Hypothalamic–Pituitary–Adrenal Dysfunction in *Apoe*$^{-/-}$ Mice: Possible Role in Behavioral and Metabolic Alterations

Jacob Raber,[1,2] **Susan F. Akana,**[3] **Seema Bhatnagar,**[3] **Mary F. Dallman,**[3] **Derek Wong,**[1] **and Lennart Mucke**[1,2]

[1]*Gladstone Institute of Neurological Disease and Departments of* [2]*Neurology and* [3]*Physiology, University of California, San Francisco, California 94141-9100*

Several neurological diseases are frequently accompanied by dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis regulates the secretion of glucocorticoids (GCs), which play important roles in diverse brain functions, including cognition, emotion, and feeding. Under physiological conditions, GCs are adaptive and beneficial; however, prolonged elevations in GC levels may contribute to neurodegeneration and brain dysfunction. In the current study, we demonstrate that apolipoprotein E (apoE) deficiency results in age-dependent dysregulation of the HPA axis through a mechanism affecting primarily the adrenal gland. *Apoe*$^{-/-}$ mice, which develop neurodegenerative alterations as they age, had an age-dependent increase in basal adrenal corticosterone content and abnormally increased plasma corticosterone levels after restraint stress, whereas their plasma and pituitary adrenocorticotropin levels were either unchanged or lower than those in controls. HPA axis dysregulation was associated with behavioral and metabolic alterations. When anxiety levels were assessed in the elevated plus maze, *Apoe*$^{-/-}$ mice showed more anxiety than wild-type controls. *Apoe*$^{-/-}$ mice also showed reduced activity in the open field. Finally, *Apoe*$^{-/-}$ mice showed age-dependent increases in food and water intake, stomach and body weights, and decreases in brown and white adipose tissues. These results support a key role for apoE in the tonic inhibition of steroidogenesis and HPA axis activity and have important implications for the behavioral analysis of *Apoe*$^{-/-}$ mice.

Key words: apoE; pituitary; adrenal gland; ACTH; corticosterone; HPA axis; anxiety; open field activity; metabolism

Apolipoprotein E (apoE) plays an important role in the metabolism and redistribution of lipoproteins and cholesterol (Mahley, 1988). In the brain, apoE has been implicated in development, regeneration, neurite outgrowth, and neuroprotection (Weisgraber and Mahley, 1996). Mice deficient in apoE (*Apoe*$^{-/-}$ mice) (Piedrahita et al., 1992; Plump et al., 1992) have been used to define the potential physiological importance of apoE in brain function. Although *Apoe*$^{-/-}$ mice have no obvious abnormalities in CNS development, they show age-dependent structural and functional alterations in the cortex and hippocampus (Masliah et al., 1995; Raber et al., 1998; Buttini et al., 1999). The mechanisms underlying these hippocampal alterations are unknown and could involve peripheral pathways. Other studies have not detected such alterations in *Apoe*$^{-/-}$ mice (Anderson et al., 1998; Fagan et al., 1998), possibly because of differences in mouse strains, husbandry conditions, diets, or functional tests used.

Although the liver and the brain are the major sites of apoE synthesis, many other tissues, and particularly steroidogenic tissues such as the adrenal gland, also express apoE (Prack et al., 1991; Nicosia et al., 1992). Adrenal apoE expression, which is highest in cortical cells that synthesize glucocorticoids (GCs), declines when steroidogenesis is stimulated and increases when it is blocked (Prack et al., 1991; Nicosia et al., 1992). However, the function of apoE synthesized by the adrenal gland is unknown.

The hypothalamic–pituitary–adrenal (HPA) axis plays an important role in many brain functions, including cognition, emotion, and feeding. Alterations in the regulation of this axis are associated with impairments in these functions. The HPA axis regulates the secretion of GCs. Although under physiological conditions GCs are adaptive and beneficial, prolonged elevations in GC levels resulting from dysregulation of the HPA axis (e.g., during chronic stress) can be detrimental (for review, see Raber, 1998). The hippocampus has the highest concentration of GC receptors (McEwen et al., 1986), and chronic HPA axis activation and GC hypersecretion are associated with disturbances in hippocampal morphology (Davis et al., 1986; Woolley et al., 1990; Watanabe et al., 1992; Magariños and McEwen, 1995; Sapolsky, 1996; Lupien and McEwen, 1997; Lupien et al., 1998; Porter and Landfield, 1998) and function (McEwen and Sapolsky, 1995). In rodents, normal hippocampal function is required for adequate exploratory behavior in a novel environment and for spatial recognition memory (Britton et al., 1982; Gray, 1982; Sutton et al., 1982; Gray and McNaughton, 1983; Koob and Bloom, 1985; Liang and Lee, 1988).

In the present study, we analyzed *Apoe*$^{-/-}$ mice to investigate the possible role of apoE in the regulation of the HPA axis and of brain functions in which the HPA axis plays an important role.

MATERIALS AND METHODS

Animals. Male *Apoe*$^{-/-}$ (C57BL/6J-Apoe^{tm1Unc}) and wild-type C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed under conditions of constant temperature (18°C), light from 6:00 A.M. to 6:00 P.M., and access to food and water

Received Nov. 17, 1999; revised Dec. 22, 1999; accepted Dec. 22, 1999.

This research was supported by a John Douglas French Alzheimer's Foundation Grant to L.M., a National Alzheimer's Association Grant to J.R., by National Institutes of Health Grants DK28172 to M.F.D. and MH057967 to S.F.A., and by a fellowship from the Medical Research Council of Canada to S.B. We thank David Sanan and Dale Newland for assistance in the adrenal histological analysis, Stephen Ordway and Gary Howard for editorial assistance, and Denise McPherson for manuscript preparation.

Correspondence should be addressed to Dr. Jacob Raber, Gladstone Institute of Neurological Disease, P.O. Box 419100, San Francisco, CA 94141-9100. E-mail: jraber@gladstone.ucsf.edu.

ad libitum. To avoid circadian variation, they were tested or killed between 10:00 A.M. and 12:00 P.M., unless indicated otherwise. To minimize the effects of social influences on behavior, mice were housed individually for 7 d before assessment of open field activity or plus maze performance (see below). Mice were also housed individually for measurements of food and water intake. Otherwise, they were group-housed.

Open field activity. Mice were placed individually into brightly lit automated activity cages equipped with rows of infrared photocells interfaced with a computer (San Diego Instruments, San Diego, CA). After a 1 min adaptation period, open field activity was recorded for 10 min on 3 consecutive days. Recorded beam breaks were used to calculate active times, path lengths, rearing times, and rearing events. After behavioral testing, the equipment was cleaned with 1 mM acetic acid to remove odors.

