may be redundant or may serve nonreproductive functions. Alternatively, higher numbers of IR-GnRH neurons might provide sufficient GnRH to support reproduction in the presence.

<table>
<thead>
<tr>
<th>No. GnRH Neurons</th>
<th>Testes Weight (g)</th>
<th>P</th>
<th>Seminal Vesicle Weight (g)</th>
<th>P</th>
<th>Body Weight (g)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. GnRH neurons</td>
<td>...</td>
<td>.49</td>
<td>~.02</td>
<td>.86</td>
<td>~.20</td>
<td>.12</td>
</tr>
<tr>
<td>Testes weight</td>
<td>...</td>
<td>.92</td>
<td>&lt;.0001</td>
<td>.13</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle weight</td>
<td>...</td>
<td>.08</td>
<td>.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Genetic correlations of male reproductive characters for 31 sibling pairs
of some inhibitory inputs, such as short photoperiod. Consistent with the suggestion that factors other than number or activity of GnRH neurons may regulate reproductive organ mass in SP is the observation that food intake was correlated with reproductive organ mass in SP, but not LP, in this population (Heideman et al. 2005). We propose the hypothesis that, in the natural source population, a higher number of IR-GnRH neurons provides sufficient GnRH secretion to maintain reproduction in spite of inhibition by winter photoperiods.

Potential sources of genetic variation that could contribute to heritable variation in IR-GnRH neuron numbers include a series of transcription factors acting differentially according to the stage of embryonic development and migrational position of developing GnRH neurons or adult function (Rave-Harel et al. 2004; Givens et al. 2005; Rave-Harel et al. 2005). For example, null mutants of the regulatory genes Msx and Dlx change the numbers and/or locations of GnRH neurons (Givens et al. 2005), suggesting that these developmental genes may normally regulate GnRH neuronal numbers. Alleles causing variation in the timing or location of expression of regulatory proteins such as these could create heritable variation in IR-GnRH numbers.

Most if not all of the genetic variation for IR-GnRH neuronal numbers in the current laboratory unselected population was likely contained in the 48 wild founders of our laboratory population. While the mean reproductive phenotype and body mass in our unselected population have not changed over time (Heideman et al. 1999a, 2005), mutation, genetic drift, and/or unintended selection in our control line might have altered frequencies of alleles affecting GnRH neurons over the seven to nine generations since founding. Testis sizes in our unselected population in generations 7–9 still matched the distribution of testis sizes of the founding males at the time of capture in the winter of 1995 (Heideman et al. 1999a). However, laboratory conditions might alter GnRH neuronal phenotype, and a different range or frequency of phenotypes might be observed under wild versus laboratory conditions. Furthermore, the broadsense heritability estimates used here include both additive and nonadditive genetic variation as well as maternal effects. Maternal effects could arise from variation in factors such as maternal body condition, milk production, or behavior (Mosseasu and Fox 1998), any of which might have effects on brain development before or after birth, possibly including development of GnRH neurons. Thus, while our results suggest that heritable variation in IR-GnRH neurons exists in the source population, study of wild individuals would be necessary to assess that variation in nature.

Previous work on bidirectionally selected lines from this population reported changes in reproductive phenotype between lines (Heideman et al. 1999a, Avigdor et al. 2005) that were correlated with differences in number of IR-GnRH neurons (Avigdor et al. 2005). However, the artificial selection on these lines was for or against gonadal development in short photoperiod (Heideman et al. 1999a) rather than on number of IR-GnRH neurons, and previous experiments did not allow us to test directly for correlations within the unselected lines. The absence of correlations in this study between IR-GnRH neurons and reproductive organ weights or body weight (Tables 1, 2) is consistent with our suggestion that the number of IR-GnRH neurons may contribute to the ability of a mouse to maintain reproductive condition in SP while not contributing to the size of the gonad that develops. The heritable variation we observed (this study) and the potential for microevolution in neuroendocrine traits related to reproductive timing (Avigdor et al. 2005) might allow, as one possibility, adaptation to local climate change or shifts in geographic range over short periods of time, as recently reported for this species (Myers et al. 2005).

Acknowledgments

We thank L. Moore for assistance with animal breeding and care and C. Jenkins for assistance with manuscript preparation. G. Gilchrist and J. Swaddle made helpful comments on an earlier version of the manuscript. This research was supported by a grant from the National Science Foundation (IBN-CAREER-9875866) to P.D.H. and by the College of William and Mary.

