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Acute Modulation of Aged Human Memory by Pharmacological Manipulation of Glucocorticoids

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In a previous longitudinal study of basal cortisol levels and cognitive function in humans, we showed that elderly humans with 4- to 7-yr cumulative exposure to high levels of cortisol present memory impairments, compared with elderly humans with moderate cortisol levels over years. Here, we measured whether memory performance in two groups of elderly humans separated on the basis of their cortisol history over a 5-yr period could be modulated by a hormone-replacement protocol in which we inhibited cortisol secretion by the administration of metyrapone and then restored baseline cortisol levels by infusion of hydrocortisone. We showed that in elderly subjects with a 5-yr history of moderate cortisol levels (n = 8), metyrapone treatment significantly impaired memory performance, a deficit that was reversed following hydrocortisone replacement. In the elderly subjects with a 5-yr history of high cortisol levels and current memory deficits (n = 9), metyrapone treatment did not have any significant effect on memory performance, but hydrocortisone treatment significantly decreased delayed memory. These results suggest that memory function in elderly humans can be intensely modulated by pharmacological manipulation of glucocorticoids, although the direction of these effects depends on the cortisol history of each individual. (*J Clin Endocrinol Metab* 87: 3798–3807, 2002)

IN THE AGED RAT, sustained exposure to elevated glucocorticoid levels is associated with hippocampal neuronal atrophy and severe memory impairments (1, 2). These results have led to the glucocorticoid-cascade hypothesis, which suggests that cumulative exposure to high levels of glucocorticoids compromises hippocampal integrity and impairs memory (1–4). A similar relationship between increases in glucocorticoid levels and impairments in memory function has been reported in aged humans (5, 6).

Using a 4- to 7-yr longitudinal study of basal cortisol levels and cognitive function in elderly human subjects, we previously reported considerable variation in plasma cortisol levels as well as clear evidence for subgroups (7) that show: 1) a progressive year-to-year increase in cortisol levels with currently high basal cortisol levels (the increasing/high cortisol group), 2) a progressive year-to-year increase in cortisol levels with currently moderate cortisol levels (the increasing/moderate cortisol group), or 3) a progressive year-to-year decrease in cortisol levels with currently moderate cortisol levels (the decreasing/moderate cortisol group). First, we measured the endocrine and metabolic correlates of these subgroups and showed that there is no change in the circadian rhythm nor corticosteroid-binding globulin levels in these three groups of subjects, and there are no any gender differences between men and women with regard to cortisol history (7). Second, we measured the neuropsychological correlates of these subgroups and showed that the increasing/high cortisol group presents significant memory impairments, compared with the other groups of subjects (5). Finally, we measured hippocampal volumes with *in vivo* magnetic resonance imaging in elderly subjects from the increasing/high and decreasing/moderate cortisol groups and reported a 14% significant decrease in the hippocampal volume of the increasing/high cortisol group, compared with the decreasing/moderate cortisol group (8). We also showed that hippocampal volume in this population is significantly correlated with both current cortisol levels as well as subjects' cortisol history (8). Altogether, these results showed that a significant increase in cortisol levels with years in the elderly is correlated with cognitive impairment only when this increase is associated with currently high cortisol levels.

Interestingly, studies in rodents report that acute or short-term variations in glucocorticoid levels exert a concentration-dependent biphasic influence on hippocampal function (9–11), long-term potentiation (12), and hippocampal-dependent forms of learning and memory (13). Situations in which glucocorticoids are significantly decreased (*e.g.* after an adrenalectomy) or increased (*e.g.* acute stress or exogenous administration) are associated with impairments in hippocampal-dependent forms of memory (14–16). Also, many authors have acutely reversed the detrimental effects of adrenalectomy on animals' behavior by subsequently administering glucocorticoids, a result that goes along with the existence of an inverted-U shape function between circulating levels of glucocorticoids and memory performance. Using this hormone replacement paradigm, many have reported that pretraining (17–19) as well as posttraining (19–22) administration of corticosterone restores an impaired learned behavior or extinction pattern induced by an adrenalectomy. Because acute modulation of cortisol levels

Abbreviations: HPA, Hypothalamus-pituitary-adrenal; HRT, hormonotherapy.

gives rise to a concomitant modulation of the learning and memory processes, direct implications of glucocorticoids in memory function have been postulated.