Elevated plus maze. Anxiety levels were assessed with an elevated, plus-shaped maze consisting of two open arms and two closed arms equipped with rows of infrared photocells interfaced with a computer (Hamilton, Poway, CA). Mice were placed individually in the center of the maze and allowed free access for 10 min. They could spend their time either in a closed safe area (closed arms) or in an open area (open arms). Recorded beam brakes were used to calculate the time spent in the open arms, the distance moved in the open arms, and the number of times the mice extended over the edges of the open arms. Reductions in these variables indicate increased anxiety. After behavioral testing, the equipment was cleaned with 1 mM acetic acid to remove odors.

Corticosterone and adrenocorticotropin measurements. To determine plasma corticosterone and adrenocorticotropin (ACTH) levels, adrenal corticosterone or pituitary ACTH content, mice were anesthetized with metofane for 2 min, decapitated, and bled into EDTA-containing tubes (Microtainer; Becton-Dickinson, Rutherford, NJ), and the adrenal glands and pituitaries were removed and placed on dry ice until extraction and assay for corticosterone or ACTH as described below. The same group of mice was used to determine basal plasma corticosterone and ACTH levels and adrenal corticosterone content. The blood was spun at 10,000 × *g* for 10 min at 4°C, and the supernatant was stored at −70°C until assayed for corticosterone or ACTH. Corticosterone was measured with a corticosterone radioimmunoassay (RIA) kit for rats and mice (ICN Biomedicals, Costa Mesa, CA). The intra- and inter-assay coefficients of variation were both 7%.

ACTH was measured with an ACTH RIA kit (Nichols Institute, Capistrano, CA). The intra- and inter-assay coefficients of variation were 3 and 7%, respectively.

Adrenal corticosterone extraction. To extract corticosterone, the adrenals were homogenized in 5 ml of 0.1 M PBS, pH 7.4, with a Polytron (Virtis, Gardiner, NY). After the addition of 5 ml of isooctane and 5 ml of ethylacetate, the samples were vortexed for 5–8 min with a multitube vortexer and centrifuged at 4,000 rpm for 5 min in an Omnifuge RT. The upper organic phase was extracted according to the protocol of Mellon et al. (1980), as modified by Akwa et al. (1993). The extraction procedure was repeated twice, and the organic phase was evaporated under a stream of nitrogen gas at 60°C. The pellets were resuspended in 400 μl of methanol and 600 μl of steroid diluent (ICN Biomedicals) and stored in the dark at 4°C until assayed for corticosterone.

Pituitary ACTH extraction. To extract ACTH, pituitary samples were placed in 500 μl of 2N acetic acid, boiled for 10 min, cooled on ice, and sonicated twice for 3 sec with a Vir Sonic 50 sonicator (Virtis). After centrifugation at 10,000 × *g* for 10 min and removal of an aliquot for protein determination (Micro BCA* protein assay reagent kit; Pierce, Rockford, IL), samples containing 450 μl were lyophilized overnight (Freeze Mobile 5SL; Virtis). The lyophilized samples were resuspended in 450 μl of RIA buffer (Raber et al., 1997) and stored at −70°C until assayed for ACTH immunoreactivity.

ACTH challenge and urinary corticosterone measurements. To determine adrenal sensitivity, naive mice were individually housed in metabolic cages, as described (Akana, 1999). After 1 week of habituation to the cages, urine was collected from 5:00 P.M. to 7:00 A.M. and from 7:00 A.M. to 5:00 P.M. for 7 consecutive days. After the 5:00 P.M. collection, the mice were challenged with saline (0.1 ml/mouse, i.p.) on the second and fourth days, and with highly purified ACTH (ACTHar; 0.4 U/mouse, 0.1 ml, i.p.; Rhone-Poulenc Rorer Pharmaceuticals, Collegeville, PA) on the seventh day. The volume of the urine samples was recorded, and the samples were stored at 4°C until assayed for corticosterone.

Adrenal histology. For histological and lipid analysis, adrenal glands were removed from perfused *Apoe*$^{-/-}$ and wild-type mice, fixed in formalin, and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin, and bright-field photographs were taken on a Leica (Nussloch, Germany) microscope. Alternatively, sections were stained with Nile Red (9-diethylamino-5H-benzo [α] phenoxazine-5-one) to identify lipid deposits (Greenspan et al., 1985) and viewed with a MRC-1024 laser scanning confocal microscope (Bio-Rad, Hercules, CA) mounted on an Optiphot-2 microscope (Nikon, Tokyo, Japan).

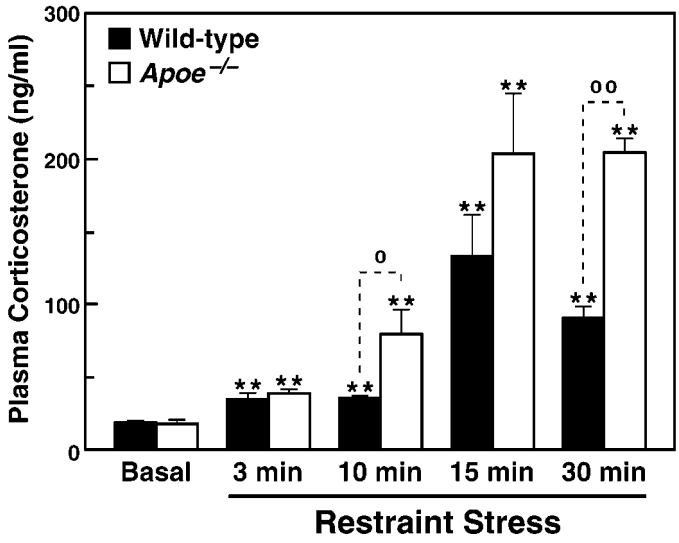

Figure 1. Plasma corticosterone levels in 6-month-old wild-type and *Apoe*$^{-/-}$ mice at baseline (*Basal*) and after 3, 10, 15, or 30 min of restraint stress. The same mice were used to determine basal plasma ACTH levels (Fig. 3), adrenal corticosterone content (Table 1), and pituitary ACTH content (see Results). $^{**}p < 0.01$ versus basal; $^{\circ}p < 0.05$; $^{\circ\circ}p < 0.01$; n = 4–9 mice per group.

Dissection of adipose tissues and plasma leptin measurements. Mice were killed by decapitation. Epididymal white adipose tissue (around the testis), mesenteric white adipose tissue (around the stomach), and interscapular brown adipose tissue were dissected. Landmarks for dissection of white adipose tissue depots followed the planes of the tissue fascia capsule. Epididymal white adipose tissue included the fat pad surrounding the testis and was dissected free of the spermatic cord, testis, and epididymis. Mesenteric fat included all the fascia enclosed fat and minor blood vessels surrounding the gastrointestinal tract and excluded the pancreas and major lymph nodes. Plasma leptin levels were measured with a leptin RIA (Linco, St. Charles, MO). The intra- and inter-assay coefficients of variation were 3 and 10%, respectively.

