Ovarian and Steroidal Influences on Neuroendocrine Aging Processes in Female Rodents*

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Abstract

SOME MAMMALIAN aging processes involve effects of steroids on the brain and pituitary. An ovary-dependent, neuroendocrine aging syndrome of laboratory rats and mice is described in this article. This syndrome can be attenuated during aging by chronic ovariectomy and can be prematurely induced in young rodents by sustained exposure to estradiol (E₂). The limited follicular stock in the ovary is proposed to be a major pacemaker of aging in this neuroendocrine syndrome; ovarian aging may interact with neuroendocrine aging. Ovary-independent neuroendocrine changes occur as well. We also discuss developmental influences on adult aging in rodents and other examples in which adult lower mammals are sensitive to long lasting effects of steroids on the brain and pituitary. Possible molecular mechanisms are considered. In view of the long lasting effects of E₂ and other steroids on lower mammals, the potential for long term effects of ovarian steroids on the human brain and pituitary warrants continued evaluation.

I. Introduction

Phenomena of female reproductive senescence have attracted much interest because many changes observed in laboratory animals and in human clinical practice can be extensively modified. Moreover, the mechanisms of aging involved might be widely generalizable, since the loss of fertility during midlife occurs in females of many mammalian species (1).

In species with regular fertility cycles, a fundamental question concerns the loss of these cycles during aging. The 4-day cycle of rodents or the 28-day cycle of women is based on an interaction of ovarian steroids and neuroendocrine peptide hormones. As ovarian follicles mature, they secrete increasing amounts of E₂. At some critical combinations of duration and level, the blood E₂ elevations trigger the pituitary to release a massive surge of luteinizing hormone (LH), which is dependent on the secretion of gonadotropin releasing hormone (GnRH) by hypothalamic neurons. This LH surge then causes ovulation of mature ovarian follicles. [Details of the postovulatory phases of the cycle, and the relative importance of the hypothalamus and pituitary in the LH surge differ between rodents and humans (2-4)]. The question arises as to what loci cause fertility cycles to cease with aging.

The possibility that hypothalamic age changes contribute to the loss of estrous cycles in aging rats and that ovarian steroids cause hypothalamic aging was suggested by Aschheim 20 years ago (5). His conclusions were puzzling at the time because much evidence indicated that irreversible or organizing effects of sex steroids on the development of neuroendocrine sex differences in rodents were limited to a critical period that ended during infancy, e.g. Barraclough's (6) demonstration that a single testosterone injection did not impair fertility of adult rats if injected on postnatal day 20, but caused permanent sterility if injected before day 10. Moreover, the irreversible loss of ovarian oocytes with aging was widely recognized (7, 8) and was considered as a sufficient explanation for the midlife decline of fertility, as suggested by Krohn's (9) well known study in which grafting of young ovaries to middle-aged mice restored regular cycles. More than a decade would elapse before hypotha-
lamic and pituitary age changes were well documented in laboratory rodents (reviewed in Section III B5). Recent studies following Aschheim’s lead (5) have led to the view that endogenous estrogens have long lasting, possibly cumulative effects on the adult rodent brain that cause a major neuroendocrine syndrome of aging; this syndrome is readily modified by ovariectomy or exposure to ovarian steroids. A major issue concerns the extent of long lasting effects from ovarian steroids during the phase of regular cycling in young rodents, as distinguished from the ensuing phases of lengthening and cessation of cycling.

This review will emphasize the role of ovarian secretions in age related neuroendocrine changes affecting reproductive function and will describe how interactions of the ovary, brain, and pituitary during aging can yield multiple etiologies of rodent reproductive aging. The experimental manipulations of reproductive aging provide important opportunities to develop detailed and quantitatively testable hypotheses about a complex aging process. Since it is beyond the scope of this review to consider all these changes in detail, major articles and recent reviews will be cited. We begin with background about age-related reproductive phenomena needed to discuss ovary-dependent neuroendocrine aging.

II. Major Phenomena of Reproductive Aging of Female Rodents

The decreasing frequency and increasing variability of estrous/menstrual cycles is a characteristic of aging in many spontaneous ovulators, including mice, rats, and humans (Fig. 1) (1, 10–12). Longitudinal studies of estrous cycles in mice and of menstrual cycles in women show a striking similarity of cycle frequency changes with age. Both species have U-shaped distributions of cycle length variance, with the greatest variability during the earlier and later phases of reproductive life. The incidence of long cycles increases during the approach to acyclicity, but short cycles also occur sporadically, even just before acyclicity. During the later phase of cycle lengthening, fertility drops strikingly despite the continued production of normal numbers of ova (1, 12). Fetal abnormalities increase in humans and rodents with maternal age; few of these come to term because of spontaneous abortions in humans or resorptions in rodents (13). In aging mice, stillbirths increase in association with delayed parturition (14). Even highly inbred rodents show remarkable individual differences in the frequency and variability of cycle length at any age, in the age when acyclicity begins, and in the types of acyclicity (10, 11, 15).

Acyclusis begins in most laboratory mice and rats between 12 and 16 months but varies according to gen-

![Fig. 1. Comparisons of age-related changes in ovulatory cycle length distributions from longitudinal studies of C57BL/6J mice (top, Reprinted with permission from Biol. Reprod. 27:327, 1982, J.F. Nelson et al.) and humans (bottom, reprinted with permission from Int. J. Fertil. 12:77, 1967, A.E. Trelaor et al.). The ages are scaled to midlife.](image-url)
the category of persistent diestrus, however, various patterns of ovarian secretion can lead to diestrous-type smears. Under our conditions, aging C57BL/6J mice rarely have repetitive pseudopregnancies unless supplied with young ovarian grafts (Section IV A). Ultimately, aging rodents enter persistent anestrus, in which ovarian secretions of E_2 and progesterone dwindle to low levels of the castrate (21); these and other similarities to menopause are discussed in Section III A. Usually C57BL/6J mice proceed directly from persistent vaginal cornification to persistent anestrus. Often, the transitions between states of acyclicity are not distinct, especially the transition to anestrus, when cornified epithelial cells gradually decrease in vaginal smears while leukocytic cells increase. Daily fluctuations in vaginal smear cell types are common and imply transient recruitment of the few remaining primary follicles to the growing pool (21, 22). The most common sequences of stages in rodent reproductive senescence are shown in Figure 2. The factors leading to these different trajectories of aging are unclear but probably include both prenatal and postnatal influences.

To identify phenotypic variations in aging and to further the possibility for future biochemical and molecular genetic approaches to mammalian aging, this laboratory has emphasized inbred mice, particularly the C57BL/6J strain which is widely used in research on aging. Neurochemical, physiological, and pathological age changes in C57BL/6J and related strains C57BL/6NNia and C57BL/6 are extensively documented (18, 23, 24). Unlike females, the male C57BL/6J does not generally undergo gonadal involution with aging (1, 25) and does not develop spontaneous pituitary tumors even at advanced ages (26). These differences give valuable comparisons between sexes to identify aging phenomena which are independent of the presence or absence of the ovary. In rodents of either sex, age-related degenerative disease, e.g. lymphoid tumors, kidney disease, and blood dyscrasias are rare or infrequent until after 20 months (24, 27–30). The major early events of reproductive senescence thus arise when C57BL/6J mice are healthy, long before the average lifespan of approximately 28 months (31, 32). The age-related degenerative diseases which are common by the average lifespan can pose major problems by their interactions with late onset aging phenomena. Effects of age-related diseases on endocrine functions are widely observed, e.g. on plasma testosterone (25) and the regulation of LH (Section IIIB2 b). We denote disease-related aging changes as pathogenic to distinguish them from other normal or eugenic changes (33).

Despite the genetic homogeneity of C57BL/6J mice, inbred since 1936 (24) considerable individual differences are observed in the timing of major events during reproductive aging. These variations are more readily attributable to developmental history or environment than to residual genetic polymorphisms. For example, the sex of fetal neighbors in the uterus influences adult neuroendocrine functions (discussed in Section VII). Also, there are wide individual differences in the ovarian store, which in 5-month-old C57BL/6J mice range from 1000 to 5000 oocytes (34). These variable ovarian oocyte stores may arise during fetal development since migration of primordial germ cells to the genital ridge is not necessarily precise (35). Fluctuating environmental factors may also influence the performance of different cohorts during aging (10, 15), including season (36), personnel, and diet composition (37) where subtle shifts in diet components by commercial sources could cause variations in aging. Nongenetic influences on individual inbred mice can arise even before embryonic implantation (38).

To analyze the details of estrous cycle patterns during manipulations of aging which are described later, we

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**Fig. 2.** Alternate trajectories that individual rodents may take through the transitional states of reproductive senescence. Cycle length usually increases in all rodents as the first age change, but short and long cycles are interspersed. In aging rats, most then enter persistent vaginal cornification (PVC) often followed by repetitive pseudopregnancy (RPP), and finally persistent anestrus [trajectory I, (54, 61, 71)]. In C57BL/6J mice, most proceed from lengthening cycles, to PVC, to persistent anestrus (trajectory II), but some go directly to anestrus (trajectory III) (10, 15, 88).
developed new quantitative criteria for analysis of vaginal smear data by microcomputer in collaboration with P.K. Randall (10, 15). Cycle regularity in C57BL/6J mice older than 6 months was maximized by individual housing (10). Although mice aged 2–3 months may not cycle regularly in single housing unless exposed to male pheromones, this (osmoleptic) influence diminishes a few months later (39). Useful parameters for characterizing age changes of estrous cycles in rodent populations include: estrous frequency (cycles/month), the fraction of mice cycling at any age, cycle length, probabilities of contiguous pairs of cycles, (e.g. 4 day-to-4 day cycles), and total number of estrous cycles (10, 40, K. Flurkey and C.E. Finch, in preparation). Cycle frequency gives a global index of cycling status in the population and represents effects from both acyclicity and lengthened cycles, thus, cycle frequency in the population is not necessarily the inverse of cycle length. The age when acyclicity begins is expectedly criterion-dependent, nonetheless, iterative variations of the criteria do not substantively change the profile of aging or relative differences among groups in their onset ages of acyclicity (15). About 40 estrous cycles occur during the lifespan of virgin C57BL/6J mice (L.S. Felicio, J.F. Nelson, and C.E. Finch, in preparation).

III. Multiple Loci in Female Reproductive Aging

(A). The ovary

The mammalian ovary acquires its fixed stock of oocytes during development. This stock is irreversibly lost during postnatal development and aging so that only 0.1–10% of oocytes remain by ages when fertility is lost and acyclicity begins (Fig. 3). The ability of ovarian grafts from old donors to support cycles in young rats is limited (5, 41). Moreover, the ovarian potential for driving estrous cycles after grafting declines strikingly with increased donor age in mice (Fig. 4) in close agreement with the histologic counts of oocyte reserves (Fig. 3).

A major issue is the contribution of this indisputable aspect of ovarian aging to physiological and cellular changes observed at the difference stages in reproductive aging. Menopause in women is widely considered to result from ovarian exhaustion (42), whereas the age-correlated loss of cyclicity in rats is widely thought to result from hypothalamic changes (43). It seemed possible at the outset of our studies that this presumed divergence of mechanisms between species could be misleading (44). Therefore, we have tried to design experiments that allowed a multiple etiologic description of these aging phenomena.

The ovarian oocyte reserves of C57BL/6J mice were characterized at 13–14 mo, during the transition to persistent vaginal cornification (34). Both cycling and acyclic mice had nearly depleted their oocyte reserves; most mice had less than 500 remaining oocytes. The subgroup of acyclic mice had 50% fewer oocytes than the cycling mice, but there was a considerable overlap be-
between the groups such that the majority of acyclic mice had as many oocytes as those that were still cycling. This observation shows that imminent oocyte depletion is not sufficient cause for the loss of cyclicity.

The hormonal mechanisms regulating the numbers of ova that are shed have an astonishing homeostatic ability. Even when near the end of fertility in most mouse strains, nearly normal clutches of ova are shed (8, 34, 45); pregnancy can result in CBA mice (8) with less than 100 follicles in their ovaries. The reduced rate of atresia in growing follicles of aging mice (34) probably contributes to the maintenance of normal numbers of Graafian follicles despite substantial reductions in the number of growing follicles (34, 46).

During persistent vaginal cornification in aging C57BL/6J mice, the growth rate of preantral follicles continues at rates normal for young cycling mice (22). The induction of ovulation by injections of human chorionic gonadotropin (hCG) during persistent vaginal cornification (47) indicates that some follicular cells continue to mature normally even in the absence of ovulatory surges of gonadotropins. As shown by follicular growth kinetics after labeling with [3H]thymidine, anovulatory Graafian follicles are replaced in 3-4 days (22). The inverse relationship between onset age of acyclicity and the duration of persistent vaginal cornification in C57BL/6J mice (15) implies a relatively constant duration when the ovary can produce growing follicles. This would be consistent with continued depletion of the limited primordial follicular pool at similar rates during cyclicity or acyclicity.