Although our previous results with elderly humans suggested that long-term exposure to increased cortisol levels (cortisol history) was associated with memory impairments, it was not clear whether the memory deficits observed in the increasing/high cortisol group were related to their acutely high levels of cortisol at the time of testing (current cortisol levels) or to their long-term history of high cortisol levels (cortisol history). Although subjects from the increasing/moderate cortisol group performed better than subjects from the increasing/high cortisol group, the former group presented a lower performance than that of elderly individuals with decreasing cortisol levels with years and currently moderate cortisol levels (the decreasing/moderate cortisol group). Still, the difference between these two later groups was related to their cortisol history and not to their current cortisol levels. Interestingly, data obtained in animals and humans suggest that the cognitive impairments associated with increased levels of glucocorticoids are both a result of long-term exposure to high levels of glucocorticoids (cortisol history) as well as currently high glucocorticoid levels and that they may interact (1–6).

To measure whether the impaired memory performance observed in the increasing/high cortisol group is related to currently high levels of cortisol, we measured whether memory performance in elderly individuals from the increasing/high and increasing/moderate cortisol groups (similar cortisol history but different current cortisol levels) could be modulated by a hormone replacement protocol in which we pharmacologically manipulated circulating levels of cortisol. In this protocol, we measured the impact of metyrapone (which blocks 11-hydroxylase and thus the conversion of 11-desoxycortisol to cortisol) on memory performance in both groups of subjects, and we further assessed whether baseline memory performance in these two groups of participants could be restored following hydrocortisone replacement.

We postulated that if the baseline memory performance of elderly humans from the increasing/moderate cortisol group is related to moderate cortisol levels, then memory performance should be impaired after metyrapone administration and restored to baseline levels after hydrocortisone replacement. In contrast, if the baseline memory deficit observed in the increasing/high cortisol group is due to currently high cortisol levels, memory performance should be improved by metyrapone-induced decrease of cortisol levels and restored to impaired performance after hydrocortisone replacement. However, if the baseline memory deficit in this group is not due to currently high cortisol levels, there should be no modulatory effect of pharmacological manipulation of glucocorticoids on memory function.

Subjects and Methods

Study subjects

The study was approved by the Human Subjects Review Committee of the Douglas Hospital, and informed consent was obtained from all subjects. Subjects for the Douglas Hospital Longitudinal Study of Normal and Pathological Aging were originally solicited from ads in the local media. The medical status of each subject was determined annually by a complete physical examination including electrocardiogram, electroencephalogram, computed axial tomography scan; a battery of laboratory tests for kidney, liver, and thyroid functions; hemogram; vitamin B12; folate levels; and a neuropsychological assessment (7). Exclusion criteria for the study were the presence of cardiovascular disease (consisting of myocardial infarction, heart block, slow cardiac conduction, heart failure, or severe hypertension), diagnosis of dementia ascertained by a complete neuropsychological assessment, presence of glaucoma, current or recent alcoholism (*e.g.* within 1 yr of the study), no general anesthesia in the last year, and no major medical illnesses. The subjects must not have taken any psychotropic medications in the month before the study and should not be using anticonvulsant drugs prior or at the time of testing because these drugs have been shown to interact with metyrapone. Subjects were tested annually for plasma cortisol levels over a continuous 24-h period with sampling each hour. Blood samples were centrifuged at 2500 rpm for 10 min at 0–4 C, frozen, and stored at −20 C until assayed. For plasma cortisol samples, a 300-μl aliquot of the extract was assayed in duplicate using a method previously described (7).

Group validation

When we performed the present study, the elderly subjects who had originally composed the groups that we previously described (5, 8) were either deceased (n = 4) or too old to be submitted to such an invasive neuroendocrine protocol (the mean age of these individuals is now 86.4 yr). Given that we were interested in measuring the impact of a hormone replacement protocol on memory performance, we decided to use a younger cohort of individuals to avoid any floor effects in drug-induced memory changes that might solely be due to the age of the participants.