Food and water intake. Naive mice were housed individually. The food and water intake of mice over 5 consecutive days was measured by weighing the remaining food and water.

Statistical analysis. Data are expressed as mean ± SEM. The statistical significance of differences between aged-matched groups was determined by ANOVA followed by Tukey–Kramer test when appropriate. $p < 0.05$ was considered significant.

RESULTS

Plasma corticosterone levels of *Apoe*$^{-/-}$ and wild-type mice

At 3 months of age, there was no significant difference in basal plasma corticosterone levels between *Apoe*$^{-/-}$ mice and wild-type controls (30.2 ± 6.8 and 23.7 ± 4.6 ng/ml, respectively; n = 4 mice per group). Ten min of restraint stress is a sensitive procedure to assess HPA axis responsivity (Raber et al., 1997). After 10 min of restraint stress, plasma corticosterone levels increased markedly, but there was no difference in plasma corticosterone levels between the *Apoe*$^{-/-}$ (140.5 ± 22.2 ng/ml) and wild-type (140.7 ± 6.2 ng/ml) mice at 3 months of age (n = 5 mice per group). In contrast, 6-month-old *Apoe*$^{-/-}$ mice had significantly higher plasma corticosterone levels than wild-type mice after 10 and 30 min of restraint stress (Fig. 1). Under basal

Table 1. Adrenal corticosterone content in *Apoe*$^{-/-}$ and wild-type mice

Group	Adrenal weight (mg)	Corticosterone content	
		ng/gland	ng/mg protein
3-Month-old mice			
Wild-type ($n = 5$)	10.8 ± 1.3	32.6 ± 6.1	2.9 ± 0.4
Apoe$^{-/-}$ ($n = 5$)	10.3 ± 0.7	32.6 ± 5.8	3.3 ± 0.6
6-Month-old mice			
Wild-type ($n = 9$)	10.8 ± 1.1	31.2 ± 6.1	2.9 ± 0.1
Apoe$^{-/-}$ ($n = 9$)	14.4 ± 1.6	74.0 ± 12.8*	5.9 ± 1.5

The same groups of mice were used to determine basal plasma corticosterone levels (see Fig. 1 and Results).

*$p < 0.01$ versus age-matched wild-type mice (Tukey–Kramer test).

conditions and after 3 min of restraint stress, *Apoe*$^{-/-}$ mice and wild-type controls had similar plasma corticosterone levels. A difference in plasma corticosterone levels after 10 min of restraint stress between *Apoe*$^{-/-}$ (109.1 ± 35.9 ng/ml) and wild-type (66.8 ± 8.0 ng/ml) mice was also detected at 18 months of age ($n = 4$ mice per group).

There were no significant differences in basal plasma corticosterone levels between wild-type and *Apoe*$^{-/-}$ mice in the late afternoon, when plasma corticosterone levels in rodents normally peak (Dallman et al., 1987), although there was a trend toward higher basal corticosterone levels in *Apoe*$^{-/-}$ (68.9 ± 13.3 ng/ml) than in wild-type (41.2 ± 10.3 ng/ml) mice ($n = 4$ mice per group).

Adrenal corticosterone content and histology

The adrenal corticosterone content was similar in *Apoe*$^{-/-}$ and wild-type mice at 3 months of age but was significantly higher in *Apoe*$^{-/-}$ mice than in wild-type mice at 6 months of age (Table 1). This difference is consistent with the increased plasma corticosterone after restraint stress in 6-month-old *Apoe*$^{-/-}$ mice. The adrenal glands in both 6-month-old groups showed similar hematoxylin and eosin staining (data not shown). However, 6-month-old *Apoe*$^{-/-}$ mice showed increases in lipid droplets in both the adrenal cortex and the medulla (Fig. 2), consistent with hypersecretion of adrenal corticosterone and increased adrenal corticosterone content (Hall and Almahbobi, 1997).

Plasma and pituitary ACTH levels

To determine the role of central HPA alterations in the stress-induced plasma corticosterone elevations of *Apoe*$^{-/-}$ mice, we analyzed plasma and pituitary ACTH levels. At 3 months of age, there was no significant difference in basal ACTH levels between *Apoe*$^{-/-}$ (65.8 ± 11.8 pg/ml) and wild-type (60.2 ± 5.3 pg/ml) mice ($n = 4$ mice per group). After 10 min of restraint stress, plasma ACTH levels increased markedly, but there was no significant difference in plasma ACTH levels between *Apoe*$^{-/-}$ (362.2.8 ± 42.8 pg/ml) and wild-type (273.3 ± 48.9 pg/ml) mice ($n = 5$ mice per group; $p = 0.096$).

There was also no significant difference in basal plasma ACTH levels between 6-month-old *Apoe*$^{-/-}$ and wild-type mice (Fig. 3). However, restraint stress-induced ACTH levels in 6-month-old *Apoe*$^{-/-}$ mice were disproportionally low (Fig. 3) compared with the increased stress-induced plasma corticosterone levels observed at this age (Fig. 1). There were no significant differences in pituitary ACTH content between *Apoe*$^{-/-}$ (0.243 ± 0.088 pg/μg protein; $n = 5$) and wild-type (0.205 ± 0.032 pg/μg protein; $n = 9$) mice.