The average duration of persistently cornified vaginal smears in C57BL/6J mice is 2-4 months (15). This phase ends as cornification diminishes and thin leukocytic vaginal smears predominate which are not associated with pseudopregnancy. Mice are then in the terminal phase of ovarian aging, persistent anestrus, which is characterized by low levels of blood concentrations of E2 and progesterone equivalent to the castrate (21). By 20-26 months, most C57BL/6J mice are in persistent anestrus with thin leukocytic smears (15). The ovary at this stage probably has few, if any, growing follicles, but no direct measurements are available. Occasional corpora lutea were found in 20-24-month-old C57BL mice (48). Circulating LH can be elevated to postovariectomy concentrations at this stage in C57BL/6J and C57BL/6NNia mice (21, 49) (Fig. 5). The increase of LH is not detected if old mice have visible pituitary tumors (lactotroph adenomas) or other gross pathologic lesions that are common in old female rodents (21). The general trend for prolactinemia, particularly observed in aging rats (50-53) may account for the failure to observe elevated LH at constant anestrus in aging rats (53, 54). Prolactinemia can inhibit the secretion of LH through several mechanisms (55-57), including direct action on the pituitary (58). The C57BL/6J family of mouse strains thus provide useful models for the relationships of elevated gonadotropins and decreased ovarian steroid production observed in human menopause (59, 60).

Many reports demonstrate that a significant subgroup of acyclic old rats in persistent vaginal cornification and repetitive pseudopregnancy may be returned transiently to ovulatory cycles by treatments with progesterone and other hormones, stress, L-DOPA and other monoaminergic drugs, dietary manipulations, or electrochemical stimulation (16, 44, 61-66) (Section III B2d). This lability, or plasticity, of aging patterns is a basic feature of female rodent reproductive senescence. However, reactivations of cycles is not always evoked and may require a minimum stock of growing follicles. Treatments of C57BL/6J mice in persistent vaginal cornification with L-DOPA have thus far failed to reactivate cycles (K. Flurkey and C.E. Finch, in preparation).

(B). The brain and pituitary

1. Performance of young ovarian grafts in aging hosts. Ovarian transplantation between hosts and donors of different ages (heterochronic transplants) provides a powerful approach to evaluating effects of age on ovarian and hypothalamic-pituitary loci. A convenient technique involves heterotopic grafting of the donor ovary beneath
the host's kidney capsule wherein the host is ovariecto-
mized in the same operation (67, 68). Under favorable
circumstances, the ovary is spontaneously vascularized
and regular cycles resume within 2 weeks (J.F. Nelson,
L.S. Felicio, and C.E. Finch, unpublished data). It should
be noted that grafting causes a substantial loss of ovarian
oocytes (68, 69), presumably because of ischemia before
revascularization. Relatively small differences in the age
of the donor can have major effects on cycling
potential (Fig. 4).

In an early application of ovarian transplantation to
reproductive aging, Krohn (9) reported that grafting of
young ovaries to old CBA mice that had irregular cycles
or were acyclic restored regular cycles in the majority of
mice; whereas in reciprocal graftings, ovaries from old
acyclic mice failed to be reactivated. These findings
suggested the primary importance of the aging ovary in
the loss of cycles of some mouse strains. Subsequent
studies on rats by Aschheim and others gave different
outcomes, in which old hosts in persistent vaginal cor-
nification or repetitive pseudopregnancy generally main-
tained the previous acyclic condition with young ovaries;
only a minority regained cycles (5, 41, 70–72). In another
study of heterochronic transplants most of the young
and old recipients became acyclic within 3 months
whether given ovaries from prepubertal or old donors
(73).

A basic concern in transplant studies is the genotypic
histocompatibility of donors and hosts. Graft rejection
due to minor genetic histoincompatibility can sometimes
take 3–4 months in rodents and can compromise cellular
functions in the grafts even without a major inflamma-
tory response (74, 75). Hence, the potential for immu-
nologic interference is a serious issue in interpreting
transplant studies with partially inbred rats such as those
of some earlier studies. Also, ovariectomy in some mouse
strains can induce estrogen secreting adrenal cortical
growths (18, 76) which also arise spontaneously during
aging in at least one strain (76). Such extraovarian
steroids could confuse interpretations of aging and
responses to ovarian grafts. Fortunately, C57BL/6J mice
are not subject to these difficulties; thin leukocytic va-
ginal smears and constricted vaginal orifice indicating
low E_2 concentrations persist more than 12 months after
ovariectomy (21). Moreover, the related C57BL mice do
not show significant output of adrenal estrogens during
aging as judged from their leukocytic vaginal smears (17,
77).

To address the above concerns and to extend the
potentially important phenomenon of ovary-dependent
neuroendocrine aging, we have begun extensive studies
on the inbred C57BL/6J mouse. We confirmed by vaginal
smear patterns that young ovaries grafted into 16 to 30
month old noncycling C57BL/6J hosts supported limited
reactivation of estrous cycles (68, 78) (Fig. 6). Nearly all
older mice receiving young grafts had persistently cor-
nified vaginal smears which indicate the presence of
circulating estrogens secreted by a successfully vascular-
ized ovary. The subset of old mice which cycled during
the first 3 months after grafting had longer cycles than
in young-to-young control transplants. The ability of old
acyclic mice to regain cycles with young ovaries seems to
be significantly greater than reported for rats, but the
poorer performance of young grafts in old rats cannot
be regarded as definitive evidence for a species difference
when the concerns of immune interference in partly
inbred rats are considered as noted above.

Thus, the limited ability of young ovaries grafted into
acyclic 16- to 30-month-old C57BL/6J mice to reactivate
cycles supports the hypothesis for age-related hypotha-
lamic-pituitary impairments in acyclic rodents. As
described below, the substantial evidence for impaired reg-
ulation of LH during aging is consistent with the limited
ability of young ovaries to reanimate cycles in old ro-
dents.

2. Regulation of LH a, LH surge. During the course of
reproductive aging, major impairments are observed in
the regulation of LH. In rats, control of the E_2-induced
LH surge and the pulse frequency of LH release resides
in the hypothalamus and preoptic regions (3), whereas,
the negative feedback of E_2 on LH also may involve the
pituitary (79, 80). Thus, age changes in the LH surge of
rodents have particular bearing on the possibility of
neural age changes.

The proestrous (nocturnal) LH surge becomes pro-
gressively altered with age in its timing and amplitude
according to most reports. In older rats, LH surges tend
to be more variable and begin later in proestrus as judged
by direct observations of LH (66, 81–85) and by indirect
evidence that injection of pentobarbitone early on proes-
trous afternoon is less likely to boc ovulation (86). The
amplitude of LH elevations also decreases during aging
to variable extents. Although serial samplings for LH
have not been done in the same individual rodent during
aging across different estrous cycles, it is possible that
LH surges with altered timing and amplitude can alter-
nate with normal surges. However, correlations between
impairments in the gonadotropin surge and the length-
ening of ovulatory cycles do not demonstrate causality
since ovulation is inducible with a small fraction (<25%)
of the normal surge of gonadotropins (87). Moreover,
since aging rats with 4-day cycles can also have smaller
proestrous LH surges (81), the lengthening of cycles may
prove to be a separate event, unrelated to initial reduc-
tions in the LH surge.

The steroid-induced LH surge in ovariectomized ro-
dents shows progressive impairments during aging that
approximate the diminishing LH surges at proestrus (Fig. 5). These findings suggest that aging rodents become less sensitive to effects of E₂ on neuroendocrine loci. We developed a protocol for inducing the LH surge in ovariectomized mice with implants replacing E₂ only (88). Because of age changes of progesterone metabolism in the pituitary (89) and evidence that the levels of circulating E₂, rather than progesterone, trigger the LH surge (88), we wished to avoid confounds from age effects on progesterone, which is often injected to induce the LH surge in E₂-primed rodents. Though a proestrus-like LH surge is induced by injecting progesterone on the morning before the nocturnal surge in E₂-primed mice (90) and though progesterone can facilitate the surge after its onset, the absence of progesterone elevations on proestrus before the LH surge (82) indicated that the LH surge induced solely by E₂ elevations most closely resembles the initial phase of the proestrous surge.

Analysis of age effects in the E₂- and progesterone-induced LH surge is also complicated by the ability of injected progesterone to reinitiate estrous cycles in old rats (16, 61, 62).

Aging mice (40; Fig. 5) and rats (91) in persistent vaginal cornification have very small LH surges, as induced after acute ovariectomy by E₂, or by E₂ and progesterone. However, when aging rats in persistent vaginal cornification subsequently enter repetitive pseudopregnancy, or if pseudopregnancy is interrupted by progesterone treatment (91, 92), the size of the LH surge induced by the combination of E₂ and progesterone is considerably increased. If rodents in persistent vaginal cornification are ovariectomized and tested after 1 month, then the induced LH surge also recovers remarkably (40, 91). The age-related impairments of the LH surge are thus labile and strongly dependent on the preceding endocrine status as well as on the mode of surge induction. The basis for maintenance of the impairments in the LH surge during persistent vaginal cornification appears to be a desensitization or down-regulation of the hypothalamic-pituitary response to E₂ in association with the sustained but moderate output of E₂ by the polyfollicular ovaries (54), (Section VI).

Impairments of the E₂-induced LH surge could arise from several factors including: inhibition by prolactinemia, an altered threshold to induction of the surge by E₂, altered neuroendocrine sensitivity to E₂, decreased secretion of LH or LH releasing hormone (GnRH) during the surge, or these in some combination. Firstly, the diminishing LH surge of aging mice does not seem to be a consequence of prolactin elevations such as are common in aging rats (52–54, 84). In 12-month-old C57BL/6J mice the proestrus surge is greatly reduced and prolactin is not elevated just after the onset of persistent vaginal cornification (82).

Hoffman (93) has suggested that age changes in the steroid-induced LH surge might account for the onset of acyclicity in aging rats. At the time of the nocturnal LH surge, rodents usually show either greatly elevated LH, presumably because the estrogenic stimulation exceeds some threshold, or have no LH elevation at all. Similar phenomena occur in monkeys (4). We recently examined (40) whether age changes occur in the threshold for LH surge. Because both the magnitude of E₂-induced LH surges and the fraction of mice with surges decrease with aging, we infer that the impairments of the LH surges are not due only to an increased threshold for surge induction by E₂. Another possible cause derives from the stringent requirements shown by mice for E₂ priming within which circulating E₂ permits surge induction after further E₂ elevations (88, 90). However, priming concentrations of E₂ slightly above or below a range of ±10 pg E₂/ml plasma prevent subsequent LH surges (88). Thus, impairments in the surge could result from either increased or decreased neuroendocrine sensitivity to E₂ during priming (Section III.B.2). A decreased capacity for GnRH secretion during the LH surge is another possibility for which there is some evidence as discussed below.
b. Postovariectomy LH secretion. Elevations of LH after ovariectomy in previously acyclic, old rodents are about 30% lower than in young ovariectomized rodents (21, 53, 94). This deficiency can be detected sometimes in middle-aged (previously) cycling rats (95). Elevations in FSH after ovariectomy, however, are generally impaired (83).

Since LH is secreted in pulses, either lower frequency or amplitude of GnRH secretion could lead to lower average LH concentrations. Age-correlated decreases in pulse amplitude would then suggest less GnRH per pulse or smaller pituitary responses to GnRH. On the other hand, lower frequencies are most likely to be due to neural changes. Reports conflict on this issue. Steger et al. (96) found that age-correlated deficits detected 5 weeks after ovariectomy are due primarily to lower pulse amplitude, whereas, Estes and Simpkins (97) found that 2 weeks after ovariectomy the impairment is due primarily to pulse frequency. Old male rats (98) and male mice (99) also have a decreased frequency of LH pulses. These discrepancies may be due to the different times after ovariectomy at which LH pulses were examined. Between 2 and 8 days after ovariectomy, LH pulse frequency increases, whereas, LH pulse amplitude remains constant (79). However, between 8 days and 21 days after ovariectomy, LH pulse frequency becomes stable, whereas LH pulses were examined. Between 2 and 8 days after ovariectomy, LH pulse frequency increases, whereas, LH pulse amplitude remains constant (79). However, between 8 days and 21 days after ovariectomy, LH pulse frequency becomes stable, whereas LH pulse amplitude increases. Therefore, older animals may have a delayed increase in the frequency of LH pulses after ovariectomy with an underlying deficit in pulse amplitude that is apparent only when the frequency component becomes stable. The rapid reinstatement of normal pulse frequency and amplitude in middle aged rats by clonidine (97), an $\alpha$-adrenergic agonist, is consistent with the ability of some centrally acting monoaminergic drugs to reactivate estrous cycles in old rats (Section III A) and supports the view that altered hypothalamic neurotransmitter functions during aging contribute to the loss of cyclicity (26, 100, 101). Furthermore, these data suggest that the GnRH-secreting neurons are not intrinsically impaired with age but that the neural signals driving them are altered.