To compose the increasing/high and increasing/moderate groups, we used data from our longitudinal study of basal cortisol and cognitive function that has been performed for the last 12 yr. Within this population, we recruited individuals for whom we had the 5 most recent years completed without any missing data and who met the inclusion criteria for this study (see below). For each individual, we calculated a cortisol slope using year as the independent variable and the 24-h area-under-the-curve plasma cortisol levels obtained on each year as the dependent variable, using a method that has been extensively described in other articles (5, 7, 8). Only those individuals with a positive cortisol slope (increasing cortisol levels with years) were included in the study. Using this method, we recruited 17 participants who met all our inclusion criteria. We then separated the increasing/high and increasing/moderate cortisol groups as a function of their current cortisol levels as obtained on the last year of the 5-yr study (current cortisol levels). Individuals with mean hourly levels above 8.5 μg/dl·h were included in the increasing/high cortisol group, and individuals with levels lower than 8.5 μg/dl·h were included in the increasing/moderate group (7). This cut-off for high *vs.* low cortisol secretion on current cortisol levels was based on a previous metaanalysis performed by our group showing that the mean cortisol levels in an elderly control population (n = 104) is 8.76 μg/dl·h (7). Using this grouping variable, nine individuals were included in the increasing/high cortisol group (five men and four women), and eight were included in the increasing/moderate cortisol group (four men and four women).

All participants were free from medications known to affect the hypothalamus-pituitary-adrenal (HPA) axis, and all subjects were within 125% of ideal body weight. All women tested were postmenopausal and two of four women in each group were using combined hormonotherapy (HRT). In accordance with previous neuroendocrine studies (23, 24), we did not find any significant differences in basal cortisol levels in women under HRT or no HRT, and we did not find any differences in the endocrine response to challenge in women under HRT. Also, we did not find any significant impact of HRT on cognitive response to challenge, so these women were collapsed in each group for subsequent analyses. Preliminary analyses assessing the existence of gender differences on endocrine and cognitive responses to challenge did not reveal any differences between men and women (all P values over 0.5), so data from men and women were collapsed over subsequent analyses. However, to ascertain that the results we obtained on the hormone replacement protocol would not be due to inclusion of women on HRT in the two groups of subjects, we analyzed all data obtained on

the hormone replacement protocol with and without inclusion of women on HRT.

Table 1 presents the demographic and endocrine data of these two groups of elderly individuals. There were no significant differences among age, education level, or score on the Geriatric Depression Scale (25) in the two groups ($P > 0.1$). However, the increasing/high cortisol group showed significantly higher cortisol levels over the 5-yr period ($P < 0.0026$) as well as higher current cortisol levels ($P < 0.0002$) and a higher cortisol slope ($P < .02$). compared with the increasing/moderate cortisol group.

The second criterion we had to meet to confirm the validation of this grouping of subjects was to show that elderly subjects from the increasing/high cortisol group presented significant memory impairments, compared with elderly subjects from the increasing/moderate cortisol group (5). All the participants of this longitudinal study have been tested annually on a test of paired-associate recall that has been described in detail elsewhere (5, 7, 8) and for which we have previously reported significant group differences as a function of basal cortisol levels (5, 8). The totality of participants in the recruited sample had received this test for the last 4 yr without interruption (but with different versions every year), so we calculated group differences in performance on this test for this 4-yr period (4-yr memory; repeated measures ANOVA with year as the within-subject factor: longitudinal analysis) as well as group differences in current memory performance as tested on the last year of the study (current memory; ANOVA on last year's memory performance: cross-sectional analysis). As presented in Table 1, current memory performance was significantly lower in the increasing/high cortisol group, compared with the increasing/moderate cortisol group ($P < 0.0003$). Memory performance measured over a 4-yr period using the same test also revealed significant impairments in the increasing/high cortisol group, compared with the increasing/moderate cortisol group ($P < 0.0016$).

These results, obtained with a different population than the one originally described (5, 8), replicated our previous finding of a significant relationship between cumulative exposure to high levels of cortisol and impaired memory performance. Moreover, these results validate the grouping of these elderly individuals within the increasing/high and the increasing/moderate cortisol groups.

Materials and methods

Hormone replacement protocol. The goal of this study was to measure the memory performance of the increasing/high and increasing/moderate cortisol groups when circulating levels of cortisol were significantly decreased and after replacement of baseline levels of cortisol in the same individuals during the same experimental day. To achieve this goal, we tested each participant on an experimental day during which memory performance was tested after administration of metyrapone (2 doses; 750 mg each) and after hydrocortisone replacement (60-min infusion of 0.06 mg/kg·h (23, 26). Thus, during the experimental day, the metyrapone condition always preceded the hydrocortisone condition. Testing for the metyrapone/hydrocortisone protocol was performed within a month of the yearly cortisol evaluation for establishment of current cortisol levels and inclusion in the increasing/high or increasing/moderate groups. Dosage for the metyrapone and hydrocortisone conditions were chosen based on results from Wilkinson *et al.* (23, 26) showing that these doses and administration time are effective in shutting off (metyrapone) or restoring (hydrocortisone) HPA activity in elderly human individuals without inducing any side effects.