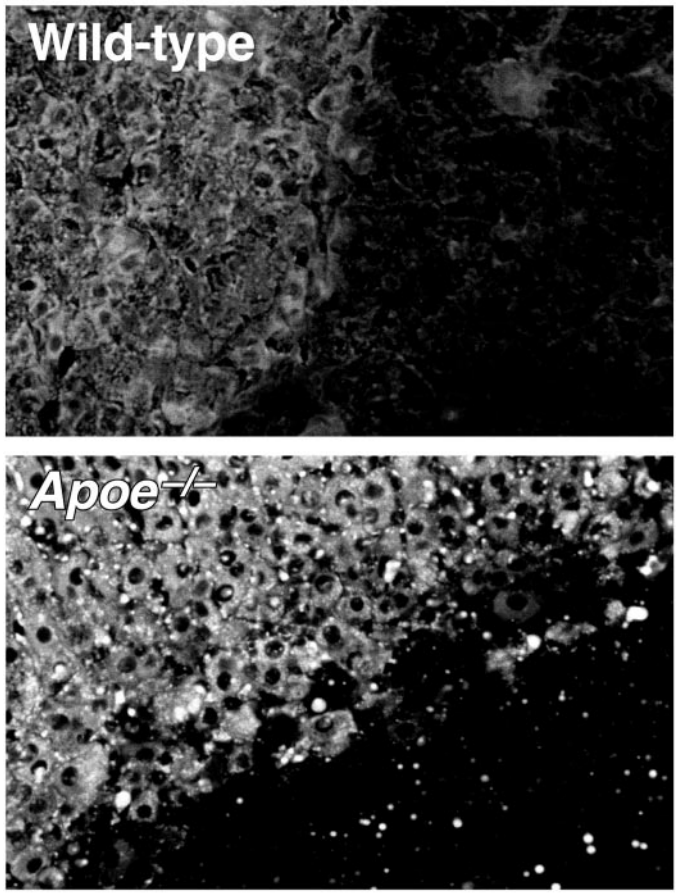

Figure 2. Nile Red staining of the adrenal glands of 6-month-old wild-type (*top*) and *Apoe*$^{-/-}$ (*bottom*) mice. Lipid deposits were visualized as described in Materials and Methods. Adrenals of *Apoe*$^{-/-}$ mice have an increased lipid content.

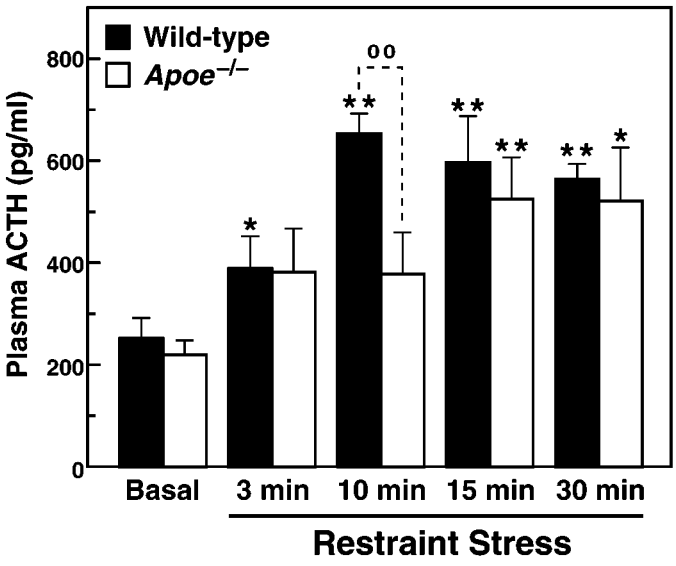

Figure 3. Plasma ACTH levels in 6-month-old wild-type and *Apoe*$^{-/-}$ mice at baseline (*Basal*) and after 3, 10, 15, or 30 min of restraint stress. *$p < 0.05$; **$p < 0.01$ versus basal; °°$p < 0.01$; $n = 4$–9 mice per group.

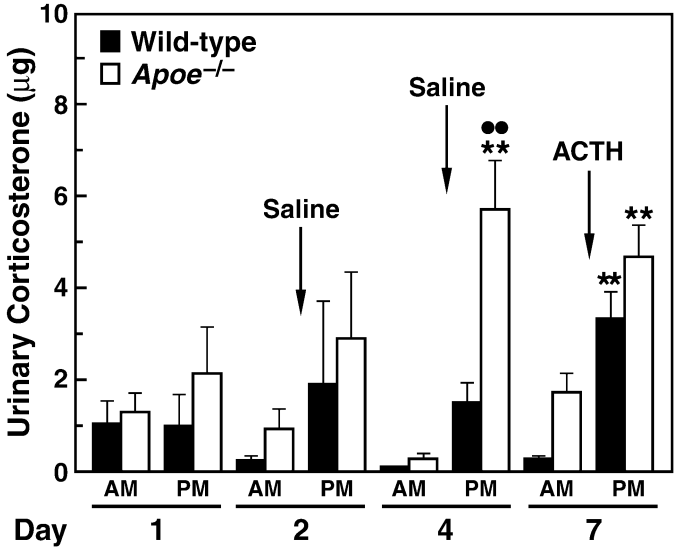

Figure 4. Adrenal responsivity to ACTH challenge in 6-month-old wild-type and *Apoe*$^{-/-}$ mice. Naive mice were housed in metabolic cages. After 1 week of habituation to the novel environment, urine was collected from 5:00 P.M. to 7:00 A.M. (*PM bars*) and from 7:00 A.M. to 5:00 P.M. (*AM bars*). After the 5:00 P.M. collection, mice were challenged with saline (0.1 ml/mouse, i.p.) on the second and fourth days and with 0.4 U of ACTHar (0.1 ml/mouse, i.p.) on the seventh day. $^{**}p < 0.01$ versus A.M.; $^{\bullet\bullet}p < 0.01$ versus wild-type; $n = 6$ or 7 mice per group.

Adrenal sensitivity to ACTH challenge

A higher sensitivity of the adrenal gland of *Apoe*$^{-/-}$ mice to stimulation with ACTH might explain the relatively low plasma ACTH levels and high plasma corticosterone levels after restraint stress (Figs. 1, 3). To test this possibility, we used metabolic cages to measure overnight urinary corticosterone excretion in 6-month-old *Apoe*$^{-/-}$ and wild-type mice challenged at 5:00 P.M. with saline or highly purified ACTH (ACTHar) (Fig. 4). After the second saline injection, the plasma urinary corticosterone excretion increased more in *Apoe*$^{-/-}$ mice than in wild-type mice ($p < 0.01$). However, there was no significant difference in the ACTHar-induced corticosterone levels between the two groups. When challenged with 0.2 or 2.0 U of ACTHar, *Apoe*$^{-/-}$ and wild-type mice also showed a similar adrenal sensitivity (data not shown). Thus, *Apoe*$^{-/-}$ mice did not exhibit an increased adrenal sensitivity to ACTH.