The size of the LH pulse resulting from direct hypothalamic stimulation by implanted electrodes, presumably causing release of GnRH, gives another measure of the neural aging. Stimulation by electrodes in the arcuate-median eminence or medial preoptic area for 60 min did not reveal age differences in LH output, whereas, with 120 min of stimulation, old rats had smaller LH elevations (102).

c. Suppression of LH by E$_2$ (negative feedback). In ovariectomized mice given a series of E$_2$ implants yielding a physiological range of E$_2$ concentrations, the ability of E$_2$ to suppress LH circulating ($\Delta$LH/$\Delta$E$_2$) decreased with age (12 and 18 months vs. 6 months). Thus, a given amount of E$_2$ reduced LH by a smaller absolute amount in old mice than in young mice$^2$. This decreased sensitivity of negative feedback to E$_2$ might be another aspect of the decreased ability of E$_2$ to induce LH surges in aging rodents. In contrast, the sensitivity to E$_2$ with respect to prolactin secretion increased with age; that is, a given increment of E$_2$ increased circulating prolactin more in old mice than in young. Similar age changes in the response of LH and prolactin secretion were observed in short term cultures of pituitary cells obtained from 6- and 24-month-old rats (103). Previous studies of age effects on negative feedback gave divergent results. Suppression of postovariectomy elevations of LH by E$_2$ in rats was impaired according to one study (95) but not in others (103, 104). E$_2$ injections did, however, provoke larger increases of prolactin in old female rats (104), as in C57BL/6J mice. The decreased sensitivity to E$_2$ with respect to LH suppression may be an aspect of the smaller E$_2$-induced LH surges in older rodents.

3. Pituitary responses to GnRH. Age effects in pituitary responses to GnRH are variable and are influenced by the particular endocrine state and probably also by spontaneous pituitary tumors of various sizes. A recent study did not detect age changes in the output of LH in response to a range of different GnRH doses in vivo if rats were matched for vaginal smear patterns (105). Thus, endocrine status is at least as important as chronologic age in determining pituitary responsiveness to GnRH. However, this same study detected impaired responses to GnRH in middle-aged rats on the afternoon of proestrus, just before the LH surge (105). Additionally, pituitary GnRH receptors may be decreased in acyclic aging rats (106). The issue of age changes in pituitary responses to GnRH thus remains open and prevents resolution of the relative contributions from hypothalamic vs. pituitary changes in the diminishing LH surge with age.

4. Pituitary tumors. A major characteristic of aging in laboratory rats and mice, particularly in females, is the increase of gross, spontaneous pituitary tumors from less than 1% before 9 months to more than 50% by 24 months (27, 107–109). Pituitary tumors grow slowly and, though not metastatic (110), can become so massive as to press upwards and distort the hypothalamus (111). In general, visible tumors first appear in aging rodent populations as they enter persistent vaginal cornification. Multiple, 

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$^2$ See note added in proof, p.497.
small nests of hypertrophic lactotrophs are common. Most tumors consist of lactotrophs, but somatotropic adenomas are 1–10% of the tumors in C57BL/6J mice as shown by specific immunocytochemical reactions (112). The microscopic adenomas may be precursors of gross tumors but the complete sequence of tumorigenesis is not known (110, 112). The sustained elevations of \( E_2 \) during persistent vaginal cornification is probably a major factor in tumorigenesis because: exogenous \( E_2 \) can induce similar tumors in young rodents (Section VA); ovariectomy of old rats in persistent vaginal cornification abolishes their prolactinemia (51), and ovariectomy of rodents when young reduces the incidence of pituitary tumors (Table 1).

The prolactin secreted by the enlarging pituitary during tumorigenesis is hypothesized to be a major cause of the repetitive pseudopregnancies which occur after some months of persistent vaginal cornification in aging rats (61, 113). Extreme hyperprolactinemia, more than 500 ng/ml, is common in 2-year-old noncyclic (anestrus) rats with gross pituitary tumors (53) but is less frequent in C57BL/6J mice (82, 114). A parallel species difference is that old male C57BL/6J mice almost never develop spontaneous gross pituitary tumors (26) and that circulating prolactin tends to be unchanged during their lifespan (115). Old male rats of some strains frequently have pituitary tumors (27) and elevated prolactin (116) but to a lesser extent than females of the same ages (117).

Lactotropic adenomas have a distinctive regional distribution in aging female C57BL/6J mice. Most visible tumors occur in the lateral tips or lateral zones of the pituitary; few occur in its center near the stalk unless they are large and have expanded in from the side (M.N. Gordon, J.E. Schechter, L.S. Felicio and C.E. Finch, in preparation). This lateral predominance is interesting because the lateral portal vessels have a lower dopamine concentration than medial vessels in rats (118). In view of the ability of dopamine to inhibit prolactin secretion (119), the higher incidence of lactotropic adenomas in the lateral pituitary regions could be related to relatively lower portal blood dopamine in the lateral vessels. We postulate that low portal blood dopamine is permissive for the initiation or growth of lactotropic adenomas and that age-related reductions in portal blood dopamine (118, 120) may be a factor in tumor growth.

5. Hypothalamus. a. Neuron number and morphology. Effects of age on hypothalamic cell number and morphology in female rodents are not well established. Most studies of the aging rodent brain agree that substantial neuronal loss is unusual and restricted to a few regions (for general reviews of aging neuronal morphology and cell loss in rodents see refs 121–123). Present data on aging female rodents give a varied picture. One study described a 30% loss of arcuate and medial preoptic nucleus neurons in 2-year-old rats (124), while others found no age change in total neurons of the medial arcuate and preoptic nucleus of 18-month-old hamsters (125, 126). Reproductive aging in hamsters may be different than in mice and rats, since most hamsters maintain regular 4-day cycles until nearly 2 years old despite major loss of fertility at 1 year as in the other laboratory rodents (127, 128). Information on GnRH-containing neurons is limited but no major cell loss was detected in acyclic aging rats (129). Similarly, the hypothalamic content of GnRH was not altered in aging ovariectomized rats as analyzed by two antibodies with different specificities (130).2

### Table 1. Manipulations of reproductive aging: ovarian- and \( E_2 \)-induced neuroendocrine aging syndromes of rodents

<table>
<thead>
<tr>
<th>Markers of reproductive aging</th>
<th>Delayed, by chronic ovariectomy in aging rodents</th>
<th>Accelerated, by chronic ( E_2 ) in young rodents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovarian cycles lengthened</td>
<td>Ref. 70*</td>
<td>Not known</td>
</tr>
<tr>
<td>2. Ovarian cycles lost</td>
<td>Refs. 5, 68*</td>
<td>Refs. 47, 162, 165</td>
</tr>
<tr>
<td>3. Smaller ( E_2 )-induced LH surge</td>
<td>Refs. 40, 94</td>
<td>Ref. 47</td>
</tr>
<tr>
<td>4. Pulsatile release of LH</td>
<td>Ref. 94</td>
<td>Not known</td>
</tr>
<tr>
<td>5. Smaller postovariectomy LH elevations</td>
<td>Refs. 88, 94</td>
<td>Ref. 47</td>
</tr>
<tr>
<td>6. Decreased negative feedback sensitivity of ( E_2 ) on LH</td>
<td>See note added in proofs, p. 497</td>
<td>Not known</td>
</tr>
<tr>
<td>7. Glial hyperactivity in arcuate nucleus</td>
<td>Ref. 137</td>
<td>Refs. 163, 165</td>
</tr>
<tr>
<td>8. Reduced pituitary stalk blood dopamine</td>
<td>Not known</td>
<td>Ref. 285</td>
</tr>
<tr>
<td>9. Increased pituitary dopamine</td>
<td>Ref. 150</td>
<td>Ref. 150</td>
</tr>
<tr>
<td>10. Increased pituitary glucose-6 phosphate dehydrogenase</td>
<td>Ref. 151</td>
<td>Ref. 151</td>
</tr>
<tr>
<td>11. Lactotroph adenomas</td>
<td>Refs. 40, 68, 114, 117</td>
<td>Refs. 163, 167, 229</td>
</tr>
<tr>
<td>12. Increased number of lactotrophs</td>
<td>Ref. 117</td>
<td>Ref. 138</td>
</tr>
<tr>
<td>13. Prolactinemia</td>
<td>Refs. 114, 117</td>
<td>Ref. 286</td>
</tr>
</tbody>
</table>

* Assayed by performance of young ovarian grafts.
Golgi staining studies of male and female C57BL/6J mice at the average lifespan indicate various degenerative changes including loss of dendritic spines, localized neuronal irregularities, swollen and distorted cell bodies, and decreased vascular branching in the hypothalamus: "neuropil impoverishment seems ... greater ... in rostral hypothalamus". No sex differences were noted (131).

b. Estradiol-binding sites. Most interesting is the 50% loss in 20-month-old hamsters of E2-binding capacity by arcuate neurons ([3H]E2 was injected into the vena cava of hamsters ovariectomized for 2 weeks). Since total neuronal density was unchanged and the density of autoradiographic grains was unaltered by aging in those neurons that did bind [3H]E2 (126), the intriguing possibility arises that E2 receptors in select arcuate neurons are lost with aging, without immediate neuronal death. A 30% decrease of in vitro E2 binding by brain nuclei was found in preoptic and in medial basal hypothalamic regions of 16- vs. 8-months-old (still cycling) Sprague-Dawley rats (132). The studies of rats and hamsters are consistent since the arcuate nucleus neurons are probably major contributors to E2-binding sites in the medial basal hypothalamus.

A major concern about E2 receptor measurements has recently arisen. Two independent approaches challenge the hypothesis that E2 binds to cytoplasmic receptors that subsequently translocate to the nucleus. The presence of major E2-binding proteins in the cytosol may be a result of nuclear leakage during preparation of nuclear fractions. The E2 receptor, whether occupied by E2 or not, appears to remain in the nucleus of pituitary cells and in some other E2 target cells (133, 134). It now must be asked if the above age-related reductions of nuclear binding for E2 result from a reduced stability of the nuclear E2 receptor complex. The equilibrium dissociation constant (Kd) for the binding of E2 to the nuclear receptor complex was not altered by age in the female rat pituitary or hypothalamus (132) but effects of age on the affinity and metabolic stability of the E2 receptor chromatin complex remain a possible explanation for reduced numbers of binding sites.

c. Glial hyperactivity. Increased glial activities are widely observed in the aging rodent brain (135, 136) and also occur in the hypothalamic arcuate nucleus of female Wistar rats and C57BL/6J mice (137). Hyperactive astrocytes and reactive microglia increase severalfold by 9–14 months in the cell-sparse region lateral to the arcuate nucleus, which contains many fine unmyelinated axons (137, 138). Although no study has yet established that spontaneous increases of glial activities during aging are a direct response to neuronal degeneration, similar changes in glial morphology are often seen in Huntingtonism and other neurodegenerative diseases (139) with more extensive neuronal loss than usually occurs during aging. The age-related increase of glial activities in the arcuate region appears to be an E2-dependent aging phenomenon, as discussed below.

d. Neurochemical changes. 1) Hypothalamus. Limited data is available on age-related changes in neurotransmitter functions in the female rodent but changes are observed in the three major hypothalamic monoamines: dopamine, norepinephrine, and serotonin. Because the metabolism of hypothalamic monamines and opioids is exquisitely sensitive to endocrine influences (3, 119, 140, 141), the differences in reproductive endocrine states between individuals during aging (Section II) make it desirable in neurochemical studies of aging to subdivide age groups carefully according to immediate hormonal status and history, where possible. The importance of the endocrine state on neurochemical age changes is suggested by brief reports that turnover of norepinephrine in medial basal hypothalamus is accelerated in the old repetitively pseudopregnant rat, but is slowed during persistent vaginal cornification (142, 143). Thus, neurochemical changes in old female rodents may be consequences, rather than causes, of acyclicity.

Persistent vaginal cornification in old rats is associated with elevated hypothalamic serotonin (20%) and with a possible damping of the daytime serotonin rhythm (65). Catecholamine levels and turnover are not uniformly altered during aging in hypothalamic regions of male or female rodents; reduced dopamine levels and synthesis are often seen in the median eminence and basal medial hypothalamus (101). Substantial deficits are observed in concentrations and synthesis of median eminence dopamine in noncycling old rats (144, 145). The reduction in concentrations of portal blood dopamine with age in male (116) and female rats (118, 120) approximates the reduction of hypothalamic dopamine synthesis (143). Moreover, aging rats have an impaired feedback of prolactin upon hypothalamic control over portal blood dopamine (146) and impairments in the short loop feedback of prolactin upon the hypothalamic control of prolactin (147). Considered together, these findings point to impairments in the responsiveness and output of the tuberoinfundibular dopaminergic neurons in the medial basal hypothalamus; these neurons secrete dopamine into the portal blood and are crucial to prolactin regulation (118, 119).

Age changes in catecholamine metabolism can be detected in rats before loss of cycles. The increase of norepinephrine turnover normally associated with the spontaneous or steroid-induced LH surge is reduced in some brain regions of middle aged rats (101). Most noteworthy is the failure of norepinephrine turnover to increase during steroid treatment in the medial preoptic
and suprachiasmic nuclei. These impairments in the regulation of hypothalamic norepinephrine by steroids are consistent with the lower activity of hypothalamic dopamine-β-hydroxylase and the smaller increases of the enzyme on proestrus in aging C57BL/6NNia mice (148) which may be linked to the diminishing LH surge described during aging in this same colony (50). We note that in male C57BL/6J mice, decreased catecholamine levels and turnover during aging are restricted to a few brain regions (medial basal hypothalamus, but not preoptic area) and are not detected until late in the average lifespan (149). Thus, changes in hypothalamic catecholamines that are independent of the ovary may not occur until long after the major events of reproductive senescence.

