The Douglas Hospital Longitudinal Study of Normal and Pathological Aging: summary of findings

Sonia J. Lupien, PhD; Georges Schwartz, MSc; Ying Kin Ng, PhD; Alexandra Fiocco, MSc; Nathalie Wan, MSc; Jens C. Pruessner, PhD; Michael J. Meaney, PhD; N.P. Vasavan Nair, MD

Douglas Hospital Research Centre, Borough of Verdun, and Department of Psychiatry, McGill University, Montréal, Que.

Introduction

Perhaps one of the most prominent features of human aging is the variability with which intellectual processes decline. Although many research avenues have been used to study the origin of the increase in variability with aging (for a review, see Lupien and Lecours1), new studies suggest that some biologic factors may be associated with normal and pathologic cognitive aging. One biologic parameter that has come under scrutiny in the past few years is related to the hypothalamic–pituitary–adrenal (HPA) axis, an endocrine closed-loop system controlling secretion of the stress hormones known as glucocorticoids (the most prominent of which are cortisol in humans and corticosterone in animals).

Important characteristics of glucocorticoids

Under basal conditions, the secretion of cortisol in humans exhibits a 24-hour circadian profile in which cortisol concentrations present a morning maximum (the circadian peak),
slowly declining levels during the late afternoon, evening and nocturnal period (the circadian trough) and an abrupt elevation after the first few hours of sleep. Circulating cortisol binds with high affinity to 2 glucocorticoid receptor subtypes: the mineralocorticoid receptor (MR, also called type I) and the glucocorticoid receptor (GR, also called type II). Although both receptor types have been implicated in mediating glucocorticoid feedback effects, there are 2 major differences between them. First, MRs bind cortisol with an affinity that is about 6–10 times higher than that of GRs. This differential affinity results in a striking difference in occupation of the 2 receptor types under different conditions and at different times of the day. Low basal cortisol levels observed during nonstressed periods or the afternoon, evening and nocturnal phase in humans serve to activate largely MRs, whereas the elevated cortisol levels characteristic of periods of stress or the morning phase in humans activate both MRs and GRs. The second major difference between these 2 receptor types is related to their distribution throughout the brain. The MRs are present exclusively in the limbic system, with a preferential distribution in the hippocampus, parahippocampal gyrus, and the entorhinal and insular cortices. In contrast, GRs are present in both subcortical structures (paraventricular nucleus and other hypothalamic nuclei, amygdala, hippocampus and parahippocampal gyrus) and cortical structures, with a preferential distribution in the prefrontal cortex. Although not the sole region involved, the prefrontal cortex has been shown to be a key structure in working memory function, whereas the amygdala plays an important role in processing the emotional significance of events. Working memory serves to store and manipulate information over short periods of time until it is transferred into long-term memory. The hippocampus is a key structure for declarative memory function (again, without being the sole region involved). Declarative memory refers to the conscious and voluntary recollection of previously learned information.

Effects of short-term increase in glucocorticoids

Given the preferential distribution of GRs in the prefrontal cortex and the presence of both MRs and GRs in the hippocampus and amygdala, it has been suggested that a short-term increase in glucocorticoids might affect various types of memory processing. In keeping with this suggestion, some studies have shown that a short-term increase in glucocorticoids impairs both the retrieval and the acquisition of information, while other studies have shown that working memory is also sensitive to a short-term increase in glucocorticoids. Finally, studies in both animals and humans have revealed that glucocorticoids have a significant impact on emotional memory.

During periods of stress or when levels of cortisol are high, both the MRs and GRs will be occupied. Many rodent studies have shown that the ratio of MR–GR occupation is the major determinant of the direction of glucocorticoid-induced cognitive changes (for a review, see de Kloet et al), which leads to an inverted U-shape function relating circulating levels of glucocorticoids and memory function. For example, in rodents, long-term potentiation (a proposed neurobiologic substrate of memory formation) is reportedly optimal when glucocorticoid levels are mildly elevated and the ratio of MR–GR occupation is high (i.e., MRs are saturated while GRs are only partially occupied). In contrast, significant decreases in long-term potentiation are observed after adrenalectomy (removal of the adrenal glands secreting glucocorticoids) and the ratio of MR–GR occupation is very low (absence of MRs or GR occupancy). The same negative impact of glucocorticoids on long-term potentiation has been reported after exogenous administration of supraphysiologic doses of synthetic glucocorticoids, which saturate both MRs and GRs, again leading to a low ratio of MR–GR occupation.