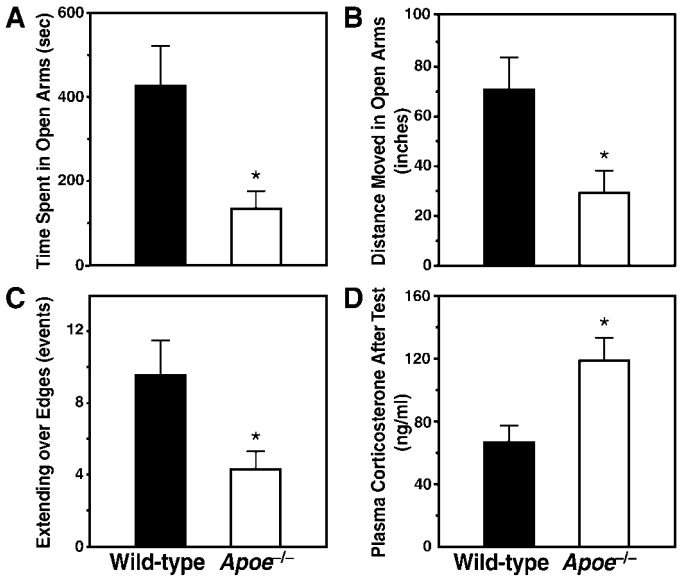

Figure 5. Anxiety levels in 6-month-old wild-type and *Apoe*$^{-/-}$ mice assessed in the elevated plus maze. Compared with the wild-type controls, *Apoe*$^{-/-}$ mice showed reductions in the time spent in the open arms (*A*), in the distance moved in the open arms (*B*), and in the number of times they extended over the edges of the open arms to explore (*C*). *Apoe*$^{-/-}$ mice also had abnormally increased plasma corticosterone levels after behavioral testing (*D*), consistent with increased anxiety. $^{*}p < 0.05$ versus wild-type; $n = 8$ mice per group.

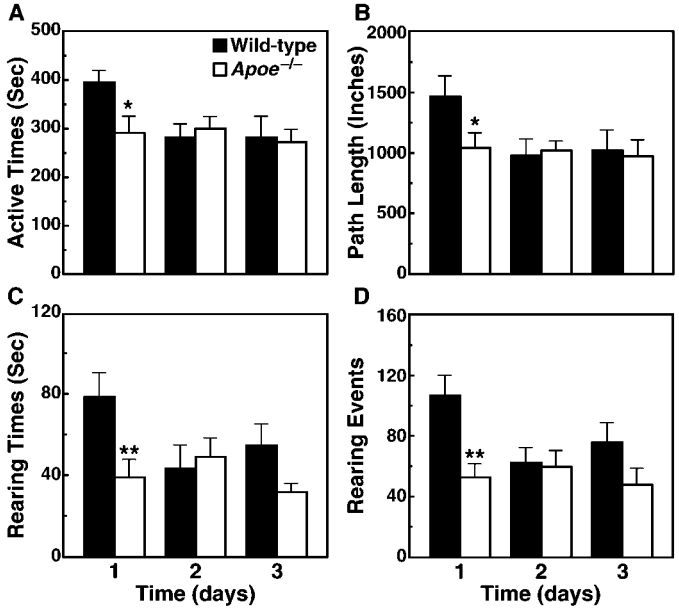

Figure 6. Open field activity of 12-month-old wild-type and *Apoe*$^{-/-}$ mice. On each of 3 consecutive days, open field activity was recorded after an initial 1 min adaptation period. In wild-type mice, horizontal and vertical activities declined significantly ($p < 0.01$, repeated measures ANOVA). On day 1, the active times (*A*), path lengths (*B*), and frequency (*C*) and duration (*D*) of rearing events were significantly reduced in *Apoe*$^{-/-}$ mice compared with wild-type controls. *Apoe*$^{-/-}$ mice showed no further decline in horizontal or vertical activity on days 2 and 3. $^{*}p < 0.05$; $^{**}p < 0.01$ versus wild-type, Tukey–Kramer test; $n = 8$ mice per group.

Behavioral alterations in *Apoe*$^{-/-}$ mice

We next determined possible behavioral consequences of HPA axis dysregulation in *Apoe*$^{-/-}$ mice. When anxiety levels were assessed in the elevated plus maze, 6-month-old male *Apoe*$^{-/-}$ mice showed more anxiety than wild-type controls (Fig. 5*A–C*). *Apoe*$^{-/-}$ mice also had higher plasma corticosterone levels than wild-type controls after behavioral testing (Fig. 5*D*). However, no significant differences in horizontal or vertical exploratory activity were detected between these groups of mice in the open field (Raber et al., 1998). In contrast, at 12 months of age, exposure to a novel open field elicited significantly less horizontal and vertical activity in *Apoe*$^{-/-}$ mice than in wild-type controls (Fig. 6). On subsequent days, *Apoe*$^{-/-}$ mice showed no decline in exploratory activity, whereas age-matched wild-type mice showed a significant decrease in horizontal and vertical activity between days 1 and 2 ($p < 0.01$) (Fig. 6).

Metabolic alterations in *Apoe*$^{-/-}$ mice

Because the HPA axis is involved in regulating energy balance (Akana et al., 1994; Dallman et al., 1994), we examined whether

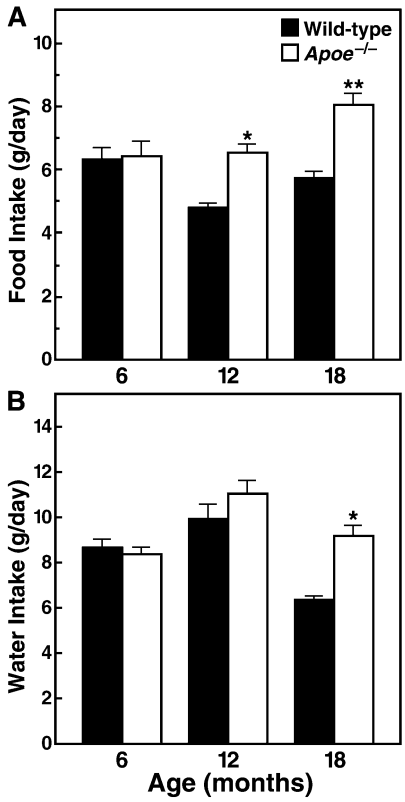

Figure 7. Food and water intake of 6-, 12-, and 18-month-old wild-type and *Apoe*$^{-/-}$ mice was measured over 5 consecutive days and averaged. $^{*}p < 0.05$; $^{**}p < 0.01$ versus wild-type; $n = 5$–11 mice per group.

the HPA axis dysregulation might be associated with metabolic alterations in *Apoe*$^{-/-}$ mice. Food and water intake was compared in *Apoe*$^{-/-}$ mice and wild-type controls (Fig. 7). At 6 months of age, there were no differences between *Apoe*$^{-/-}$ mice and wild-type controls. However, *Apoe*$^{-/-}$ mice showed significant increases in food intake at 12 and 18 months of age and in water intake at 18 months of age (Fig. 7).

Next we determined whether the increased energy intake in *Apoe*$^{-/-}$ mice was associated with other metabolic alterations (Table 2). Compared with age-matched wild-type mice, *Apoe*$^{-/-}$ mice showed significant increases in stomach and body weight and decreases in interscapular brown adipose tissue at 18 but not at 6 months of age. Plasma leptin levels and epididymal white adipose tissue were significantly lower in *Apoe*$^{-/-}$ mice than wild-type controls at both 6 and 18 months of age.