2) Pituitary. Dopamine concentration increases manyfold during aging in the anterior pituitary of female rats (144) and C57BL/6J mice (150). A much smaller increase also occurs at later ages in male mice (149). The increased dopamine concentration in superficially normal pituitaries (150) generally parallels the 40% increase of lactotrophs between 3 and 12 months in Wistar rats without gross tumors (117). Similar trends for increased numbers of lactotrophs are indicated in C57BL/6J mice (107). Another marker is the activity of pituitary glucose-6-phosphate dehydrogenase which increases with age by about 20% in 6- vs. 20-month-old female C57BL/6J mice but not in aging males (151). A relationship of these changes to tumorigenesis seems possible but is not known. These biochemical changes are useful markers for steroidal manipulations (Section VI A).

IV. Ovary-Dependent Age Changes in the Hypothalamus and Pituitary

A. Delay of age-related change by long term ovariectomy

Aschheim’s original observations on a limited number of rats suggested that persistent estrus rats aged 2 years did not regain cycles with young grafts unless the old hosts were ovariectomized when young and allowed to age in the absence of ovaries (5). Since old ovaries could support some cycles when grafted to young hosts, this study set two cornerstones for the neuroendocrinology of aging by providing a basis for the hypotheses: 1) that an age change occurs in the hypothalamic sensitivity to ovarian steroids which impairs its capacity to drive ovarian cycles, and 2) that in the absence of the ovary, hypothalamic aging is attenuated “C’est donc l’hypothalamus qui est reste ‘jeune’ sans doute grace a la castration . . . “.

In further confirmation of Aschheim, we observed that long term ovariectomy of C57BL/6J mice dramatically increased their ability at midlife or later to cycle with young ovarian grafts as judged from vaginal smear cytology (68) (Fig. 6). Histologic studies proved that ovulation occurred during these cycles, evidenced by the presence of ova in the fluid filled space between graft and the renal capsule as well as by recent corpora lutea and growing follicles in the graft itself. Normal numbers of corpora lutea were formed in those long term ovariectomized mice that regained cycles with young ovaries. Additionally, elevations of plasma LH on proestrus evening were similar in young-to-young controls and in long term ovariectomized, 24-month-old mice with young ovarian grafts (68). Thus, long term ovariectomy can preserve the capacity for cyclic LH release until the average lifespan, i.e. 6 to 12 months beyond the usual age of its disappearance in intact C57BL/6J mice. The number of cycles in long term ovariectomized mice that were given young ovaries when 16 months old was close to (80%) the number of cycles achieved by young-to-young controls (Fig. 6). Moreover, in long term ovariectomized hosts observed during the first month after grafting, the cycles were short as seen in the young controls (68, 70; L.S. Felicio, J.F. Nelson, C.E. Finch, in preparation). This result shows that the neuroendocrine potential for supporting short ovulatory cycles is preserved by long term ovariectomy, at least beyond middle age.

An extensive list of neuroendocrine age changes that are retarded or prevented by long term ovariectomy is compiled in Table 1. We propose that the hypothalamic and pituitary age changes of Table 1 constitute an ovary-dependent neuroendocrine syndrome of aging, since their appearance during aging in female rodents is dependent on the presence of the ovary for some number of preceding months. As described in Section VI B, most features of the ovary-dependent neuroendocrine aging syndrome can be induced prematurely in young rodents by sustained exposure to E₂. We caution that there may be important species and genotypic differences in these phenomena; nonetheless, impairments of LH regulation and pituitary tumors are widely found during aging in most laboratory rodent populations.

The time course of the ovary-dependent changes is partially known. In C57BL/6J mice, dysfunctions in the regulation of LH and glial hyperactivity are concurrent with the lengthening and loss of cycles (10-14 months). Visible pituitary tumors and prolactinemia are a later event, arising after persistent vaginal cornification has lasted 3-5 months in the population. As the time course of the onset and progression of these aging changes becomes known in greater detail, it should be possible to pose detailed hypotheses with quantitative predictions about the potential interrelationships of these changes. For example, biochemical and morphological changes in
the pituitary and subsequent appearance of gross tumors may be a function of specific durations of E2 receptor occupancy.

B. Hypotheses about the cumulative impact of steroids during the ovary-dependent neuroendocrine syndrome of aging

The effects of long term ovariectomy in attenuating hypothalamic-pituitary age changes in Table 1 suggest that some or all of these are dependent on long term exposure to ovarian steroids. Since most of these changes are precociously induced in young rodents by E2 treatment (described in Section VI A), we proposed that the ovarian-steroid dependent neuroendocrine syndrome of aging in rodents results from the cumulative impact of ovarian steroids on the hypothalamus-pituitary during some or all of the estrous cycles (152). It seemed likely a priori that: a) the presence of the ovary is required for considerably more than one cycle or 1 day to cause these effects, or b) that a cumulative property of the strength-duration product for E2 exposure could be determined.

We initially considered an exploratory hypothesis (Fig. 7) that a cumulative effect of ovarian steroids on neuroendocrine loci is integrated over some or all cycles to cause a decreasing sensitivity of LH regulation to E2 (152). Experiments to test features implied by this initial hypothesis are described below.

Effects of long term ovariectomy are predicted by this model. If the removal of ovarian steroids by ovariectomy attenuates ovary-dependent neuroendocrine aging, then replacement of the ovaries at later ages should reactivate these processes. Therefore, young ovaries were grafted to middle aged, long term ovariectomized hosts, which were 16 months old, an age when cycles would normally have ceased. As predicted, cycles of normal length (4-6 day) were reinitiated and continued for about 5 months but eventually lengthened and ceased (Fig. 6) (L.S. Felicio, J.F. Nelson, and C.E. Finch, in preparation). Pituitary tumors occurred in some of the grafted long term ovariectomized mice a few months later at the termination of cycling (68) but were many fewer than intact old controls (109). Moreover, ovariectomy at puberty eliminated the occurrence of pituitary tumors in rats aged 23 months (117). Thus replacement of ovaries appeared to reactivate the ovary-dependent aging syndrome.

The above findings that ovary-dependent neuroendocrine aging can be interrupted and can be reactivated later then lead us to examine whether the ovary-dependent neuroendocrine aging processes are cumulative over all estrous cycles. The exploratory cumulative steroid hypothesis (Fig. 7) predicts that the number of cycles would be limited and not extendable by replacing the mouse's aging ovaries at the approach of acyclicity. We chose a subgroup of cycling 12-month-old mice as hosts to avoid confounds from the noncycling status, such as the neuroendocrine desensitization in some responses of LH regulation to E2 which arises from sustained presence of E2 associated with persistent vaginal cornification or chronic E2 treatments (40, 88, 91; Section VI A). Therefore, ovaries from 3-month-old donors were grafted to 12-month old, still cycling mice. A striking result was obtained. The middle-aged hosts with young ovaries continued to cycle for 8 months or more, thereby nearly doubling the normal number of cycles achieved by intact mice (Fig. 8); similar extensions of regular cycling were observed in a separate study (C.V. Mobbs, L.S. Kannegieter, and C.E. Finch, in preparation). By contrast, a previous study (Fig. 6, replotted in Fig. 8) showed that slightly older acyclic hosts had very limited ability to support cycles with young grafted ovaries. This result conclusively negates the possibility that an absolute neuroendocrine counting mechanism limits the number of estrous cycles and determines the normal onset of acyclicity during aging in this strain. In turn, this result suggests further hypotheses: a) that the capacity for cycling in aging C57BL/6J mice is ultimately limited by the number of ovarian follicles, as is consistent with the imminent exhaustion of ovarian follicles by 13-14 months (34; Fig. 1); and b) that a major phase of ovary-dependent neuroendocrine damage occurs between 12 months when most mice are still cycling, and 16 months when most mice have been acyclic for 1–2 months.

The early changes of the ovary-dependent neuroendocrine syndrome leading to acyclicity may require only
FIG. 8. Examination of whether or not ovary-induced hypothalamic damage is cumulative over all cycles in C57BL/6J mice. If so, replacement of ovaries in currently cycling mice at midlife with young ovarian grafts (just before loss of cyclicity) should yield few additional cycles. The top panel shows the limited ability of young ovaries to reactivate cycles in 16-month-old hosts as determined from longitudinal studies of daily vaginal smears (from Fig. 6, ref. #68). The bottom panel from a subsequent study shows that slightly younger 12-month-old currently cycling hosts nearly doubled their normal number of cycles and duration of cycles with ovarian grafts from 3-month-old donors (L.S. Felicio, J.F. Nelson, and C.E. Finch, in preparation). We propose that ovary-induced neuroendocrine damage is not cumulative over all cycles and is limited to the phase of ovarian aging when cycles are prolonged.

2-4 months out of the usual 8-12-month-long duration of cycling. The transition to lengthening cycles and persistent vaginal cornification during aging usually takes 2-4 months (10). During this time, there are increases in the ratio of plasma $E_2$:progesterone which may be important in the mechanism of ovary-induced neuroendocrine aging (Fig. 9; Section IX). The increased ratio of $E_2$:progesterone during aging includes deficits of progesterone at proestrus and may arise from ovarian age changes since transplant studies suggest that the deficit of progesterone at proestrus in 12-month-old mice is reversed by grafting of a 3-month-old ovary (L.S. Felicio, J.F. Nelson, C.E. Finch, in preparation). Thus, we hypothesize that aging changes in the ovary are a major factor in the ovary-dependent neuroendocrine aging syndrome. However, the ovary also appears to induce neuroendocrine changes during the phase of regular cycling, since young ovarian grafts do not restore the incidence of short cycles in 12-month-old cycling hosts (40, 70) unless they were ovariectomized when young (70).

The major increases of $E_2$ and other steroids during pregnancy may also interact with neuroendocrine aging since multiparous C57BL/6J females ceased cycling 3 months before virgin mice, as observed from parallel groups (36). In contrast, the loss of ovarian oocytes during aging in other strains of mice is little influenced by multiparity (8).

Finally, we note that effects of chronic tryptophan restriction may also be compared with chronic ovariectomy. Extreme chronic deficiencies of this essential amino acid preserve the potential for fecundity during aging in rats (153). When tryptophan deficient rats were given a normal diet at middle age, they soon achieved the appearance of a normal young adult; upon mating, they could become pregnant even when 28 months old and reared litters of 3-7 pups. Controls lost fertility at 17 months as expected. Extreme deficiencies of tryptophan resemble effects of hypophysectomy on growth (154). Like surgical hypophysectomy (155), extreme dietary restriction may retard ovarian oocyte loss and also may reduce the secretion of ovarian steroids.

C. Reversal of age-related changes

A potential for reversing the age-related neuroendocrine syndrome was suggested by the recovery of the LH surge in aging rats after ovariectomy for several months or after the transition from persistent vaginal cornification to repetitive pseudopregnancy (Section III B2). Since a single experimentally-induced LH surge cannot assay the potential for supporting a series of estrous cycles.

FIG. 9. The ratio of plasma estradiol ($E_2$)/progesterone (P) of C57BL/6J mice that are cycling (4 months old) and or middle aged (12 months old) in persistent vaginal cornification. (Redrawn from Biol. Reprod. 24:784, 1981, J.F. Nelson, et al.).
cycles, we therefore also investigated the effects of ovariectomizing aging mice on their ability to cycle with young ovarian grafts (40). As expected, ovariectomy of acyclic, 18 month old, persistently cornified hosts for 2 months greatly enhanced their ability to cycle with young ovarian grafts as judged by the total number of cycles (Fig. 10) and the incidence of short cycles (40). However, ovariectomy for 2 months had little effect on cycling 12-month-old hosts as monitored by the fraction of mice that regained regular estrous cycles, by the incidence of short cycles, and by the total number of cycles (Fig. 10).3 Because the 18-month-old mice were acyclic, whereas those 12 months old were cycling when given young ovaries, we cannot determine if the partial recovery of cyclicity by ovariectomy for 2 months derives only from age, or from the acyclic endocrine state of persistent vaginal cornification. The E2-induced LH surge of 18-month-old acyclic mice also recovered considerably after ovariectomy for 2 months and was indistinguishable from LH surges of 16-month-old mice ovariectomized when 6 months old. Since ovariectomy for 1–2 months also substantially increased the E2-induced LH surge of young mice (40, 88), the duration of ovariectomy is crucial in choosing controls for such studies.

These results suggest that ovary-dependent neuroendocrine impairments occur in two stages. Exposure to ovarian secretions during estrous cycles causes some neuroendocrine impairments by middle age that may be a cause of cycle lengthening. Cycle lengthening at middle age is not reversed by ovariectomy for 2 months (40), but does not occur if 12-month-old hosts are ovariectomized when young (70). After estrous cycles cease, further exposure to secretions (probably E2, discussed in Section VI A) from the polyfollicular, acyclic ovaries causes additional neuroendocrine impairments. Some of these additional impairments are reversed by prolonged ovariectomy, as shown by recovery of the LH surge and the ability to support cycles with young ovarian grafts.

There is also a major influence of ovariectomy on pituitary tumors. Eighteen-month-old mice, ovariectomized for 2 months before grafting with young ovaries, subsequently had 70% fewer pituitary tumors when 24 months old than those given young ovarian grafts without delay (40). Thus, even temporary removal of ovarian influences can greatly reduce pituitary tumorigenesis, long after ovarian replacement.