The memory performance measured after metyrapone and hydrocortisone conditions was compared with that of a placebo day during which the same participants were measured for memory after two placebo conditions applied at the exact same time as the experimental conditions. Each placebo condition was then used as a specific control for the metyrapone and hydrocortisone conditions. Thus, performance on the first placebo condition was compared with that of the metyrapone condition (both treatments applied at the same time), and performance on the second placebo condition was compared with that of the hydrocortisone condition (both treatments applied at the same time). The use of this within-subject design permitted us to assess drug-induced influences in cognitive function in both groups, taking into account the differences in baseline memory performance in each group (Table 1). This experimental design also controlled for any possible effects of practice or fatigue on the placebo or treatment day (27). The order of placebo and treatment days was counterbalanced across subjects, and both participant and experimenter were blind with regard to subject's group and treatment. Studies were separated by at least 2 wk for all subjects.

Procedure. Figure 1 presents a schematic representation of the experimental protocol. Subjects fasted from midnight before study and were maintained at bed rest throughout the study. In the active drug condition, metyrapone 750 mg po was administered at 0600 h. At 0630 h, an iv catheter was inserted into the subject's arm and used for blood sampling and cortisol infusion. Blood samples were taken at 0730 h and 0800 h. At 0805 h, a small snack (yogurt and fruits) was given to participants, another blood sample was taken at 0830 h, and a second dose of 750 mg po metyrapone was administered at 0900 h. After a third blood sample obtained at 0915 h, the memory testing was performed at 0920 h and lasted for 30 min. Further blood samples were obtained after the first memory testing at 0950, 1030, and 1040 h, and a light breakfast was given at 0955 h. At 1045 h, hydrocortisone infusion began and was maintained at a rate of 0.06 mg/kg·h for 60 min. The second memory testing began 30 min after the start of the cortisol infusion (1115 h) and lasted 30 min. An additional blood sample was obtained at 1200 h at which time subjects were served with a lunch and were free to go home after being checked by the medical supervisor of the study (N.P.V.N.). Blood samples were not obtained during the time of neuropsychological testing to not disturb participants in their performance. The placebo condition protocol was identical to the active drug condition except that subjects received placebo tablets and a placebo normal saline infusion equivalent in volume to the cortisol infusion.

Blood samples (10 ml) were collected in Vacutainer tubes containing the anticoagulant EDTA and immediately centrifuged at 900 g at 4 C. The isolated plasma samples were divided into two equal aliquots, one for cortisol, 11-desoxycortisol, and glucose measurements and the other for ACTH determination. In the latter, 50 ml protease inhibitor, N-ethylmaleimide (0.1 M solution) were added. The plasma samples were stored in polypropylene tubes at −80 C. All hormone assays were carried out within 6 months.

Measures of memory. Declarative memory was measured using a free recall test of a 12-word list presented over three trials. All the elderly

TABLE 1. Demographic, endocrine, and neuropsychological data for the increasing/moderate cortisol group and the increasing/high cortisol group

	Increasing/moderate cortisol group	Increasing/high cortisol group	Difference (*P* values)
Age (mean ± SD)	68.4 ± 7.8	71.3 ± 8.2	0.29
Education level (mean ± SD)	10.7 ± 3.2	10.9 ± 4.1	0.7
Geriatric Depression Scale Score (mean ± SD) (threshold for score suggestive of depression: 11)	2.22 ± 1.16	2.62 ± 1.37	0.75
5-yr cortisol level (cortisol history) (μg/dl · h ± SEM) measured over a period of 24 h)	7.02 ± 0.34	10.1 ± 0.39	0.0026
Current cortisol levels (μg/dl · h ± SEM measured over a period of 24 h)	6.83 ± 0.24	9.56 ± 0.40	0.0002
Cortisol slope (μg/dl · h · yr ± SEM)	0.71 ± 0.09	2.33 ± 0.87	0.02
4-yr memory (% correct recall ± SEM)	76.12 ± 5.11	53.47 ± 5.88	0.0016
Current memory (% correct recall ± SEM)	72.5 ± 4.01	42.36 ± 7.06	0.0003