Effects of long-term increase in glucocorticoids

In the rat and the monkey, chronic hypercortisolemia with or without stress leads to severe memory impairments and increases the vulnerability of the hippocampal neurons to subsequent insults, although neuronal loss has been reported in some but not all experimental paradigms. Interestingly, in the rat, less severe forms of chronic stress have been shown to produce a different pattern of hippocampal morphologic changes, which are reflected in atrophy of the neuronal dendrites. This type of atrophy is characterized by atrophy of the CA3 pyramidal neurons and features a less complex branching pattern and a decrease in the total length of the apical dendrites. It generally appears after 21 days of corticosterone therapy or 6 hours/day of restraint stress. Interestingly, neurons exhibiting dendritic atrophy do not necessarily proceed to stress-induced neuronal death, which could explain recent rodent results showing that the hippocampal atrophy induced by exposure to either chronic stress or high levels of corticosterone is reversible. However, given that no other brain regions have been studied in the context of long-term exposure to stress or high levels of corticosteroids, it is difficult to ascertain whether the impact of corticosteroids on brain atrophy is specific to the hippocampus.

For obvious ethical reasons, most of the human studies assessing the impact of chronic hypercortisolemia on memory performance and hippocampal volume are correlational, although some have prospectively measured the impact of treatment on hippocampal volume. In general, researchers have found that patients with Cushing’s disease (which leads to chronic oversecretion of cortisol) and other patients taking exogenous glucocorticoids (as an anti-inflammatory treatment) on a long-term basis present both memory impairments and reduced hippocampal volume. Together, these results led to the hypothesis that long-term exposure to high levels of glucocorticoids might have a negative impact on both memory performance and hippocampal volume in humans.

Previous studies showing that aging in animals is associated with increased secretion of glucocorticoids and others reporting that older adults with dementia have hypersecretion of cortisol raised the questions of whether variability in cortisol secretion could be observed in older adults and whether older adults with increased levels of cortisol would present with memory impairments and/or reduced hippocampal volume.
Douglas Hospital Longitudinal Study of Normal and Pathological Aging

To address these important questions, one of us (N.P.V.N.) initiated the Douglas Hospital Longitudinal Study of Normal and Pathological Aging in 1988 (with approval from the Research Ethics Committee of the Douglas Hospital). The main goals of this study were to assess whether there are significant interindividual differences in the secretion of cortisol in later life and whether there is an association between basal cortisol levels, memory performance and hippocampal volumes in older adults. We examined a sample of older healthy control subjects (60–87 years of age at the time of entry into the study), with hourly sampling of cortisol levels over a 24-hour period performed once yearly. Seventeen women and 34 men participated in this longitudinal study, providing informed consent each year. The inclusion criteria for entry into the study were as follows: no history of head trauma or cerebral vascular accident, no alcohol abuse or use of drugs that could interfere with performance, no signs of dementia or depression on standardized tests and questionnaires, and no general anesthesia in the previous year.

Each year, subjects underwent a complete physical examination, including electrocardiography, electroencephalography, CT, and a battery of laboratory tests for kidney, liver and thyroid functions, vitamin B12 and folate levels, as well as a neuropsychologic assessment by a certified neuropsychologist. For determination of cortisol levels, samples were obtained hourly over a 24-hour period using an indwelling forearm catheter, which was kept patent with a 0.3% heparin saline solution. Throughout the course of sampling, illumination was maintained at 300 lx during the “daytime” (7 am to 11 pm) and at 50 lx during the “nighttime” (11 pm to 7 am). Blood samples were taken each hour, centrifuged at 2500 rpm for 10 minutes at 0-4°C, frozen and stored at –20°C until assayed.