Pseudopregnant-like smear sequences occurred in about 20% of 13–21 month old mice with young ovarian grafts, whereas intact controls rarely (<4%) showed pseudopregnancy (40). Pseudopregnancy is very common in aging rats (Section II) and its rarity in aging C57BL/6J mice may be consequent to their greater depletion of oocytes, compared with rats at the onset of acyclicity (73, 156). In many regards, the neuroendocrine aging changes of female C57BL/6J mice and laboratory rats seem similar, e.g. impaired LH surges, smaller postovariectomy LH elevations, and lactotroph adenomas. However, in view of differences between young mice and rats in the extent of sex differences of hypothalamic anatomy (157) and in the regulation of LH (88), it is not surprising to find some species differences in neuroendocrine aging phenomena. Another example is the greater effects of age on the tuberoinfundibular dopaminergic neurons (A12 group) of old male Fischer 344 rats vs. C57BL/6NNia mice (158).

V. Ovary-Independent Age Changes

The above studies demonstrate phenomena of neuroendocrine aging that depend on the presence of the ovary during aging for their development or their maintenance. However, long term ovariectomy does not completely attenuate neuroendocrine aging since 24- to 30-month-old hosts (at the average lifespan) that were ovariectomized when young can support only a few ovulatory cycles with young ovarian grafts as described above. We do not know what causes this ovary-independent neuroendocrine change but note the likely influence of age-related diseases which can impinge on neuroendocrine impairments.
functions. Most mice or rats at the average lifespan have some gross pathologic lesions or nonspecific frailty (Section II). With the exception of pituitary tumors, age-related pathologic lesions probably are not greatly reduced by chronic ovariectomy but little information is available. Also adrenal estrogens might still be sufficient to cumulatively influence the hypothalamus even though vaginal cells may remain hypotrophic.

VI. Estrogen-Induced Infertility Syndromes of Adults

The concept of a critical period during development for the organizing influences of steroids on the sexual differentiation of the rodent nervous system is well established (6, 159–161). E$_2$ and aromatizable androgens, whether endogenous or injected, can influence hypothalamic neuronal number and connections during a critical period that extends from late fetal development until about 10 days after birth. As a result, males and females have distinct differences in hypothalamic neuroanatomy and in the regulation of LH, for example. However, it is now clear that chronic exposure of adult rodents and sheep to estrogens can also have irreversible effects on neuroendocrine functions as manifested by infertility syndromes that are similar in some regards to aging changes.

A. Rodents

Depot injections of 2.5 mg E$_2$-benzoate caused young adult (70 day old) rats to become anovulatory and remain in a persistent vaginal cornification lasting for 2 to 5 months (161). These effects of E$_2$ were permanent in some individuals and led Brown-Grant (161) to conclude “...that the concept of an absolute 'critical period' during development after which the neural mechanisms cannot be permanently affected by steroid hormones needs to be modified". This conclusion is also supported by the early study of Kawashima (162). During daily sc injections of E$_2$ in minute 0.01 µg doses, young adult rats maintained regular 4 day cycles for several months; however, cycles eventually ceased prematurely after 3–4 months and rats became persistently estrous or diestrous. Subsequent studies by Brawer et al. demonstrate directly that treatment of adult rats with chronic E$_2$ alters hypothalamic cell structures causing glial hyperactivities in the arcuate nucleus region with accompanying degenerated axons and axonal endings, myelin figures, and lipofuscin in scattered neurons of the arcuate nucleus (163).

Several studies showed that sc depot injection of E$_2$-valerate initially lead to supraphysiologic E$_2$ levels which returned to basal values by 4–8 weeks in rats (164) and mice (47). Histopathologic changes in rats were seen in the hypothalamus at sacrifice 6 months later (165). Sustained but physiological levels of E$_2$ (20 pg E$_2$/ml plasma) for 1.5 month also cause a permanent anovulatory syndrome in about 50% of adult mice (47). It is noteworthy that the 50% of mice which regained cycling after removal of the E$_2$ implant had about 40% fewer cycles than sham-implanted controls (47). Glial hyperactivity in the arcuate region of rats is also caused by a 3-month exposure to E$_2$ in the physiological range (165). Thus, sustained physiological levels of E$_2$ can cause cryptic changes in mice which have a delayed effect leading to premature loss of cycles and onset of persistent vaginal cornification. These changes are primarily neuroendocrine rather than ovarian since transplant studies showed that the ovary was not damaged by E$_2$-valerate treatment and cycled at least as well as control ovaries of the same age when grafted to young mice (47). In contrast, young ovaries did not cycle in hosts that were treated with E$_2$-valerate 8 months before; also, hCG readily induced ovulation (47). The concern about ovarian damage by the E$_2$-valerate is based on observations that exposure of neonates to E$_2$ or androgens can cause long lasting ovarian impairments in adults (161; C.V. Mobbs, L.S. Kannegieter, and C.E. Finch, in preparation).

In addition to histopathologic changes, the E$_2$-induced LH surge was markedly impaired, even 6 months after the E$_2$-valerate depot injection had cleared (Fig. 11); at this time, no mice had elevated blood prolactin concentrations or gross pituitary tumors. Pituitary responses to a single injection of GnRH were slightly greater than in controls, when tested 4 months after E$_2$ valerate; mice had been ovariectomized and given E$_2$ implants to achieve equivalent hormone levels (47). Similar conclusions about hypothalamic functional damage were drawn from the small LH surge induced by electrochemical stimulation of the preoptic region in E$_2$-valerate treated rats, in contrast to unimpaired responses to a GnRH analog (166). Thus, sustained exposure to elevated E$_2$ in rats or mice can cause an immediate or delayed onset of a permanent anovulatory syndrome that is strikingly similar to that arising spontaneously in the neuroendocrine syndrome of aging, including the eventual induction of pituitary tumors (Table 1).

Irreversible impairments of LH regulation in mice occur after about 6 weeks of sustained physiological E$_2$ (47), whereas gross pituitary tumors may not be visible in this species until 5 months later (167). These events approximate the time interval between the lengthening of cycles and onset of pituitary tumors observed in mice during normal aging. Since ovariectomy before treatment with E$_2$-valerate prevents the glial reaction (168), the E$_2$ secreted by the polyfollicular ovaries in the initial anovulatory state produced by these treatments appears to be required for the full syndrome. Evidently, the initial,
FIG. 11. Effects of prior chronic estrogen treatment on the subsequent capacity of C57BL/6J mice to display the induced LH surge. Mice, aged 4 months, were given E2-valerate (EV) in one of two doses (low dose EV, 0.05 mg/kg; high dose EV, 0.2 mg/kg) which initially caused supra-physiological plasma E2 concentrations and rapidly interrupted estrous cycles. The EV-treated mice maintained a permanent state of persistent vaginal cornification even though the EV depot had disappeared two months later. When 8 months old, mice were ovariectomized and 1 month later were given E2 injections that induced a nocturnal LH surge in controls of the same age but not in EV-treated mice. Thus, effects of chronic estrogen elevations permanently impaired neuroendocrine responses to E2; the impairments were not restored by ovariectomy for 1 month (47).

Supraphysiologic concentrations of E2 after E2-valerate injection are sufficient to inhibit cycles, perhaps by desensitizing the hypothalamus to E2 in a process that is analogous to the agonist-induced down regulation of monoaminergic receptors (88, 170). The initial elevations may not be sufficient to cause the full syndrome unless sustained by E2 secreted by the polyfollicular ovaries during the continuing acyclic phase that continues after the E2-valerate depot is absorbed (170). However, E2-valerate given to ovariectomized mice reduced by 50% the number of cycles obtained with young ovaries when grafted 6 months later (C.V. Mobbs and C.E. Finch, in preparation), a result which indicates cryptic damage from E2-valerate.

Adult male rats develop similar histopathologic changes in the arcuate nucleus after repeated injections of E2-valerate (168). Thus, susceptibility to some neuro-pathologic effects of E2 is not restricted to adult rodents with the X,X genotype. Moreover, chronic exposure of adult male mice to estrogens will induce pituitary tumors (167).

The steroidal specificity for inducing these changes is partly known. Neither progesterone nor testosterone in daily injections caused permanent acyclicity in mice (162), but testosterone implants in ovariectomized rats did cause mild glial hyperactivity in the arcuate region (165). Importantly, testosterone and 5α-dihydrotestosterone protect against the E2-induced glial changes if given concurrently with E2 (165); and so may progesterone (H. Schipper and J.R. Brawer, cited in Refs. 137, 138). Brawer notes (165) that the ability of testosterone to block the effects of E2 explains why orchietomy has little influence on the somewhat increased glial activity observed during aging in male rats and on the mild stimulation of glial activities by exogenous testosterone in young rats. Although testosterone is converted to E2 by brain aromatase, testosterone is also converted in the brain to 5α-dihydrotestosterone which blocks these effects of E2. Thus, testosterone in this case is a precursor to both a neurotoxin and its antidote.

Constant light also causes loss of cycles and suppression of surges in (e.g. 65, 169) and eventually hypothalamic glial hyperactivity after 4 months (168). The chain of events probably involves neuroendocrine desensitization from the sustained production of E2 by the polyfollicular ovaries during the constant light-induced anovulatory syndrome, since ovariectomy before exposure to constant light prevented the glial reaction (168), just as ovariectomy prevents glial reactions after depot injection of E2-valerate (168, 169).

No conclusions can be drawn at present about the primary sites of steroidal actions, and transneuronal effects may be anticipated. It seems likely that more than one cell locus is altered by chronic E2, given the diversity of cellular and neurotransmitter changes in the aging female hypothalamus (Section III B 5). For example, estrogens can induce lactotrophic adenomas in rat pituitaries ectopically transplanted to the kidney (171). Thus, hypothalamic changes need not be primary factors in the pituitary changes associated with age or E2 treatment (Table 1), although the deficits of portal blood dopamine in old rats (Section III B 5 d) suggest a plausible factor in lactotroph tumorigenesis. Walker (65) suggests that the loss of serotonin rhythm during aging or chronic E2 is directly linked to loss of the LH surge. If so, the suprachiasmatic nucleus may be a key locus in the early stages of the E2 effects, with consequences that could cascade transynaptically to alter the regulation of GnRH-secreting neurons in the arcuate nucleus and elsewhere. Moreover, glial hyperactivity during aging is seen in the region lateral to the arcuate nucleus where fibers that are crucial to the LH surge transit from the rostral hypothalamus (3).

The evidence is thus unambiguous that sustained exposure to supraphysiological or physiological concentra-
tions of E2 causes long lasting hypothalamic and pituitary damage in adult rats and mice according to functional and morphologic criteria. The potential reversibility of these changes by prolonged ovariectomy or drugs remains to be evaluated.

The dose-time (strength-duration) relationships of E2 required for impairing the LH surge in adult rodents are partly defined, but the transitions between reversible and irreversible changes are still uncharted. Strength-duration relationships are well established in electrically excitable functions of nerve and muscle, as illustrated by the familiar excitability curve for myelinated fibers, in which the product, intensity x duration of stimulus, shows a commutative relationship. Thus, within some boundary conditions, strength or duration can vary inversely without affecting output. Strength-duration relationships for effects of E2 on priming or inducing the LH surge are also known in rats (172) and monkeys (4).

The short-term inhibition of LH surges by E2 is clearly reversible. Administration of E2 to ovariectomized adult rats (173) or hamsters (174) induces sequential daily LH surges in the afternoon. With sustained E2, the daily LH surges in rats and hamsters gradually diminish and disappear in about 2 weeks, but will recover if the E2 implant is removed within several weeks. There are interesting similarities between these phenomena and the impaired LH surges of aging rats in persistent vaginal cornification (65). The LH surge recovers after reduction of plasma E2 by ovariectomy of old rats (20) or mice (40). Walker (65) suggests that the absence of the LH surges results from damping of the hypothalamic, diurnal serotonin rhythm by sustained E2. Moreover, LH surges can be reactivated by drug treatments with chlorophenylalanine with 5-hydroxytryptophan on the next day, a procedure which mimics the natural serotonin rhythm.

The exposure of E2 required for irreversible loss of cyclicity in young mice (≥ 6 weeks at average levels for the estrous cycle) (47) is close to the duration of ovary-induced changes leading to acyclicity, 2–4 months (Fig. 8). The E2 dose time product required for the irreversible loss of cyclicity can be estimated. At the first approximation, about 1000 pg-days E2/ml plasma of constant exposure to E2 will cause permanent acyclicity in adult C57BL/6J mice. These early data suggest that the strength-duration product of E2 can be determined for specific aspects of the ovary-dependent neuroendocrine syndrome (Table 1) and that it will be possible to define the boundary values within which the E2 strength-duration product has commutative properties, e.g. does 10 days of E2 at 100 pg E2/ml plasma have the same effect as 50 days at 20 pg E2/ml plasma.

B. Clover disease of sheep

Domestic sheep are subject to a permanent infertility syndrome called clover disease, when grazed as adults for several years on estrogenic clovers. Several strains of clover (Trifolium subterraneum) introduced to Western Australia about 1931 were subsequently found to cause infertility in association with hyperplasia in the uterus, cervix, adrenal cortex, and with neuronal damage and gliosis in the hypothalamus (175–177). The active agents appear to be phytoestrogens (isoflavones) which can interact with trace elements in the diet, e.g. cobalt enhances clover disease whereas selenium protects against effects of cobalt (175). A series of studies by Adams demonstrates neuroendocrine alterations in clover disease that last at least 5 yr after return to normal pasturage including: impaired E2-induced LH surges (178), impaired female mating behavior, and a tendency for male-like courting behavior observed after ovariectomy and treatment with testosterone (179). Despite clover disease, ovulation is relatively normal (180) and the infertility is attributed to impaired transport of sperm through the cervix (176, 181). Although the functional significance of the neuroendocrine changes is less clear for clover disease than for the E2-induced anovulatory syndromes of rodents, the case is also strong for long lasting, probably irreversible effects of estrogens on the adult sheep brain.