DISCUSSION

This study shows that apoE deficiency in mice results in an age-dependent dysregulation of the HPA axis through a mechanism affecting primarily the adrenal gland. *Apoe*$^{-/-}$ mice had an age-dependent increase in adrenal corticosterone content at baseline and abnormally increased plasma corticosterone levels after restraint stress, whereas their plasma and pituitary ACTH levels were either unchanged or decreased compared with those in wild-type controls. The dysregulation of the HPA axis in *Apoe*$^{-/-}$ mice was associated with behavioral and metabolic alterations.

The function of apoE synthesized by the adrenal gland is unknown. Our findings of increased adrenal corticosterone content and stress-induced corticosterone hypersecretion in *Apoe*$^{-/-}$ mice suggest a key role for apoE in the tonic inhibition of steroidogenesis and adrenal cortical activity. These data are consistent with the inverse relationship between the levels of apoE mRNA and adrenal steroidogenesis (Prack et al., 1991; Nicosia et al., 1992). ApoE may exert regulatory effects on steroidogenesis by altering cholesterol metabolism or cholesterol trafficking within cells. ApoE expression in murine adrenocortical Y1 cells promotes cholesteryl ester storage and reduces cholesterol utilization for either steroidogenesis or efflux from the cell (Prack et al., 1991). Intracellular roles for apoE are supported by immunocytochemical studies detecting apoE in intracellular locations besides those expected for the secretory or endocytic pathways (Hamilton et al., 1990).

The dysregulation of the HPA axis in *Apoe*$^{-/-}$ mice is age-dependent and parallels the time course of the development of structural alterations in the hippocampus (Masliah et al., 1995; Buttini et al., 1999). Chronic stress or corticosterone induces dendritic atrophy in hippocampal neurons (Woolley et al., 1990; Watanabe et al., 1992). Adrenalectomy and basal level corticosterone replacement attenuated the hippocampal pathology in aged rodents (Landfield et al., 1981), supporting an important role for elevated GC levels in hippocampal atrophy (Porter and Landfield, 1998). Thus, HPA axis dysregulation in *Apoe*$^{-/-}$ mice might contribute to the age-dependent loss of microtubule-associated protein-2-positive neuronal dendrites and synaptophysin-positive terminals in the hippocampus found in these mice (Masliah et al., 1995; Buttini et al., 1999).

The hippocampus may be a site of GC-mediated negative feedback on the HPA axis. Damage to the hippocampus (Sapolsky et al., 1985) or prefrontal cortex (Diorio et al., 1993) increases the corticosterone response to restraint stress. This raises the possibility that the neuropathological changes in *Apoe*$^{-/-}$ mice are either primary alterations that dysregulate the HPA axis or secondary alterations that exacerbate this dysregulation. The following observation suggests that the neuropathological changes in *Apoe*$^{-/-}$ mice are not the main cause of the HPA axis dysregulation. In *Apoe*$^{-/-}$ mice, in which human apoE isoforms were expressed in the brain at matching levels directed by the neuron-specific enolase promoter, apoE3 prevented age-dependent neuropathology in the cortex and hippocampus, but apoE4 did not (Buttini et al., 1999). However, restraint stress-induced plasma corticosterone levels in *Apoe*$^{-/-}$ mice expressing apoE3 or apoE4 were not significantly different and comparable with those in *Apoe*$^{-/-}$ mice without human apoE expression (*Apoe*$^{-/-}$ mice, 203 ± 43 ng/ml; apoE3 mice, 192 ± 44 ng/ml; apoE4 mice, 238 ± 34 ng/ml; $n = 5$–7 mice per group).

The age-dependent stress-induced increase in plasma corticosterone levels in *Apoe*$^{-/-}$ mice was paralleled by increased anxiety in the elevated plus maze and decreased exploratory behavior in the open field on day 1 (Figs. 5, 6). Because in their home cages, mice also experience stressful situations, plasma corticosterone levels may repeatedly increase to abnormal levels in *Apoe*$^{-/-}$ mice. Consistent with this hypothesis, plasma corticosterone levels in 6-month-old *Apoe*$^{-/-}$ mice were higher than in age-matched controls after testing in the plus maze (Fig. 5). Repeated exposure to the open field on consecutive days was associated with a decline in horizontal and vertical activity in wild-type but not *Apoe*$^{-/-}$ mice (Fig. 6). This could indicate that *Apoe*$^{-/-}$ mice have impairments in spatial habituation learning, which is characterized by decreased responses to repeated presentation of the same spatial stimuli, independent of muscle fatigue or receptor adaptation. However, the horizontal explor-

Table 2. Metabolic alterations in *Apoe*$^{-/-}$ mice

	6-Month-old		18-Month-old	
Characteristic	Wild-type	*Apoe*$^{-/-}$	Wild-type	*Apoe*$^{-/-}$
Body weight (gm)	33.9 ± 0.8	33.2 ± 2.0	31.2 ± 1.0	41.5 ± 3.7*
Stomach[a]	172.8 ± 20.0	229.6 ± 33.2	205.5 ± 7.1	286.3 ± 44.2*
Adipose Tissues[a]				
Interscapular brown	33.6 ± 0.3	30.7 ± 1.2	38.0 ± 3.8	24.2 ± 0.4*
Mesenteric white	161.7 ± 15.6	108.4 ± 19.1	226.1 ± 14.2	197.4 ± 19.2
Epididymal white	141.8 ± 15.6	59.3 ± 4.7**	105.0 ± 10.4	74.0 ± 8.3*
Leptin (ng/ml)	6.3 ± 0.9	1.7 ± 0.5**	4.3 ± 0.8	2.6 ± 0.6*

$n = 4–8$ mice per group.

[a]Values represent mg/10 gm body weight to correct for differences in body weight.

*$p < 0.05$; **$p < 0.01$ versus wild-type (Tukey–Kramer test).

atory activity of the wild-type mice on day 3 and of the *Apoe*$^{-/-}$ mice on day 1 was similar, and this may be the minimal horizontal activity C57BL/6J mice attain in this paradigm. Therefore, differences in the activity of *Apoe*$^{-/-}$ and wild-type mice in the open field may be attributable primarily to the increased anxiety of *Apoe*$^{-/-}$ mice in a novel environment.