To determine the change in cortisol levels over a period of years for a particular subject, a simple regression analysis on plasma cortisol levels for each subject was conducted, with year as the independent variable and the integrated 24-hour cortisol concentration for each year as the dependent variable. The direction and amplitude of the slope of the regression line served as the measure of cortisol history for each subject. Indeed, the direction of the slope gave us an indication of the change (increase or decrease) in cortisol levels with time, while the magnitude of the slope gave us an indication of the rapidity of these changes over time.23

Using this measure, we have found considerable variation in plasma cortisol levels, as well as clear evidence for 3 subgroups: the first showing a progressive year-to-year increase in cortisol levels with currently high levels (the Increasing/High cortisol group; n = 12), the second showing a progressive year-to-year increase in cortisol levels with currently moderate levels (the Increasing/Moderate cortisol group; n = 29), and the third showing a progressive year-to-year decrease in cortisol levels with currently moderate cortisol levels (the Decreasing/Moderate cortisol group; n = 10) (Table 1). We measured the endocrine and metabolic correlates of these subgroups and found that there was no change in the circadian rhythm or the corticosteroid-binding globulin levels in the 3 groups, nor were there any differences between men and women with regard to cortisol history or any other variables tested.30 These results confirmed animal studies showing that about 30% of older animals exhibit significant increases in glucocorticoids during aging.27

In the second phase of this longitudinal study, we measured the neuropsychologic correlates of the subgroups and found that the Increasing/High cortisol group experienced significant impairments in declarative memory and selective attention, relative to the other 2 groups.31 These results showed that the cognitive deficits associated with increased secretion of cortisol in older humans are not specific to hippocampal function, since they also target the process of selective attention, which is sustained by the frontal lobes. There were no differences between groups for other cognitive measures, such as verbal fluency, picture naming, divided attention, and immediate and short-term memory.

In the third phase of the study, we tested the effects of chronic exposure to high levels of cortisol on the human hippocampus. MRI of the brain in a subset of subjects showed that those in the Increasing/High cortisol group (n = 6) had a 14% smaller hippocampal volume than those in the Decreasing/Moderate group (n = 5).32 These results are in accordance

Table 1: Summary of findings to date in the Douglas Hospital Longitudinal Study of Normal and Pathological Aging

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cortisol group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increasing/High</td>
</tr>
<tr>
<td>No. (% of total sample) (n = 51)</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Memory performance</td>
<td>Impaired (n = 8)</td>
</tr>
<tr>
<td>Mean hippocampal volume (SEM), mL</td>
<td>4.00 (0.08)</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Memory performance after metyrapone</td>
<td>No change (n = 9)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEM = standard error of the mean.
with animal studies showing that cumulative exposure to high glucocorticoids has functional and structural consequences for the hippocampus.33,34

Studies of brain changes associated with dementia in later life have reported an anatomically specific relationship between hippocampal volume and memory.35 These observations have been extended to elderly people showing mild cognitive impairments (MCI).36 Interestingly, the magnitude of the decrease in hippocampal volume in the Increasing/High cortisol group was comparable to that previously reported for elderly subjects with age-related MCI (i.e., 14%).37 Given that our subjects were originally selected on the basis of differences in cortisol secretion,38 rather than differences in cognitive performance, it could be that increases in cortisol secretion in later life mark the genesis of MCI and/or MCI-related hippocampal atrophy.

However, as summarized above, previous results obtained in our longitudinal study of basal cortisol levels and cognitive function during human aging showed that participants with an increase in cortisol levels over time and elevated cortisol levels at the time of testing (the Increasing/High cortisol group) presented significant memory impairments relative to participants with a similar increase in cortisol levels over time but only moderate cortisol levels at the time of testing (the Increasing/Moderate cortisol group). Given that the only difference between these 2 groups was their current cortisol levels, it was not clear whether the glucocorticoid-related memory deficits observed in the Increasing/High cortisol group were related to the acutely high levels of glucocorticoids at the time of testing (leading to a low MR–GR occupation ratio), or to the cumulative exposure to high glucocorticoid levels (leading to hippocampal dysfunction and related memory impairments).

To examine this question, we performed a hormone removal and replacement study involving the participants in the Increasing/High and Increasing/Moderate cortisol groups.37 Only subjects who were in good health and who reported no medication use were included in this study (9 participants from the Increasing/High cortisol group and 8 participants from the Increasing/Moderate cortisol group).

In a hormone removal and replacement protocol, the hormone of interest is suppressed, and behaviour resulting from the absence of the hormone is measured; hormonal levels are then restored to normal baseline values, and the same behaviour is measured again. It is postulated that if the hormone of interest has a real impact on the behaviour tested, then that behaviour should be restored to normal after replacement of the hormone.