VII. Influences from Development on Adult Neuroendocrine Functions

A. Prenatal influences from fetal neighbors

The organizing influences of endogenous steroids on the rodent brain during late prenatal and early postnatal development also extend to influences from neighboring fetuses, such that female fetuses flanked by males, 2M females, are more masculine as adults in their physiologic and behavioral responses than are female fetuses flanked by females, OM females (182, 183). The 2M females have longer estrous cycles as young adults when compared with OM females: 6–7 days vs. 5 days, respectively (34). Males are also modified as adults by the sex of their fetal neighbors (184). There are major puzzles about the active agent in this shading of gender and how fetal influences are transmitted to their neighbors in view of the separate amniotic circulations. The acting steroid might be testosterone or E2 (39, 184, 185).

The OM and 2M female mice (CF-1, Charles River, Breeding Lab, Wilmington, MA) develop important differences during reproductive aging. If 2M females are mated when young, they become infertile during aging about 2 months before OM females. Pregnancy begins normally, but parturition in older 2M females is delayed, leading to a high incidence of stillbirths (186). Thus, the 50% subgroup of 12-month-old C57BL/6J mice whose parturition was delayed by more than 2 days (14, 187) may have included 2M females; however, the role of
neuroendocrine age changes in the loss of fertility and the delayed parturition is not defined. Important behavioral changes occur during aging as well. Old 0M virgin females become more like 2M females in mounting and aggression after exposure to testosterone (188). These important findings support our hypothesis (152) for a lifelong continuum of steroid influences on the rodent brain, in which steroid exposure during development determines the subsequent amount of steroid exposure required to cause specified age changes. In this case, 0M females would have less prenatal steroid exposure, but can catch up to 2M females by exposure to endogenous ovarian or adrenal steroids (possibly aromatizable androgens, 188) during adult life to achieve the same end point of changes in brain regions mediating male-type behavior (188). These observations also support the view that reproductive aging in female rodents includes partial or mosaic masculinizing processes (discussed in 152, 188).

B. Delayed anovulatory syndromes

Large doses of $E_2$ or testosterone given within the neonatal critical period for sexual differentiation of the hypothalamus in mice or rats result in complete failure of estrous cycles and infertility of the adult (6, 189, 190). However, if neonatal rodents are given smaller submasculinizing doses, a limited number of cycles occur after puberty, but acyclicity soon follows with an anovulatory polyfollicular state, persistent vaginal cornification, and impaired LH surges. These phenomena constitute the delayed anovulatory syndrome (DAS) (190), which can be induced by testosterone (190–192) or $E_2$ (C.V. Mobbs, Kannegieter, and C.E. Finch, in preparation). The steroid-induced LH surge is progressively impaired during the approach to acyclicity (C.V. Mobbs, L.S. Kannegieter, and C.E. Finch, in preparation; 193). The DAS differs from the full anovulatory syndrome or complete neonatal masculinization, since unlike the latter, rats showing the DAS display normal lordosis when ovariectomized and treated with $E_2$ and progesterone (192).

The DAS has been long recognized to show important similarities with subsequent age changes. For example, Swanson and van der Werff ten Bosch (191) noted the possibility "... that the influence of aging on the ovaries is partially due to aging of the central nervous system, and that the presence of androgen shortly after birth promotes the latter process." Alternatively, Gorski (190) suggested that "... the (delayed)-anovulation syndrome develops ... because of a subsequent and independent modification of a neonatally partially differentiated system. This modification presumably is produced by postpubertal ovarian steroid feedback ... ". At present, no choice between these two mechanisms is possible.

Aging rodents (194–196), as well as those in DAS (197), retain lordosis responses. Ovulation is also induced in aging (47) and in DAS rodents (see above) by exogenous gonadotropins. Although neuroendocrine aging in female rodents as well as in the DAS could be considered as a mosaic masculinization (152), it cannot yet be concluded that the failure of the steroid-induced gonadotropin surge results from long lasting steroid effects at the same anatomic loci in both aging and the DAS. For example, lesions of adult rats at different loci in the preoptic or anterior hypothalamus can differentially affect responses of LH secretion to $E_2$, or progesterone (198, 199).

An important similarity between the DAS and reproductive aging is the required presence of the ovaries for subsequent impairments of the LH surge. Several studies show that ovariectomy at various times after weaning can postpone the onset of acyclicity when tested with ovarian grafts (200, 201), as well as postponing the impairments of the gonadotropin surge (192). However, another study failed to observe this effect of ovariectomy (197). The ovarian requirement for inducing the DAS can be substituted for in ovariectomized DAS rats by daily injections of $E_2$ or testosterone between 30 and 90 days; the $E_2$-induced LH surge then became greatly impaired (192). This result argues against a neuroendocrine mechanism in the DAS that simply counts LH surges and indicates that a definable exposure to aromatizable steroids is required.

Because of the effects of $E_2$ on adult rodents (described above, Section VI A), we also characterized the DAS induced in mice by $E_2$. A DAS was induced in mice by injection on postnatal day 6 with 0.1 $\mu$g $E_2$-benzoate (C.V. Mobbs, L.S. Kannegieter, and C.E. Finch, in preparation). Regular cycles were achieved when most mice reached 3 months of age. We emphasize that the $E_2$-injected mice had longer cycles. The majority of $E_2$-injected mice stopped cycling by 5 months. The prompt disappearance of cornified smears after ovariectomy of DAS mice ruled out possible confounds from ovary-independent vaginal cornification as observed in adult mice that were treated as neonates with $E_2$ (202). The ability of DAS mice to ovulate with hCG and the normal length (4–5 day) of cycles obtained after transplantation of DAS ovaries to young controls proved that the ovaries were unimpaired. These results establish the important point that cycle lengthening can result in the absence of ovarian impairments because of neuroendocrine alterations. Moreover, ovaries from DAS donors supported about 25% more cycles than age-matched control donors. Conversely, mice in DAS did not regain cycles with young ovarian grafts. We conclude that the $E_2$-induced DAS in mice provides a model for neuroendocrine aging in which the ovary is not compromised.

The effects of $E_2$ given to neonates may decrease the amount of ovarian $E_2$ experienced during subsequent
cycling that is required to cause neuroendocrine changes leading to acyclicity. In terms of the cumulative impact model (Fig. 7), DAS rodents would start adult life with a higher base line. The E₂-induced DAS thus provides evidence for a continuum of cumulative E₂ effects during development into adult life, as predicted (154).

The DAS poses a major puzzle. Assuming that the aging ovary is the major factor in causing neuroendocrine damage that leads to acyclicity in normal mice between the ages of 12 to 16 months (Fig. 8) and that the ovary is undamaged after the onset of DAS (see above), then what other factors cause the rapid loss of cycles during the DAS? As shown above, one possibility concerns the long cycles observed before the onset of DAS in which cycles of 6 days or longer occur 2 times more frequently than in controls. The levels of steroids during the initial cycles are unknown but could conceivably include increased ratios of E₂:progesterone which is a probable factor in the etiology of neuroendocrine aging (Section IV B). If so, progesterone implants might further retard the loss of cycles in DAS.

In sum, the DAS shares many similarities with the neuroendocrine deficits that arise during normal aging, but occurs without ovarian deficits. The normal potential of the ovaries and the requirement for ovaries or injections of ovarian steroids during the postpubertal period before the loss of cycles suggests the hypothesis that the DAS involves a vicious feedback cycle, in which neuroendocrine disturbances during the early cycling phases of the DAS then alter ovarian steroid secretion, and in turn, cause further neuroendocrine change.

Until we know the primary cellular sites of change in the brain during normal reproductive aging, during the E₂-induced anovulatory syndrome of adult rodents, and during the DAS, we can only speculate that these phenomena share some or all of the same neural mechanisms. Nonetheless, these phenomena share one major manifestation, impairment of the E₂-induced LH surge. Speculations that aging (152) and the DAS (193) result in partial or mosaic masculinization of the brain pertain mainly to the sex difference in LH regulation. Lordosis is not impaired in the DAS or during aging (see above). Moreover as noted earlier, 0M female mice become more sensitive to the induction of male type behaviors by testosterone during aging (188). Thus, a partial masculinizing influence of ovarian steroids may occur widely in aging adult rodents.

Another curious phenomenon may be mentioned here. During aging, hens sometimes develop masculine behavior and plumage, an apparent result of the activation of undeveloped testicular tissues that are present in adult female avians (203).

VIII. Synopsis

The steroid-dependent neuroendocrine phenomena described in Sections IV, V, and VII are clearly complex and do not promise reduction to simple mechanisms. They probably involve multiple sites of action in steroid target cells in different brain regions as well as in the pituitary. Nonetheless, some generalizations can be considered.

First, long lasting or organizing effects of sex steroids on the rodent brain are not restricted to the perinatal critical period (5 days before birth to 10 days after) when neurogenesis is most active. This is demonstrated by the ovary-dependent neuroendocrine aging syndrome (Section V), the ability of estrogens to prematurely induce aging-like changes in adult rodents and possibly sheep (Section VI) and the ovary-dependence of the DAS (Section VII B). Another example is the effects of testosterone in sensitizing adult female mice to male type behavior. Several studies show that the ability of testosterone to sensitize a female mouse to the aggression-inducing effects of a subsequent, smaller injection of testosterone extends into early adult life (185, 204, 205). The active steroid(s) and sites of neural action are unknown. Recall also that OvM female mice become more sensitive during aging to the induction of male type behavior by testosterone (Section VII C). The concept of a strictly limited, critical period for the organizing effects of sex steroids on the rodent brain has often been challenged (161, 165, 190). The present discussion merely notes the extensive data supporting the view that the adult rodent brain remains sensitive to long lasting effects of steroids, but adds the example that this sensitivity continues at least through middle age.

Second, exposure to sex steroids in adults or during development can cause cryptic changes with delayed consequences that are manifested later after subsequent steroid exposure. For example, if adult mice are given E₂ implants yielding sustained physiological elevations of E₂ for 6 weeks, regular cycles can resume for several months when the implants are removed, but then cease prematurely (Section VI A). A formally similar phenomenon is the DAS, in which rodents given partial masculinizing doses of testosterone or E₂ as neonates initially have ovulatory cycles which cease prematurely (Section VII B). As in normal aging, the onset of impairments in the LH surge during the DAS is delayed by ovariectomy. In both cases, cryptic effects of exogenous sex steroids are retained for several months and appear to reduce the amount of subsequent steroid exposure needed to impair the LH surge. These delayed effects could be viewed as resulting from the partial completion of a (putative masculinizing) process by the initial treatment, which then requires less subsequent exposure to ovarian steroids to
reach an endpoint, the loss of the E2-induced LH surge. Alternatively, these effects may be viewed as activating a different mechanism which sensitized rodents to cumulative effects of ovarian steroids during regular cycling. Further study of these phenomena seem likely to identify new aspects of neuroendocrine responses to steroids and other hormones.

IX. Possible Mechanisms

Despite the early stage of this subject, sufficient data is available to consider specific hypotheses about the mechanisms of ovary-dependent neuroendocrine aging and the effects of E2 on the adult rodent brain.

A. Cellular clocks

Does the brain contain cellular pacemakers for aging which register discrete events? For example, some types of proliferating diploid cells differentiate after a fixed number of mitotic cycles during embryonic development (206). The limited proliferative potential of diploid human fibroblasts and many other cell types in vitro (207) has been widely discussed as an intrinsic pacemaker system for aging. It should be noted that postmitotic diploid fibroblasts can remain metabolically active for months in vitro (208) and are as effective as host cells for viral infections as are early passage fibroblasts (207). In these regards, they may be considered like postmitotic neurons of the adult mammal in which some cell types maintain undiminished functions for 9 or more decades in the case of humans without neurological disease.

The loss of proliferative capacity with serial passages in vitro is not simply a result of passing time since the same number of population doublings can be achieved after arresting proliferation through deep freezing (207) or by maintaining at confluence (209), with subsequent restoration to proliferative conditions. Moreover, the proliferative capacity (number of population doublings of diploid fibroblasts in vitro) is not absolutely fixed, and can be considerably increased by the addition of glucocorticoids to the culture medium (209). Thus in vitro aging diploid fibroblasts do not provide a cell model for strict counting. As described above, there is no evidence for a counting mechanism in the brain that registers the number of ovulatory cycles during aging. For example, we ruled out the possibility that age-related acyclicity occurs because the brain possesses only a fixed number of LH surge events, since the cycling lifespan is doubled by replacing ovaries of cycling, middle-aged mice with young ovaries (Fig. 8). Moreover, there is a sporadic recovery of the LH surge during the transitions between repetitive pseudopregnancies (113) and a reemergence of the steroid-induced LH surge after ovariectomy of rodents in persistent vaginal cornification (40, 91). Despite recovery

of the LH surge to near normal levels in old acyclic mice after 2 months of ovariectomy, neuroendocrine recovery was still incomplete since young ovarian grafts supported only limited numbers of cycles (Section IV C).