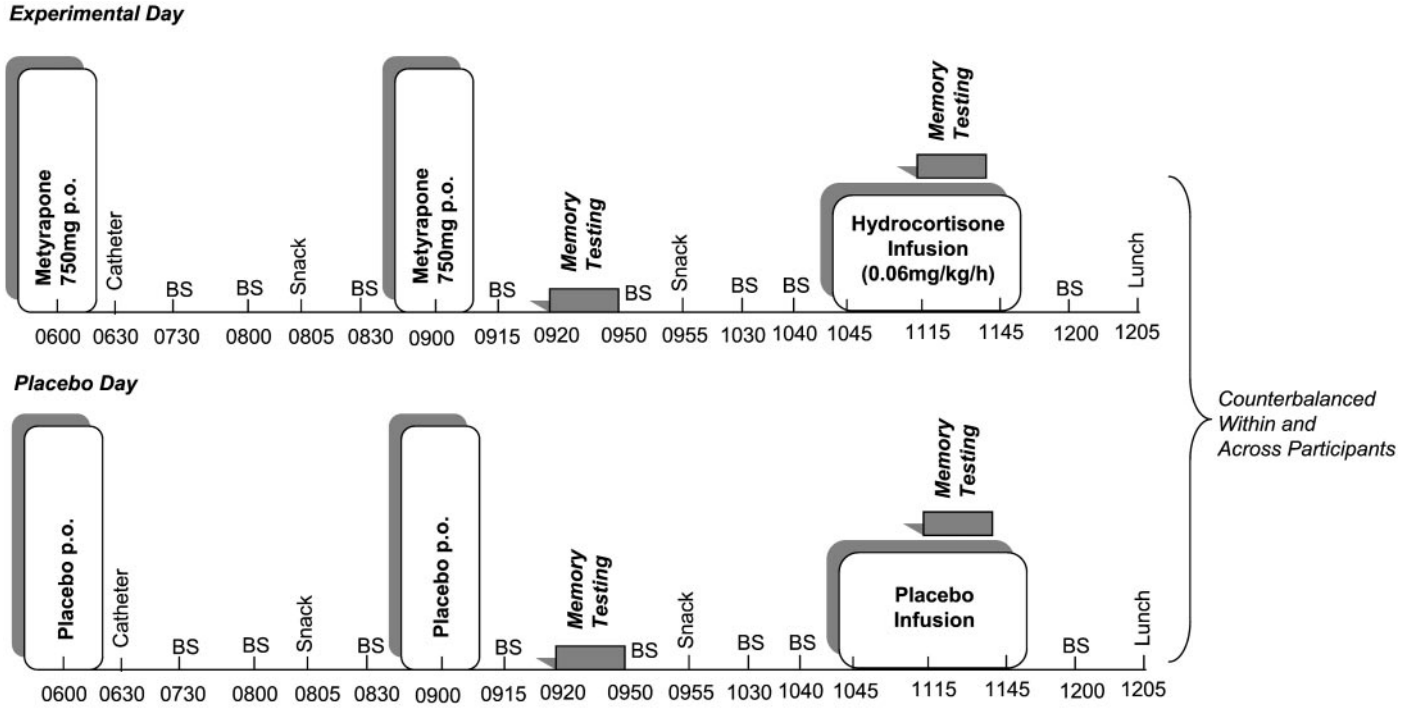

FIG. 1. Schematic representation of the hormone replacement protocol.

individuals tested in the present study had never been exposed to this test before because it had never been used for our annual neuropsychological testing with this population. Each subject was presented with a list of 12 imageable and concrete words. The subject had to read each word aloud and try to memorize them for subsequent declarative free recall. Each list was presented three times and free recall was assessed after each trial. Free recall performance over the three trials constituted the measure of learning capacity for each subject. Delayed (20 min) memory performance was also tested on a fourth recall during which subjects were asked to recall the previously presented words. The percentage of correctly recalled words over the three immediate trials served as a measure of learning capacity (declarative memory), and recall of the same words after the 20-min delay served as a measure of the rate of forgetting. These two components have been shown to be dependent on the integrity of the hippocampus in both animals and humans (28). Four lists of words were created to measure learning and memory capacity after each experimental condition. Preliminary pilot studies with normal elderly subjects (n = 10) were performed to ensure that each list was equivalent in terms of recall performance. List order was counterbalanced within and across subjects.