For this study, we used a within-subject, double-blind experimental design in which we first lowered cortisol levels by administration of metyrapone, a potent inhibitor of cortisol synthesis, and then (later the same day) restored circulating cortisol levels to baseline levels with infusion of hydrocortisone. Participants’ memory performance under each of these conditions was compared with that measured on a placebo day. We predicted that if the memory impairments of the Increasing/High cortisol group were due to acutely high levels of cortisol at the time of testing, then memory performance should increase after a short-term decrease in circulating levels of cortisol (which would restore the MR–GR balance), and memory performance should be restored to impaired values after hydrocortisone replacement. However, if the memory impairment observed in this group was due to long-term exposure to high levels of cortisol (and thus possibly a glucocorticoid-induced brain dysfunction), the pharmacologic manipulations should have no modulatory effect on memory function. We also postulated that in the Increasing/Moderate group, a decrease in circulating levels of cortisol (low MR–GR occupation ratio) should lead to memory impairments, and that normal memory function would be restored after hydrocortisone replacement.

The results confirmed the long-term exposure hypothesis of memory impairments in the Increasing/High cortisol group. Metyrapone treatment did not have any effect on memory performance, but hydrocortisone replacement further increased the memory impairments in this group (see Table 1). In contrast, inhibition of cortisol production in the Increasing/Moderate cortisol group significantly impaired memory performance; this pattern of cognitive impairments was completely reversed by subsequent administration of hydrocortisone.37

We also found that the endocrine response to metyrapone and hydrocortisone replacement was similar in the 2 groups. Thus, the metyrapone-induced suppression of glucocorticoid synthesis resulted in comparable elevations of plasma levels of adrenocorticotropic hormone (ACTH) in the Increasing/High and Increasing/Moderate cortisol groups. Likewise, subsequent infusion of hydrocortisone suppressed plasma ACTH levels to the same extent in both groups. These results suggest that sensitivity to either removal or replacement of circulating glucocorticoids in the Increasing/High cortisol group is comparable to that observed in the Increasing/Moderate cortisol group. Finally, to determine whether the effects of metyrapone on memory performance might be due to the drug’s impact on other hormones involved in cortisol secretion (11-deoxycortisol, ACTH) or memory performance (glucose), we performed correlational analyses in each group between percent change in memory performance after metyrapone and hydrocortisone treatment and percent change in 11-deoxycortisol, glucose and ACTH after metyrapone and hydrocortisone treatment. All of the correlation coefficients were nonsignificant, which indicates that the memory changes observed after the hormone removal and replacement protocol were not due to metyrapone-induced changes in other hormones.37

These results allowed 2 general conclusions: first, that the memory impairments observed in the Increasing/High cortisol group were mostly due to cumulative exposure to high levels of cortisol, and second, that memory performance in older adults with moderate circulating levels of cortisol can be modulated by pharmacologic manipulations of cortisol. This latter result suggests that there might exist a window in the course of human aging during which at-risk individuals could be receptive to therapeutic interventions to prevent glucocorticoid-induced cognitive impairments.

Altogether, the results of the Douglas Hospital Longitudinal Study of Normal and Pathological Aging have provided...
evidence for a link between increased secretion of stress hormones in later life, memory impairments and reduced hippocampal volume. Although the longitudinal design of the study has provided fruitful data on the association between basal cortisol levels, cognitive function and hippocampal volume in a population of older adults, it must be emphasized that the sample size for each of the component studies was small, which might have affected the results. However, our finding of an association between increased cortisol levels over time and impaired memory performance has been replicated in the MacArthur studies of successful aging, which had a larger sample size.39

The results of the Douglas Hospital Longitudinal Study of Normal and Pathological Aging must be understood in the context of other studies showing an increase in circulating levels of cortisol in patients with depression and/or Alzheimer’s disease. At present, we have no data supporting the potential predictive value of cortisol for further development of depression or dementia in later life (for a complete review of this issue, see Lupien et al39). Over the past 10 years, 23 of the subjects have dropped out of the study: 4 participants died, 14 dropped out because of other commitments or development of physical disorders, and 5 dropped out because of development of Alzheimer’s disease, as diagnosed by a neurologist. In addition, major depression has developed in 4 participants, who are now being treated by a family doctor or a psychiatrist. These participants have agreed to continue their participation in the longitudinal study, and their data are being stratified from the larger group.