Literature Cited


| Table 2: Phenotypic correlations of male reproductive characters for 62 individuals |
|---------------------------------|---------|--------|--------|---------|--------|--------|
| No. GnRH Neurons                | Testes Weight (g) | P   | Seminal Vesicle Weight (g) | P   | Body Weight (g) | P   |
| No. GnRH neurons                | …      | −.04  | .49   | −.04   | .78   | .0     | 1.0   |
| Testes weight (g)               | …      | …     | …     | …      | …     | …     | …     |
| Seminal vesicle weight (g)      | …      | .92   | <.0001| .27    | .03   | …     | …     |
| Body weight (g)                 | …      | .19   | .14   | …      | …     | …     | …     |
to selection for photoperiod responsiveness on the density
and location of mature GnRH-releasing neurons. Am J Physi-
iol Regul Integr Comp Physiol 288:1226–1236.
Bartness TJ., J.B. Powers, M.H. Hastings, E.L. Bittman, and
B.D. Goldman. 1993. The timed infusion paradigm for mel-
atonin delivery: what has it taught us about the melatonin
signal, its reception, and the photoperiodic control of sea-
Enterprises, Pullman, WA.
Bittner G.D. and B.X. Friedman. 2000. Evolution of brain struc-
tures and adaptive behaviors in humans and other animals:
role of polymorphic genetic variations. Neuroscientist 6:241–
251.
Ebling F.J.P., I.H.M. Alexander, H.F. Urbanski, and M.H. Has-
tings. 1995. Effects of N-methyl-D-apsartate (NMDA) on
seasonal cycles of reproduction, body weight, and pelage col-
our in male siberian hamsters. J Neuroendocrinol 7:555–
566.
Eleftheriou B.E. and A.J. Zolovick. 1965. The forebrain of the
deermouse in stereotaxic coordinates. Kans State Univ Agric
Givens M.L., N. Rave-Harel, V.D. Goonewardena, R. Kurotani,
S.E. Berdy, C.H. Swan, J.L.R. Rubenstein, R. Benoit, and P.
Mellon. 2005. Developmental regulation of gonadotropin-
releasing hormone gene expression by the MSX and DLX
19165.
that underlie complex traits. Science 298:2345–2349.
Goldman B.D. 2001. Mammalian photoperiodic system: formal
properties and neuroendocrine mechanisms of photoperi-
dodic time measurement. J Biol Rhythms 16:283–301.
Association of vasopressin 1a receptor levels with a regulatory
odic time measurement. J Biol Rhythms 16:283–301.
Koning, and L.A. Sternberger. 1984. High affinity monoclo-
nal antibodies to luteinizing hormone-releasing hormone:
preparation and binding studies. J Neuroimmunol 6:361–
371.
Brotherstone, and F.E. Guinness. 2000. Heritability of fitness
in a wild mammalian population. Proc Natl Acad Sci USA
97:698–703.
Lynch M. and B. Walsh. 1998. Genetics and Analysis of Quan-
titative Traits. Sinauer, Sunderland, MA.
short-day responsive and short-day nonresponsive white-
footed mice (Peromyscus leucopus) do not affect reproductive
Molenaar G.I., C. Lugard-Kok, R.H. Meloen, R.B. Oonk, J. De
Koning, and C.J.G. Wensing. 1993. Lesions in the hypo-
thalamus after active immunisation against GnRH in the pig.
Mosseaus T.A. and C.W. Fox, eds. 1998. Maternal Effects as
Myers P., B.L. Lundrigan, and R. Vande Kopple. 2005. Climate
change and the distribution of Peromyscus in Michigan: is
global warming already having an impact? Pp. 101–125 in
E.A. Lacey and P. Myers, eds. Mammalian Diversification:
From Chromosomes to Phylogeography (A Celebration of
the Career of James L. Patton). Vol. 133. University of Cal-
ifornia Press, Berkeley.
sible variable in microtine population-density fluctuations.
Paxinos G. and C. Watson. 1986. The Rat Brain in Stereotoxic
Coordinates. 2nd ed. Academic Press, San Diego, CA.
toperiodic polyphenisms in rodents: neuroendocrine mech-
isms, costs and functions. Q Rev Biol 76:293–325.
seasonal rhythms: behavioral and neuroendocrine substrates.
Pp. 93–156 in D.W. Pfaff, A. Arnold, A. Etgen, S. Fahrbach,
Academic Press, San Diego, CA.
Rave-Harel N., M.L. Givens, S.B. Nelson, H.A. Duong, D. Coss,
TALE homeodomain proteins regulate gonadotropin-releas-
ing hormone gene expression independently and via inter-