Ovary-dependent neuroendocrine aging may be like the DAS in regard to the limited number of cycles supported once the LH-surge loci are masculinized to a sufficient extent, either by small doses of E2 in neonates, or by ovary-dependent changes as cycles lengthen and cease during normal aging. This hypothesis would then predict that the strength-duration requirements for E2 to cause permanent impairments of the LH surge would be markedly less in young DAS mice before the onset of acyclicity than for normal middle aged mice at the onset of cycle lengthening.

B. Steroid-induced cell death

During development in female rats, elevations of testosterone (and presumably E2) promote enlargement of the sexually dimorphic nucleus, with increased numbers of neurons and different synaptic distributions in the preoptic region (210, 211). It is unclear if the steroids rescue neurons that would otherwise die, or if neuroblast proliferation is stimulated.

In young adult rats, the E2-induced glial hyperactivity in the arcuate nucleus is associated with degenerating neurons as described in an ultrastructural study (163). Sustained prolactin elevations from prolactinomas also cause arcuate neuron shrinkage in adult rats (212). However, there is as yet no quantitative evidence for loss of arcuate neurons during chronic treatment of young rodents with E2 or prolactin. Recall also that not all studies detected neuronal loss during aging in the arcuate nucleus (Section III B 5).

Glucocorticoids are also implicated in neuronal invo-

lution. Elevations of corticosterone in male rats for 3 weeks initially cause a reversible loss (down-regulation) of corticosterone binding sites in hippocampal pyramidal neurons (213) before irreversible damage and death of select neurons (214). These effects of corticosterone suggest a similar sequence in neurotoxic effects of E2 in which neuronal E2 binding sites are first reversibly down-regulated, with subsequent irreversible neuronal damage and then possibly cell death. One class of nuclear E2 receptors in the uterus is down-regulated by continuous exposure to physiological E2 for 96 h (215).

Lymphocytes show cytotoxic responses to glucocorticoids, including major impairments in the transport of amino acids and glucose, and inhibition of the synthesis of RNA and lymphokines (216, 217). At least 2 genetic loci are required which encode a functional glucocorticoid receptor and a lysis function (218). E2 also is a powerful suppressor of immune functions which may be mediated
by thymic E₂ receptors (219). The molecular mechanisms remain to be elucidated, but important clues to the central actions of E₂ and corticosterone may be obtained from peripheral cell responses.

C. Synaptic remodeling

The short-term effects of E₂ on hypothalamic synaptic structures are well demonstrated in adult rats by morphologic studies of synaptic contacts (220) and ligand-binding studies of hypothalamic neurotransmitter receptors (221). The concept of E₂-induced synaptic remodeling in adult rodents is consistent with the extensive capacity for remodeling of synapses that adult rodents manifest after lesions (222). In adult female canaries, sex steroids can induce extensive growth of dendrites (223).

Once the cellular foci of ovary-dependent aging and the postmaturational effects of E₂ are better known, detailed quantitation of synaptic profiles will be important in designing hypotheses about altered neuronal circuits during reversible and irreversible phases of these changes. The present evidence suggests, but does not establish, the possibility that E₂ causes irreversible changes of synaptic contacts in adult rodents. There is no evidence yet to evaluate the hypotheses that a certain fraction of hypothalamic synapses are destroyed as a result of E₂ action during aging and that the LH surge is lost when a critical number of synaptic connections is damaged (168). In view of the experiments described in Figure 8, it seems unlikely that such a mechanism occurs during most cycles. Postsynaptic modifications could also be considered as observed in the stable increase of glutamate receptors associated with long term potentiation in the hippocampus (224). E₂ effects also could be mediated via the cell nucleus, e.g. by altering the synthesis or secretion of GnRH without altering synaptic connectivity.

The reversible and irreversible effects of E₂ and other steroids on neuroendocrine functions of adults have some interesting parallels with mechanisms attributed to memory. For example, long term potentiation can increase with repetition of electrical or sensory stimuli. These effects can last for many months (224, 225), perhaps even for the lifespan. The conclusion that “the post-synaptic face of neuronal connections is quite plastic, and can be changed by physiologic activity” (224) might be extended to include long term effects of E₂ on the hypothalamus. Another potential similarity is suggested by the likely role of glutamic acid in hippocampal pathways involving long term potentiation (224) and the ability of glutamatergic excitotoxins to damage the arcuate nucleus of neonates and adults (226). Thus, the ovary-dependent and E₂-dependent neuroendocrine ag-

D. Actions on different E₂ targets and receptors

Information on how the effects of E₂ vary with stimulus strength (blood concentrations) may be useful in identifying mechanisms of the ovary-dependent neuroendocrine syndrome. In view of the more than 10-fold greater E₂ concentration required to occupy nuclear receptors in the hypothalamus vs. pituitary (80), it may be possible to determine if the minimum circulating concentrations of E₂ required for irreversible neuroendocrine damage involves only the pituitary E₂ receptors, or if hypothalamic E₂ receptors must also be occupied. At the other extreme, if the E₂-induced damage continues to increase progressively with increases in circulating E₂ above that required for occupancy of both hypothalamic and pituitary receptors, then other nonclassical sites of E₂ action can be considered such as those involving the very rapid effects of E₂ on electrical activities in the basal ganglia (227). Another mechanism of E₂ independent of the cell nucleus is shown by the ability of E₂ to alter turnover of hepatic vitellogenin mRNA in minutes without immediate effects on transcription (228).

Rodent genotypes with variations of responses to E₂ might be useful in identifying relationships between various parameters of ovary-dependent neuroendocrine changes. For example, lactotroph adenomas are readily induced by chronic estrogens in the Fischer 344, but not the Holtzman rat (229); the genotypic influence was proven to be at the pituitary level. After transplantation of pituitaries to the kidney of F1 hybrids, only the susceptible strain developed tumors with chronic estrogen treatment (170). Mouse strains with different uterine growth and pituitary tumorigenic responses to E₂ (18, 167) may also be useful for such studies.

Effects of E₂ and other steroids could be indirectly mediated through other molecules. For example, E₂ acts on the rodent brain and other organs to induce heat-labile nondialyzable factors that enhance growth of tumor cells in vitro (230). Such steroid-induced growth factors could influence neural or glial functions independent of the classic steroid-receptor mechanism. E₂ also has a well-known effect of stimulating prolactin secretion among other hormones which act on the hypothalamus (231).

Although hyperactivity of glia, particularly microglia, is often secondary to neuronal degeneration (139, 232), some steroids can act directly to enhance glial activities. For example, glucocorticoids induce glycerol-3-phosphate dehydrogenase (233), and E₂ induces nerve growth factor secretion (234).
ing aging (Fig. 9) can be considered in terms of the antagonism of E₂ and progesterone. In uterine cells, the translocation of the progesterone receptor to the nucleus can cause rapid dissociation of the E₂ receptor complex from the nucleus of that same cell (235). Also, injection of progesterone at proestrus reduces the amount of E₂ bound to nuclear receptors in uterine nuclei. Conversely, these results would suggest that the reduced progesterone concentrations in aging rodents could result in prolonged retention of the E₂-receptor in the nucleus. Effects of progesterone on E₂ receptors in the hypothalamus could account for the antagonism of neonatal androgenization by progesterone (236). Moreover, the induction of pituitary tumors by chronic estrogen treatment in rats was reduced by concurrent administration of progestins (237). The low circulating progesterone and sustained E₂ that prevail during persistent vaginal cornification might also perpetuate an abnormally continuous nuclear retention of the E₂ receptor complex.

We propose that there is a class of aging phenomena that includes neuronal damage from unremitting receptor stimulation which arises because of extrinsic influences on target cells or molecules. In addition to the E₂-driven neuroendocrine syndrome, sustained elevations of glucocorticoids or stress can damage brain neurons (214). The effects of continuous nuclear receptor occupation in nongrowing cells are unknown but might interfere with the normal turnover of receptor proteins. If this occurs, molecular damage to the receptor and chromatin might accumulate. Recalling that the E₂-induced damage in the arcuate nucleus can be modified by progesterone, an antagonist of E₂ (Section V IA), other experimental and clinical modifications of steroid-dependent aging processes should therefore be possible.

**E. E₂-induced DNA demethylation**

Another molecular mechanism is suggested by the memory effects of E₂ on inducible protein synthesis in the avian liver. After the affects from an initial injection of E₂ on inducing phosphoprotein synthesis have subsided, a subsequent E₂ injection will induce more rapid and larger responses. This memory effect lasts for many weeks (238) and is associated with alterations in stable nucleasie hypersensitive sites in chromatin and with demethylation of specific cytosine residues in the DNA of the E₂-induced vitellogenin gene (239). Some evidence suggests that spontaneous DNA demethylation occurs during aging in E₂-sensitive genes of adult roosters (240) as a consequence of sustained exposure to the relatively low estrogen levels of male birds. Although there is no proof that the inducibility of genes requires demethylation at general or specific sites (239), the demethylation of hormonally regulated genes might still provide a useful marker for cumulative effects of E₂ and other steroids on gene expression in neurons. Such demethylation might be a type of steroidal memory in brain cells and could be involved in the slow, ovary-independent neuroendocrine aging processes (Section V). An analogous concept was proposed by Holliday and Pugh (241) in which demethylation coupled to cell division might be a biological clock. This idea is consistent with observations that DNA is progressively demethylated during diploid fibroblast senescence in vitro (242). The above discussion illustrates some new possibilities for E₂ effects on neurons which can now be studied at the molecular level.

**X. Questions About Menopause**

**A. Potential neural mechanisms**

Neuronal mechanisms have at least one clearly demonstrated role in menopause. The hot flashes that commonly arise after E₂ concentrations become very low during menopause are known to result from hypothalamic and autonomic events in which α-adrenergic receptors are implicated. Hot flashes are clearly steroid-dependent and remit rapidly upon treatment with E₂ (243).

A potential neuroendocrine component in menopause concerns the frequency of gonadotropin pulses which has a major influence on follicular development in the rhesus monkey. Experimentally retarding the frequency of GnRH pulses from once/60 min to once/120 min causes anovulatory cycles with lower peak E₂ values. Further slowing to once/180 min did not support any follicular development, despite normal circulating FSH levels (244). In view of the reduced frequency of LH pulses observed by middle age in rodents of both sexes (Section III B 2), and the similar patterns of cycle variance with aging in mice and women (Fig. 1), we propose that the lengthening of cycles during the approach to menopause could result from a slowed frequency of GnRH pulses. Thus, Knobil's (4) suggestion that small changes in the frequency of GnRH pulses have a major influence on ovarian function and initiation of puberty could be extended to consider a role of GnRH pulse frequency during aging to the lengthening of cycles. It is also possible that altered rates of ovarian steroid secretion during the approach to menopause could influence the frequency of GnRH pulses thereby compounding effects on folliculogenesis. No data are available on how aging may influence the frequency of gonadotropin pulses in women. Since preovulatory-like surges of LH and FSH can be induced after menopause by treatment with estrogens and progesterone (245), at least some aspects of GnRH control and pituitary responses to steroids remain intact at menopause. Such single surges do not establish the capacity to sustain ovulatory cycles.
Because of the importance of the arcuate nucleus in controlling the GnRH pulses in monkeys (4) and the morphologic changes in the arcuate nucleus region of aging rodents (Section III B 5), efforts should be made to do more anatomic studies on postmortem brain tissue of pre- and postmenopausal women. Virtually nothing is known about morphologic changes in the human hypothalamus during menopause. Limited observations in two rarely cited reports are mentioned to encourage further studies. Neurons in the paraventricular nucleus of three postmenopausal women showed altered fluorescent emission patterns with acridine orange (longer wave length) as compared to five younger women (246). Also, in two-thirds of postmenopausal women sampled, neuronal hypertrophy occurred in the subventricular hypothalamic nucleus which is distinct from the arcuate nucleus and which is not generally prominent in young women (247). This heterogeneity recalls that some menopausal women do not suffer from hot flashes (243).

B. Issues in evaluating possible E₂-induced neuroendocrine damage to humans

Do ovarian steroids also have long term effects on the human hypothalamus and pituitary? The use of sex steroids by humans as contraceptives and in replacement of ovarian steroids after oophorectomy or menopause is widespread and the associations with malignant disease are complex and controversial. For example, a recent study suggests that the risks of breast cancer from combination oral contraceptives (containing both estrogens and progestins) appear to be increased, but only if use was begun before age 25, or in older postmenopausal women (248). Both groups have longer and less frequent ovulatory cycles (11).

Although chronic E₂ readily induces pituitary tumors in rodents, there is little evidence that chronic estrogens do so in primates or humans (249). In women with prolactinomas, the history of oral contraceptive usage was not different than in controls (250, 251). However, lactotroph adenomas and hyperprolactinemia occasionally occur in polycystic ovarian disease (252), possibly as a consequence of unremitting stimulation by elevated estrone (253).