Because it strongly reflects the potential association among increased circulating levels of cortisol, depression and Alzheimer’s disease in later life, we briefly report here the case of an older woman who participated in the longitudinal study. This subject was born in 1923 and entered the study in 1990 at the age of 67 years; Alzheimer’s disease was diagnosed. At the beginning of 1997, she started to present abnormal performance on standardized neuropsychologic tests used to detect dementia (Mini-Mental State Examination), and Alzheimer’s disease was diagnosed. At that time, depression was diagnosed, and antidepressant treatment was initiated. The woman was not responsive to antidepressant treatment, and there were no obvious effects of the antidepressants on memory function (Fig. 2). In 1997, declarative memory performance on our test of paired-associated words31 started to decline significantly, reaching a minimum in June 1998. At that time, Alzheimer’s disease had not been diagnosed, since most of her results on standardized neuropsychologic tests were attributed to her depression. In September 1999, she finally started to present abnormal performance on standardized neuropsychologic tests used to detect dementia (Mini-Mental State Examination), and Alzheimer’s disease was diagnosed. Structural analyses of the brain were performed in 1994 (inclusion in the Increasing/High cortisol group), 1998 (diagnosis of depression) and 1999 (diagnosis of dementia). MRI in 1994 and 1998 showed a decline of 5% in the left perirhinal and entorhinal cortex and a decline of 45% in the right perirhinal and entorhinal cortex, as well as a 10% decline of the left hippocampus and a 23% decline of the right hip-

![Fig. 1: Area under the curve (AUC) for 24-hour cortisol levels obtained from 1990 to 1998 for a female patient who entered the study at the age of 67 years.](image)

![Fig. 2: Declarative memory performance on the paired-associated word test (see Lupien et al31) from 1994 to 1999 in the same patient. In both 1998 and 1999, memory was assessed twice within the calendar year.](image)
pocampus (Fig. 3). Between 1998 and 1999, further rapid decline in all of these brain structures was reported (perirhinal + entorhinal cortex, 30% decline on the left and 15% decline on the right; hippocampus, 20% decline on the left and 13% decline on the right).

Three points are worth noting. First, the total volume loss for this patient was up to 60% over a period of 5 years. Second, the rate of decline might be different for different brain structures and for the left and right brain. Finally, these results confirm studies suggesting that depression in later life can be a predictor of and even a causative factor in dementia. Buntinx et al used a retrospective cohort study to test this hypothesis and reported a significant relation between old-age depression and subsequent dementia in patients 50 years of age or older but born after 1910. Lawlor et al reported that in patients with Alzheimer’s disease who were assessed at 6-month intervals, early onset of the disease significantly predicted the development of greater behavioural disturbances and depression during the course of the illness.

These data have important implications for the study of pathologic brain processes, since patients with both dementia and depression may represent a different subgroup of disease that could be amenable to pharmacologic therapy, or they may represent a different point on a continuum relating depression to dementia in old age. Although the hypothesis of a continuum of evolving pathology between depression and dementia in later life has not yet received any experimental confirmation, it is important to note that this suggestion would fit with the neuropsychoendocrine experimental framework that has been summarized in the present review. Indeed, increased cortisol secretion during depression has been described and has been related to a lower incidence of cognitive deficits than that reported for dementia (for a complete review, see Lupien et al). Because of the detrimental effects of long-term exposure to increased cortisol levels on hippocampal function and/or structure, it might be suggested that depression in the older adult is associated with increased cortisol levels, and that with the passage of time, an older depressed individual with high cortisol levels might be at greater risk of subsequent dementia. Further studies assessing the origin of the increase in secretion of cortisol in about 30% of the older population should provide valuable data on the environmental and physiologic factors that may lead to pathological aging in humans.

Acknowledgements: The Douglas Hospital Longitudinal Study of Normal and Pathological Aging has been supported by a grant from the Canadian Institutes of Health Research (CIHR grant 15000) to S.J. Lupien, a grant from the Alzheimer Society of Canada to S.J. Lupien and N.P.V. Nair and a Research Scholar Award from the E.J.L.B. Foundation to S.J. Lupien. Dr. Lupien’s work is also supported by an Investigator Award from the CIHR Institute of Aging.

Competing interests: None declared.

Contributors: All authors contributed substantially to drafting and revising the article, and each gave final approval for the article to be published.

References


Fig. 3: Changes in the volume of the hippocampus (top) and the posterior part of the entorhinal and perirhinal cortex (bottom) in the same patient. The percent values below the image for each year represent volume relative to the volume in 1994. In these images, the value at the left of each panel represents the left hemisphere, and the value at the right of each panel represents the right hemisphere.