Endocrine and glucose assays. Cortisol levels were determined using validated RIA kits (Johnson & Johnson, Mississauga, Ontario, Canada). In this assay, cortisol is released from corticosteroid-binding globulin by a chemical blocking agent contained in the kit and the total cortisol determined with the ^{125}I-labeled cortisol and polymer-bound sheep anti-cortisol antibody provided. The intra- and interassay coefficients of variation were 4.3% and 7.7% for mean concentrations of 8.15 μg/dl and 8.28 μg/dl, respectively. The sensitivity of the assay was 0.1 μg/dl. For the determination of 11-desoxycortisol levels, validated RIA kits were used (ICN Pharmaceuticals, Inc., Costa Mesa, CA). This is a double-antibody assay using ^{125}I-labeled 11-desoxycortisol. The intra- and interassay coefficients of variation were 2.1% and 13.7% for mean concentrations of 2.55 ng/ml and 2.63 ng/ml, respectively. The sensitivity of the assay was 0.02 ng/ml. The ACTH levels were determined using a validated immunoradiometric assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay uses a ^{125}I-labeled monoclonal antibody binding the N-terminal regions of ACTH and a polyclonal antibody binding the C-terminal region. It measures the amount of intact ACTH in plasma samples. The intra- and interassay coefficients of variation were 3.0% and 7.8% for mean concentrations of 35 pg/ml and 36 pg/ml, respectively. The sensitivity of the assay was 1 pg/ml. Plasma glucose levels were determined on an automated chemistry analyzer, Beckmann LX20, at St. Mary's Hospital (McGill University). The assay is based on the glucose oxidase method.

Statistical analyses. Group comparisons with regard to baseline nontransformed endocrine measures (cortisol, 11-desoxycortisol, ACTH, and glucose) were performed using an ANOVA with group (increasing/moderate cortisol group *vs.* increasing/high cortisol group) as the between-subjects factor and treatment (placebo *vs.* treatment) and samples (1 through 8) as the within-subject factors. Significant interactions were decomposed using Tukey HSD unequal sample size tests. Metyrapone-induced effects on memory were analyzed by comparing performance of each group after metyrapone and first placebo condition (paired with the metyrapone condition) using ANOVA with group (increasing/moderate cortisol group *vs.* increasing/high cortisol group) as the between-subjects factor and treatment (metyrapone *vs.* first placebo) and memory trial (percent correct recall on first, second, third, and delayed recall trial) as the within-subject factors. Hydrocortisone-induced effects on cognition were measured by comparing performance of each group after hydrocortisone and second placebo condition (paired with the hydrocortisone condition) using the same type of ANOVA as above. Performance on the delayed recall trial was included in the same ANOVA as performance on the first, second, and third immediate recall trials to ascertain drug effects on memory, taking into account the delay between the third and delayed recall trial (20 min). However, significant effects on delayed memory were further confirmed by a univariate analysis on the delayed recall trial. Significant interactions were decomposed using Tukey HSD unequal sample size tests using the nontransformed data. Data were checked for assumption of sphericity and Greenhouse and Geisser correction (29) was applied if sphericity was not met.

Inclusion of all four conditions in a single omnibus F test was precluded for two main reasons. First, the two treatments applied on the experimental day were not orthogonally independent (*i.e.* cognitive performance under hydrocortisone treatment totally depended on previous response to metyrapone treatment; modulatory protocol), but the two treatments applied on the placebo day were orthogonally independent (*i.e.* cognitive performance under the second placebo condition was not dependent on response to the first placebo condition). Second, this modulatory drug protocol was applied to two populations that did not start the experiment at the same level of cognitive performance. Given

this latter fact, any modulatory actions of the drug on memory function would be obscured by these baseline differences among groups (30).

Results

Endocrine

Figure 2 presents the cortisol, ACTH, 11-desoxycortisol, and glucose response to metyrapone and hydrocortisone treatments in both groups of elderly participants, compared with placebo conditions. Results are presented as means and SE (SEM). Placebo condition baseline plasma ACTH, 11-desoxycortisol, and glucose did not differ among groups (all P values >0.7), although cortisol levels were significantly higher in the increasing/high cortisol group, compared with the increasing/moderate cortisol group [$F(1,15) = 19.5$; $P < 0.001$].