Our behavioral data are consistent with studies using glucocorticoid and mineralocorticoid receptor antagonists, which support a role for GCs in exploring novel environments and anxiety-related behavior (Oitzl et al., 1994; Korte et al., 1995; Smythe et al., 1997; Bitran et al., 1998; Ströhle et al., 1998). *Apoe*$^{-/-}$ mice have been the subject of numerous behavioral investigations (for example, see Gordon et al., 1995; Masliah et al., 1997; Oitzl et al., 1997; Anderson et al., 1998; Fisher et al., 1998; Raber et al., 1998). The findings of the current study underline the need to carefully consider the altered HPA axis and increased anxiety of these mice in the interpretation of behavioral alterations identified in these animals. Although the increased stress response in 6-month-old male *Apoe*$^{-/-}$ mice had no effect on their performance in a water maze task or on their behavior in the open field (Raber et al., 1998; data not shown), male *Apoe*$^{-/-}$ mice showed decreased activity in the open field at 12 months of age (Fig. 6). The metabolic alterations in *Apoe*$^{-/-}$ mice (Fig. 7, Table 2) can also have important implications for their behavioral assessment, because they could confound the interpretation of certain tests, such as the hole board test, in which mice must learn to locate a food or water reward.

In the current study there were no differences at 3 months of age in plasma corticosterone response after restraint stress between *Apoe*$^{-/-}$ and wild-type mice. These results differ from the decreased plasma corticosterone response after restraint stress reported for 1.5- and 4-month-old *Apoe*$^{-/-}$ mice (Gordon et al., 1996; Zhou et al., 1998). The reason for this discrepancy is unclear. The stress-induced increases in plasma corticosterone levels in 6-month-old *Apoe*$^{-/-}$ mice (Fig. 1) were associated with a disproportionately low increase in the ACTH response (Fig. 3). Yet, there was no significant difference in the ACTH-induced urinary corticosterone levels between *Apoe*$^{-/-}$ and wild-type mice, indicating that *Apoe*$^{-/-}$ mice did not exhibit an increased adrenal sensitivity to ACTH. It is conceivable that increased levels of other factors that can directly activate the adrenal gland (Raber et al., 1997) might contribute to the stress-induced GC hypersecretion in *Apoe*$^{-/-}$ mice.

The increase in stress-induced plasma GC levels in *Apoe*$^{-/-}$ mice preceded the increase in food intake and the decrease in adipose tissue, consistent with previously reported effects of chronic stress (Akana et al., 1996). The reduced amount of adipose tissue in *Apoe*$^{-/-}$ mice is consistent with their reduced plasma levels of leptin, which is secreted by adipocytes in proportion to the amount of adipose tissue. The stress-induced hypersecretion of GCs might also reduce leptin levels by stimulating leptin clearance (Arvaniti et al., 1998). The reduced leptin levels in turn could contribute to stress-induced GC hypersecretion, because leptin has been reported to reduce the plasma GC response to stress and fasting (Ahima et al., 1996; Heiman et al., 1997) and the secretion of GCs in primary adrenal cultures (Kruse et al., 1998). Interestingly, reduced plasma leptin and increased plasma total cholesterol levels predict increases in body mass index, even when adjusted for body fat, in children (Byrnes et al., 1999). The *Apoe*$^{-/-}$ model may relate to this situation, because it also combines weight gain with decreased leptin (this study) and increased plasma cholesterol levels (Zhang et al., 1992).

HPA axis dysregulation with chronic GC hypersecretion appears to exert detrimental effects in normal human aging (Lupien et al., 1998; Porter and Landfield, 1998) and in several human diseases, including Alzheimer's disease (AD), AIDS dementia, Cushing's syndrome, and depression. In patients with AD and Cushing's syndrome and in children with AIDS, HPA axis activity, as determined by plasma cortisol levels, correlates with the severity of hippocampal atrophy. There are different patterns of response to chronic HPA axis activation (Aguilera, 1994). The pattern of HPA axis dysregulation in *Apoe*$^{-/-}$ mice may be relevant to human disease. Chronic activation of the HPA axis in patients with AD, multiple sclerosis, and depression (Hatzinger et al., 1995; Gold et al., 1995) also blunts the ACTH response but not the cortisol response, resembling diminished ACTH responses during chronic stress (Aguilera, 1994). Our study suggests an important role for apoE in the regulation of adrenal steroidogenesis and raises the intriguing possibility that alterations in the level or activity of apoE could be involved in disorders with GC hypersecretion.

REFERENCES

Aguilera G (1994) Regulation of pituitary ACTH secretion during chronic stress. Front Neuroendocrinol 15:321–350.

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252.

Akana SF (1999) Assessment of integrated endocrine responses to cold challenge in mice. Endocr Soc Abstr V139A:364.

Akana SF, Strack AM, Hanson ES, Dallman MF (1994) Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a