At the hypothalamic level in primates, there is little evidence for permanent organizing effects of sex steroids on brains of the neonate or fetuses in regard to the regulation of LH (2-4, 159, 254). In adult primates, dependency of the preovulatory LH surge on major changes in the hypothalamic regulation of GnRH is much less than in rodents (3, 4). Moreover, the induction of progesterone receptors by E₂ is restricted to fewer hypothalamic regions in monkeys than in rats (255). The effects from E₂ fluctuations on adult human behavior are generally difficult to establish and cannot be compared with the major behavioral changes during the rodent fertility cycle (254, 256). Taken together, these data suggest that E₂-induced neuroendocrine damage in adult humans is less likely than in rodents. However, careful consideration should be given to this possibility in future postmortem studies of women with different histories of hormone treatment after menopause or hysterectomy.

In contrast to the uncertain effects of sex steroids on the perinatal or postnatal primate brain, steroids do appear to act on the brain during postnatal maturation of the neural mechanisms which cause pulsatile LH release. The frequency of episodic LH pulses increases during puberty, particularly at night (257, 258). Hypothalamic changes leading to the reawakening of the arcuate nucleus oscillator are important in the onset of puberty (4, 257). Ovarian steroids do not seem crucial to maturation of the hypothalamic oscillator controlling GnRH release since near normal LH secretion patterns are observed in adults with gonadal dysgenesis despite extremely low sex steroids. Adrenal steroids, however, are implicated in hypothalamic maturation of humans (257).

Another rarely noted feature of puberty is the development of the capacity for hot flashes which are triggered in normal adults by low postmenopausal concentrations of E₂. Prepubertal girls have circulating E₂ as low as at menopause (259) yet are not known to have hot flashes. Moreover, hot flashes are not reported in gonadally dysgenetic adults, e.g. Turner's syndrome (260).

A few cases suggest that hot flashes will occur in gonadally dysgenetic adult Turner's syndrome patients who were treated with estrogens for osteoporosis and were withdrawn subsequently from E₂ (260). Thus, the capacity for hot flashes during low E₂ is an example of a steroid-induced postnatal hypothalamic (maturational) change in humans.

It is intriguing that hot flashes spontaneously disappear in many postmenopausal women after 1-5 years even in the absence of steroid replacement therapy (260). Individuals differ widely in the age when hot flashes fade away but some may suffer them for more than 5 yr (261). Because hot flashes are a central nervous system coordinated event, their spontaneous extinction strongly implies a change in the hypothalamus. The spontaneous disappearance of hot flashes in some women after menopause might result from alterations such as the loss of dendritic spines (131) or slowed norepinephrine turnovers (26, 149) observed in the hypothalamus of aging mice as indicated by Yen (260). Perhaps the sustained neuronal hyperactivity implied by sustained high levels of LH secretion eventually causes burnout of some neurons associated with the control of tonic or episodic GnRH secretion. Gradual decreases in plasma LH are
observed in some women several decades after meno-
pause (262) but the relationship to the incidence of hot
flashes is not known. As noted in Table I, female rodents,
if ovariectomized when young, can maintain elevated LH
for most of their lives. This contradicts a simple limit in
the amount of GnRH or LH that can be secreted (see
calculations in Ref. 21).

XI. Mechanisms of reproductive senescence in
relation to general theories of aging

A major theoretical issue concerns the localization of
pacemakers for aging processes. According to one view,
the complex, multi-level changes during mammalian ag-
ing are governed by subsets of pacemaker cells which
influence many other cells through interacting cascades
of changes in neural and humoral factors (263). Many
other cellular changes of aging can be shown to involve
altered neural and endocrine regulatory factors rather
than intrinsic cell aging changes (263). Regulatory net-
works have also been proposed in aging processes (263–
271). A key feature of such regulatory cascades is the
interaction between the various regulators which can
drive the system to change progressively. Progressive
interactions are illustrated by the ovary-dependent neu-
roendocrine aging syndrome of laboratory rodents in
which ovarian steroids and the loss of follicles appear to
be pacemakers of the cascade. Yet, aging of the ovary is
also influenced by the pituitary hormones and is atten-
uated by hypophysectomy (155). Thus, there is a funda-
mental reciprocity between the aging of the ovary and
hypothalamic-pituitary aging since removal of the ovary
or of the pituitary attenuates aging of the other partners
in this regulatory system.

Another case of cascading interactions discussed above
is the adrenal cortical-hippocampal interaction in aging
rats in which elevations of plasma corticosteroids can
down-regulate hippocampal corticosterone receptors
(213) and eventually cause hippocampal neuronal dam-
age and loss (214). In turn, the feedback control of ACTH
may be desensitized permitting elevated corticosterone
and perpetuating a feedforward cascade (270, 271). A
further example of cascading interactions is the hyper-
adrenocorticism of spawning Pacific salmon, as described
in the classic studies of Robertson, Wexler, and col-
leagues. Spawning salmon manifest histopathologic
changes (272) which resemble the effects of Cushing’s
disease in humans. These usually fatal changes can be
prevented by castration of salmon when young (273),
whereby growth continues and lifespan is then nearly
doubled. Conversely, hydrocortisone treatment preoc-
ciously induces the same pathologic changes in immature
fish (274).

The immune system may also participate in aging
cascades through its regulatory networks of antibodies
and anti-idiotypic antibodies (275–276) and through the
interactions of the thymus with neuroendocrine hor-
mones (218, 219, 268, 277–279) many of which have
altered regulation with aging. Thus, a wide variety of
aging phenomena in vertebrates may involve cascading
interactions which are mediated through neural and hu-
mal factors and which are related to irreplaceable cells
whose number is determined during development, in-
cluding neurons and oocytes.

The altered regulation of neural and humoral factors
during aging may cause altered expression of genes in
some target cells since the levels of hormones as well as
the temporal pattern of hormones (280) can cause major
changes in gene activity. Continuing the analysis of
physiological age changes would appear to lead toward
an understanding of mammalian aging at a molecular
 genetic level which may reveal the mechanisms control-
ing genes whose expression determine the onset of age-
related diseases and other determinents of longevity.

The change in sensitivity to E2 with age may be viewed
in a developmental context. A pleiotropic view of aging
proposed by Williams (281) suggests that aging results
from the continuance of developmental processes which
eventually become deleterious to the organism. The con-
tinuation of these processes may not be selected against
during evolution if the deleterious effects occur after
reproduction. This view would predict that age-corre-
lated changes would be in the same direction as devel-
opmental changes and that the origins of some neuroen-
docrine age changes may be traced to early development
(263). A key developmental event early in puberty is a
decreased feedback sensitivity of E2 on LH which occurs
just after the first LH surge in rats (282). Thus the age-
correlated change in sensitivity of E2 is consistent with
a pleiotropic view of aging. A decrease in negative feed-
back sensitivity necessary for the onset of puberty may
continue after puberty and eventually lead to loss of
reproductive function. The onset of puberty in rats can
be accelerated by daily injection of E2 (283, 284). This
effect of E2 to decrease sensitivity to E2 in rodents is
consistent with the ovarian-dependency of the age-cor-
related changes in sensitivity. In this context, it is of
interest to determine if the increased prolactin secretion
which occurs at puberty (282) results from increased
sensitivity to E2 since such an increase in sensitivity
during development would also be consistent with sub-
sequent further age-correlated enhancement of the re-
lease of prolactin by E2 (Section III A 2 C).

Taken together, the evidence seems strong for the view
that most cells in the organism do not age uniformly and
that the mere passage of time does not inevitably lead to
cellular dysfunction. The experimental manipulations
possible in the ovary-dependent neuroendocrine aging
syndrome and in the other systems mentioned above provide powerful opportunities to test the levels of causality in aging. If an age-correlated change can be accelerated, retarded, or reversed by specific treatments, then a case is made for the greater importance of extrinsic regulatory factors of aging. In general terms, such studies probe a deep feature of biological time that concerns the distinction between event-dependent age changes vs. time-dependent age changes. The present analysis of the ovary-dependent neuroendocrine aging thus may be considered to illustrate more general issues in aging and age related disease, even though reproductive aging phenomena may not play a major role in limiting the lifespan.

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References

17. Thung PJ, Boot LM, Mühlebock O 1956 Senile changes in the estrous cycle and in ovarian structures in some inbred strains of mice. Acta Endocrinol 23:8
20. Lu JKH, Gilman DP, Meldrum DR, Judd HL, Sawyer CH 1981 Relationship between circulating estrogens and the central mechanisms by which ovarian steroids stimulate luteinizing hormone secretion in aged and young female rats. Endocrinology 108:836
29. Cotchin E, Roe FJC 1967 Pathology of Laboratory Rats and Mice. FA Davis Co, Philadelphia, Pennsylvania
30. Simms HS, Berg BN 1957 Longevity and the onset of lesions in male rats. J Gerontol 12:244
32. Finch CE 1971 The comparative biology of senescence: some evolutionary and developmental considerations. In: Animal Models for Biomedical Research, IV. Published by National Academy of Sciences (USA), p 47
34. Gosden RG, Laing SC, Felicio LS, Nelson JF, Finch CE 1983 Imminent oocyte exhaustion and reduced follicular recruitment mark the transition to acyclicity in aging C57BL/6J mice. Biol Reprod 25:55
39. vorn Saal FS, Bronson FH 1980 Variation in the length of the estrous cycle in mice due to former intrauterine proximity to male fetuses. Biol Reprod 22:777
40. Mobbs CV, Gee DM, Finch CE 1984 Reproductive senescence in female C57BL/6J mice: ovarian impairments and neuroendocrine impairments that are partially reversible and delayable by ovariectomy. Endocrinology 115: in press
63. Wuttke W, Meites J 1973 Effects of electrochemical stimulation of medial preoptic area on prolactin and luteinizing hormone release in old female rats. Pfugers Arch 341:1
64. Cooper RL 1983 Pharmacologic and dietary manipulations of reproductive aging in the rat. Significance to central nervous system aging. In: Walker RF, Cooper RL (eds) Experimental and Clinical Interventions in Aging, Dekker, New York, p 27
72. Huang H-H 1977 Relation of Neuroendocrine System to Loss of Reproductive Function in Aging Female Rats, Ph.D. Dissertation, Department of Physiology, Michigan State University, East Lansing, MI
75. Dernott-Friberg B, Beals TF, Schultz JS 1979 H2 and background influences on tissue grafts across the H-Y barrier. Immunogenetics 9:369
79. Leipheimer RE, Gallo RV 1983 Acute and long-term changes in central and pituitary mechanisms regulating pulsatile luteinizing hormone secretion after ovariectomy in the rat. Neuroendocrinology 37:421
82. Flurkey K, Gee DM, Sinha YM, Finch CE 1982 Age effects on luteinizing hormone, progesterone, and prolactin in proestrus and proestrus and cyclic C57BL/6J mice. Biol Reprod 26:835
83. Smith WA, Conn PM 1983 Causes and consequences of altered gonadotropin secretion in the aging rat. In: Walker RF, Cooper...
null

179. Adams NR 1983 Sexual behavior of ewes with clover disease treated repeatedly with oestradiol benzoate or testosterone propionate after ovariecotomy. J Reprod Fertil 68:113


183. Meisel RL, Ward II 1981 Fetal female rats are masculinized by male littermates located caudally in the uterus. Science 213:239


186. vom Saal FS, Moyer CL 1984 Prenatal effects on reproductive capacity during aging in female mice. Biol Reprod (in press)


188. Rines J, vom Saal F 1984 Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and testosterone. Horm Behav 18:117


194. Sarkar DK, Gottschall PM, Meites J 1982 Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting tumors. Proc Natl Acad Sci USA 218:684


197. Markaverich BM, Roberts RR, Alexandro M, Clark JH 1984 The effect of low dose continuous exposure to estradiol on the estrogen receptor (Type I) and nuclear type II sites. Endocrinology 114:814


207. Bliss TVP, Gardner-Medwin AT 1973 Long lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:357


211. Wicklund JA, Gorski J 1982 Genetic differences in estrogen-induced deoxyribonucleic acid synthesis in the rat pituitary: cor-
relations with pituitary tumor susceptibility. Endocrinology 111:1140


244. Pohl CR, Richardson DW, Hutchinson JS, Germak JA, Koobil E 1983 Hypophysiotropic signal frequency and the functioning of the pituitary-ovarian system in the rhesus monkey. Endocrinology 112:2076


256. Beach FA 1961 Hormones and Behavior, Cooper Square Publ, New York


Note Added in Proof

Page 474: Mobbs CV, Cheyney D, Sinha YN and Finch CE Age-correlated and ovary-dependent changes in relationships between plasma estradiol and luteinizing hormone, prolactin, and growth hormone in female C57BL/6J mice. Endocrinology; in press.

Page 475: No loss of hypothalamic neurons containing GnRH or tyrosine hydroxylase occurs in female C57BL/6J mice, 4 vs. 14 months (G. Hoffman-Small and C.E. Finch, in preparation), nor was a loss of tyrosine hydroxylase containing neurons found in hypothalamus of 4 vs. 20-month-old Long-Evans rats (Raymond MJ, Arita J, Dulles CA, Moss RL, Porter JC 1984 Dopaminergic neurons in the medial basal hypothalamus of old rats: evidence for decreased affinity of tyrosine hydroxylase for substrate and cofactor. Brain Res 304:215


Page 488: Another example of damage induced by sustained stimulation is the renal impairments that result from chronic exposure to elevated vaspressin (Miller M 1984 Chronic exposure to vasopressin in the aging rat impairs renal responsiveness to vasopressin. Seventh Int. Congress Endocrinology, Abstracts, p 823, Exerpta Medica Ser. 652.