Metyrapone treatment substantially reduced initial plasma cortisol concentrations [increasing/moderate cortisol group: 5.4 ± 0.3 μg/dl; increasing/high cortisol group: 5.8 ± 0.6 μg/dl; $F(1,15) = 228.3$; $P < 0.0001$] and increased plasma 11-desoxycortisol [all values >25 ng/ml; $F(1,15) = 1864.16$; $P < 0.00002$] in both groups. As presented in Fig. 2, ACTH levels increased significantly after the second metyrapone treatment in both groups [$F(1,15) = 79.6$l $P < 0.0001$], and there were no significant group differences in ACTH response to metyrapone ($P = 0.76$). No impact of metyrapone administration was observed on glucose levels ($P = 0.8$) in either group. Although glucose levels at time points 0950 and 1030 h showed a tendency toward a group difference, t tests performed on these particular sampling times did not revealed any significant differences (all $P > 0.3$).

After hydrocortisone replacement, cortisol significantly increased in both groups and reached levels above those measured on the placebo day [12.08 μg/dl during placebo and 17.57 μg/dl after hydrocortisone treatment; $F(1,15) = 210.7$; $P < 0.001$]. ACTH levels significantly decreased in both groups [$F(1,15) = 8.97$; $P < 0.009$], and there were no significant group differences in ACTH response to hydrocortisone ($P = 0.41$). Deoxycortisol levels did not show any change ($P = 0.7$) because of the short period of sampling after hydrocortisone replacement. No impact of hydrocortisone replacement was observed for either group on glucose levels ($P > 0.8$). Finally, all analyses were performed a second time by taking out the two women on HRT in each group, and this *a posteriori* method did not modify the previously obtained results.

Memory

Figure 3 presents memory performance in each group after metyrapone and hydrocortisone treatment, compared with their appropriate placebo conditions. Results are presented as mean percent correct recall and SE (SEM). Preliminary analyses taking into account the order of presented lists (comparison of cognitive performance within and among groups as a function of received list of words on the placebo and treatment days) as well as the order of treatment (comparison of cognitive performance within and among groups as a function of the order of treatment received across the two testing days) did not reveal any significant effect of list or treatment order on memory performance in both groups, so data were collapsed across these two variables for subsequent analyses.

Placebo condition baseline memory was significantly lower in the increasing/high cortisol group, compared with the increasing/moderate cortisol group, for each of the four memory trials [$F(1,15) = 9.4$; $P < 0.007$]. When comparing the memory performance of the increasing/high cortisol group with that of the increasing/moderate cortisol group after placebo and metyrapone administration, we found a significant third order interaction among group, treatment, and memory trial [$F(3,45) = 3.4$; $P < 0.026$]. In the increasing/moderate cortisol group, metyrapone significantly decreased memory for the third immediate trial recall ($P < 0.003$; 12% decrease in memory performance) as well as on the delayed recall trial ($P < 0.0004$; 18% decrease in memory performance), compared with the placebo condition (Fig. 3, *top left*). No significant impact of metyrapone was observed in the increasing/high cortisol group ($P = 0.454$; Fig. 3, *top right*).

Hydrocortisone replacement significantly restored memory performance to the level of placebo in the increasing/moderate cortisol group (no difference between memory performance under placebo and hydrocortisone treatment; $P = 0.84$; Fig. 3, *bottom left*), and it had a tendency to decrease memory performance in the increasing/high cortisol group (Fig. 3, *bottom right*). To assess the exact influence of hydrocortisone on each recall trial in this particular group, we performed *a posteriori* mean comparisons (t test) over the four recall trials (placebo *vs.* hydrocortisone). Results of these *a posteriori* mean comparisons revealed a significant impairment of hydrocortisone replacement on delayed recall ($P < 0.05$; 14% decrease in memory performance). All analyses were performed a second time by taking out the two women on HRT in each group, and this *a posteriori* method did not modify the previously obtained results.

Finally, to assess whether there exist significant correlations between the magnitude of changes in hormone levels and the magnitude of changes in cognition after each treatment, we performed correlational analyses in each group between percent change in immediate (average of the first three immediate memory recall trials) and delayed memory recall trials after metyrapone and hydrocortisone treatment (calculated as the percentage of changes, compared with their appropriate placebo-controlled condition) and percent change in both cortisol and ACTH after metyrapone and hydrocortisone treatment. As present in Table 2, all the correlation coefficients were nonsignificant.