larger hypothalamic system that determines caloric flow. Endocrinology 135:1125–1134.
Akana SF, Hanson ES, Horsley C, Strack AM, Bhatnagar S, Bradbury MJ, Milligan ED, Dallman MF (1996) Clamped corticosterone (B) reveals the effect of endogenous B on both facilitated responsivity to acute restraint and metabolic responses to chronic stress. Stress 1:33–49.
Akwa Y, Schumacher M, Jung-Testas I, Baulieu EE (1993) Neurosteroids in rat sciatic nerves and Schwann cells. C R Acad Sci III 316:410–414.
Anderson R, Barnes JC, Bliss TVP, Cain DP, Cambon K, Davies HA, Errington ML, Fellows LA, Gray RA, Hoh T, Stewart M, Large CH, Higgins GA (1998) Behavioral, physiological and morphological analysis of a line of apolipoprotein E knockout mouse. Neuroscience 85:93–110.
Arvaniti K, Ricquier D, Champigny O, Richard D (1998) Leptin and corticosterone have opposite effects on food intake and the expression of UCP1 mRNA in brown adipose tissue of *lep*^{ob}/*lep*^{ob} mice. Endocrinology 139:4000–4003.
Bitran D, Shiekh M, Dowd JA, Dugan MM, Renda P (1998) Corticosterone is permissive to the anxiolytic effect that results from the blockade of hippocampal mineralocorticoid receptors. Pharmacol Biochem Behav 60:879–887.
Britton DR, Koob GF, Rivier J, Vale W (1982) Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci 31:363–367.
Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T, Mucke L, Mahley RW (1999) Expression of human apolipoprotein E3 or E4 in the brains of *Apoe*$^{-/-}$ mice: isoform-specific effects on neurodegeneration. J Neurosci 19:4867–4880.
Byrnes SE, Baur LA, Bermingham M, Brock K, Steinbeck K (1999) Leptin and total cholesterol are predictors of weight gain in prepubertal children. Int J Obes Relat Metab Disord 23:146–150.
Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N (1987) Regulation of ACTH secretion: variations on a theme of B. Recent Prog Horm Res 43:113–173.
Dallman MF, Akana SF, Levin N, Walker C-D, Bradbury MJ, Suemaru S, Scribner KS (1994) Corticosteroids and the control of function in the hypothalamo-pituitary-adrenal (HPA) axis. Ann NY Acad Sci 746:22–32.
Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathθ AA, Johns CA, Horvath TB (1986) Cortisol and Alzheimer's disease, I: basal studies. Am J Psychiatry 143:300–305.
Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J Neurosci 13:3839–3847.
Fagan AM, Murphy BA, Patel SN, Kilbridge JF, Mobley WC, Bu G, Holtzman DM (1998) Evidence for normal aging of the septohippocampal cholinergic system in apoE ($^{-/-}$) mice but impaired clearance of axonal degeneration products following injury. Exp Neurol 151:314–325.
Fisher A, Brandeis R, Chapman S, Pittel Z, Michaelson DM (1998) M1 muscarinic agonist treatment reverses cognitive and cholinergic impairments of apolipoprotein E-deficient mice. J Neurochem 70:1991–1997.
Gold PW, Licinio J, Wong M-L, Chrousos GP (1995) Corticotropin releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. Ann NY Acad Sci 771:716–729.
Gordon I, Grauer E, Genis I, Sehayek E, Michaelson DM (1995) Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. Neurosci Lett 199:1–4.
Gordon I, Ben-Eliyahu S, Rosenne E, Sehayek E, Michaelson DM (1996) Derangement in stress response of apolipoprotein E-deficient mice. Neurosci Lett 206:212–214.
Gray JA (1982) The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system. New York: Oxford UP.
Gray JA, McNaughton N (1983) Comparison between the behavioural effects of septal and hippocampal lesions: a review. Neurosci Biobehav Rev 7:119–188.
Hall PF, Almahbobi G (1997) Roles of microfilaments and intermediate filaments in adrenal steroidogenesis. Microsc Res Tech 36:463–479.
Hamilton RL, Wong JS, Guo LS, Krisans S, Havel RJ (1990) Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. J Lipid Res 31:1589–1603.
Hatzinger M, Z'brun A, Hemmeter U, Seifritz E, Baumann F, Holsboer-Trachsler E, Heuser IJ (1995) Hypothalamic-pituitary-adrenal system function in patients with Alzheimer's disease. Neurobiol Aging 16:205–209.
Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS (1997) Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 138:3859–3863.
Koob GF, Bloom FE (1985) Corticotropin-releasing factor and behavior. Fed Proc 44:259–263.
Korte SM, de Boer SF, de Kloet ER, Bohus B (1995) Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze. Psychoneuroendocrinology 20:385–394.
Kruse M, Bornstein SR, Uhlmann K, Paeth G, Scherbaum WA (1998) Leptin down-regulates the steroid producing system in the adrenal. Endocr Res 24:587–590.
Landfield PW, Baskin RK, Pitler TA (1981) Brain aging correlates: retardation by hormonal-pharmacological treatments. Science 214:581–584.
Liang KC, Lee EHY (1988) Intra-amygdala injections of corticotropin releasing factor facilitate inhibitory avoidance learning and reduce exploratory behavior in rats. Psychopharmacology 96:232–236.
Lupien SJ, McEwen BS (1997) The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res Rev 24:1–27.
Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NPV, Thakur M, McEwen BS, Hauger RL, Meaney MJ (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1:69–73.
Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience 69:89–98.
Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–630.
Masliah E, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD (1995) Neurodegeneration in the central nervous system of apoE-deficient mice. Exp Neurol 136:107–122.
Masliah E, Samuel W, Veinbergs I, Mallory M, Mante M, Saitoh T (1997) Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. Brain Res 751:307–314.
McEwen BS, Sapolsky RM (1995) Stress and cognitve function. Curr Opin Neurobiol 5:205–216.
McEwen BS, De Kloet ER, Rostene W (1986) Adrenal steroid receptors and actions in the nervous system. Physiol Rev 66:1121–1188.
Mellon SH, Nussbaum S, Hochberg RB (1980) Biosynthesis of lipidal derivative of pregnenolone and hydroepiandrosterone by the adrenal. J Biol Chem 255:5566–5572.
Nicosia M, Prack MM, Williams DL (1992) Differential regulation of apolipoprotein-E messenger RNA in zona fasciculata cells of rat adrenal gland determined by in situ hybridization. Mol Endocrinol 6:288–298.
Oitzl MS, Fluttert M, de Kloet ER (1994) The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticosteroid receptors. Eur J Neurosci 6:1072–1079.
Oitzl MS, Mulder M, Lucassen PJ, Havekes LM, Grootendorst J, De Kloet ER (1997) Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. Brain Res 752:189–196.
Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N (1992) Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 89:4471–4475.
Plump AS, Smith JD, Hayek T, Aalto-Setala A, Walsh A, Verstuyft JG, Rubin EM, Breslow JL (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71:343–353.
Porter NM, Landfield PW (1998) Stress hormones and brain aging: adding injury to insult? Nat Neurosci 1:3–4.
Prack MM, Nicosia M, Williams DL, Gwynne J (1991) Relationship

between apolipoprotein E mRNA expression and tissue cholesterol content in rat adrenal gland. J Lipid Res 32:1611–1618.
Raber J (1998) Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation—from obesity to memory deficits. Mol Neurobiol 18:1–22.
Raber J, O'Shea RD, Bloom FE, Campbell IL (1997) Modulation of hypothalamic-pituitary-adrenal function by transgenic expression of interleukin-6 in the CNS of mice. J Neurosci 17:9473–9480.
Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pitas RE, Mahley RW, Mucke L (1998) Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: increased susceptibility of females. Proc Natl Acad Sci USA 95:10914–10919.
Sapolsky RM (1996) Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. Stress 1:1–19.
Sapolsky RM, Krey LC, McEwen BS (1985) Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5:1222–1227.
Smythe JW, Murphy D, Timothy C, Costall B (1997) Hippocampal mineralocorticoid, but not glucocorticoid, receptors modulate anxiety-like behavior in rats. Pharmacol Biochem Behav 56:507–513.
Ströhle A, Poettig M, Barden N, Holsboer F, Montkowski A (1998) Age- and stimulus-dependent changes in anxiety-related behaviour of transgenic mice with GR dysfunction. NeuroReport 9:2099–2102.
Sutton RE, Koob GF, Moal ML, Rivier J, Vale W (1982) Corticotropin releasing factor produces behavioural activation in rats. Nature 297:331–333.
Watanabe Y, Gould E, Cameron HA, Daniels DC, McEwen BS (1992) Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. Hippocampus 2:431–435.
Weisgraber KH, Mahley RW (1996) Human apolipoprotein E: the Alzheimer's disease connection. FASEB J 10:1485–1494.
Woolley CS, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 531:225–231.
Zhang SH, Reddick RL, Piedrahita JA, Maeda N (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258:468–471.
Zhou Y, Elkins PD, Howell LA, Ryan DH, Harris RBS (1998) Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. Brain Res 788:151–159.