Discussion

In the present study, we duplicated our previous finding of a significant relationship between cumulative exposure to high levels of cortisol and impaired memory performance (5), using a different population of aged individuals measured over a 5-yr period. This result is consistent with another independent report by Seeman *et al.* (6), who also replicated our finding in a sample of 90 elderly individuals measured over a 2-yr period. It is important to note in this context that the memory impairment observed in the increasing/high cortisol group is associated with basal cortisol

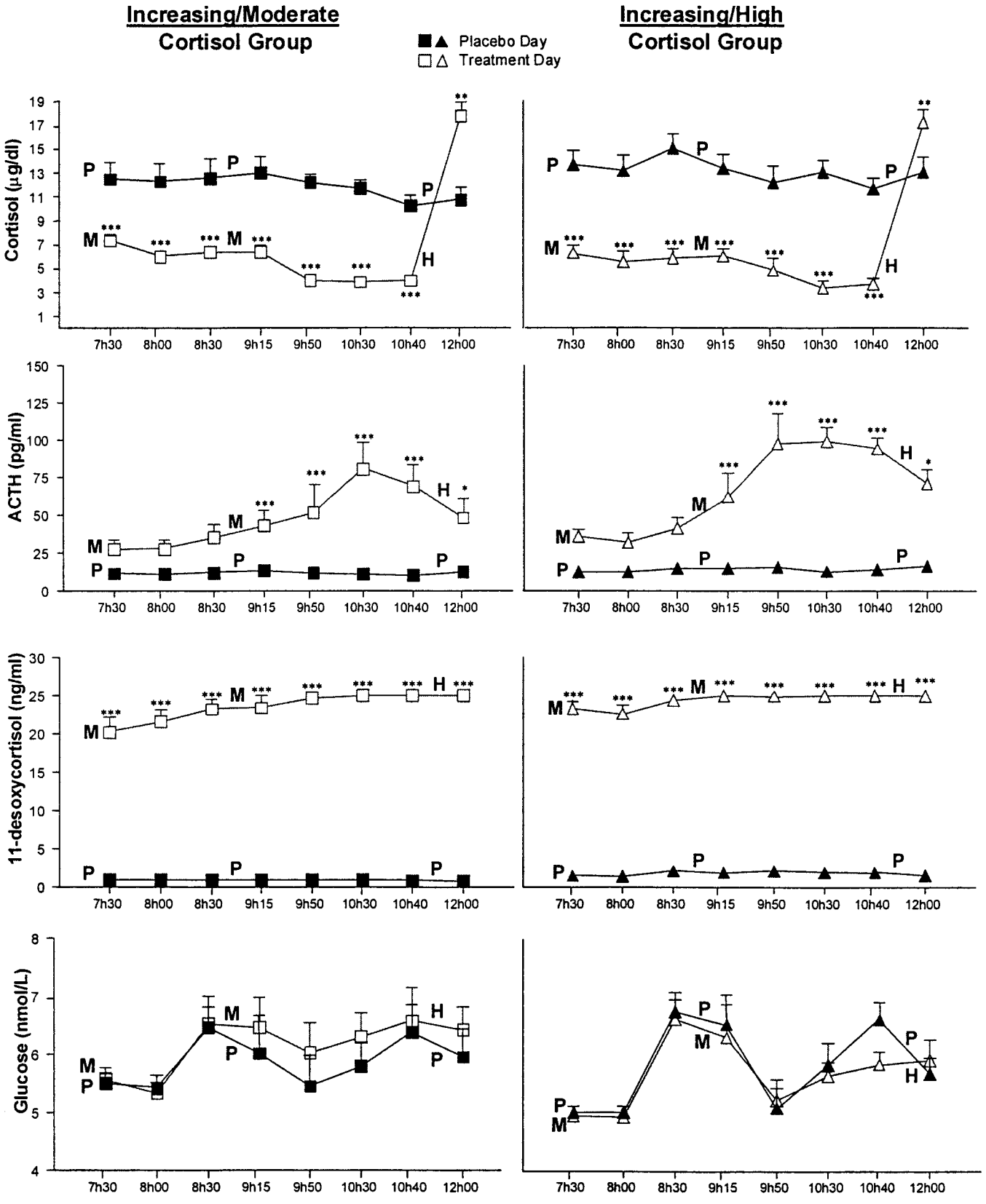

FIG. 2. From *top* to *bottom,* cortisol, ACTH, 11-desoxycortisol, and glucose levels during placebo day and treatment day in the increasing/moderate cortisol group (*left*) and the increasing/high cortisol group (*right*). P, Time of placebo administration; M, time of metyrapone administration; H, time of hydrocortisone administration. Hormone levels during treatment significantly different from hormone levels during placebo at *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

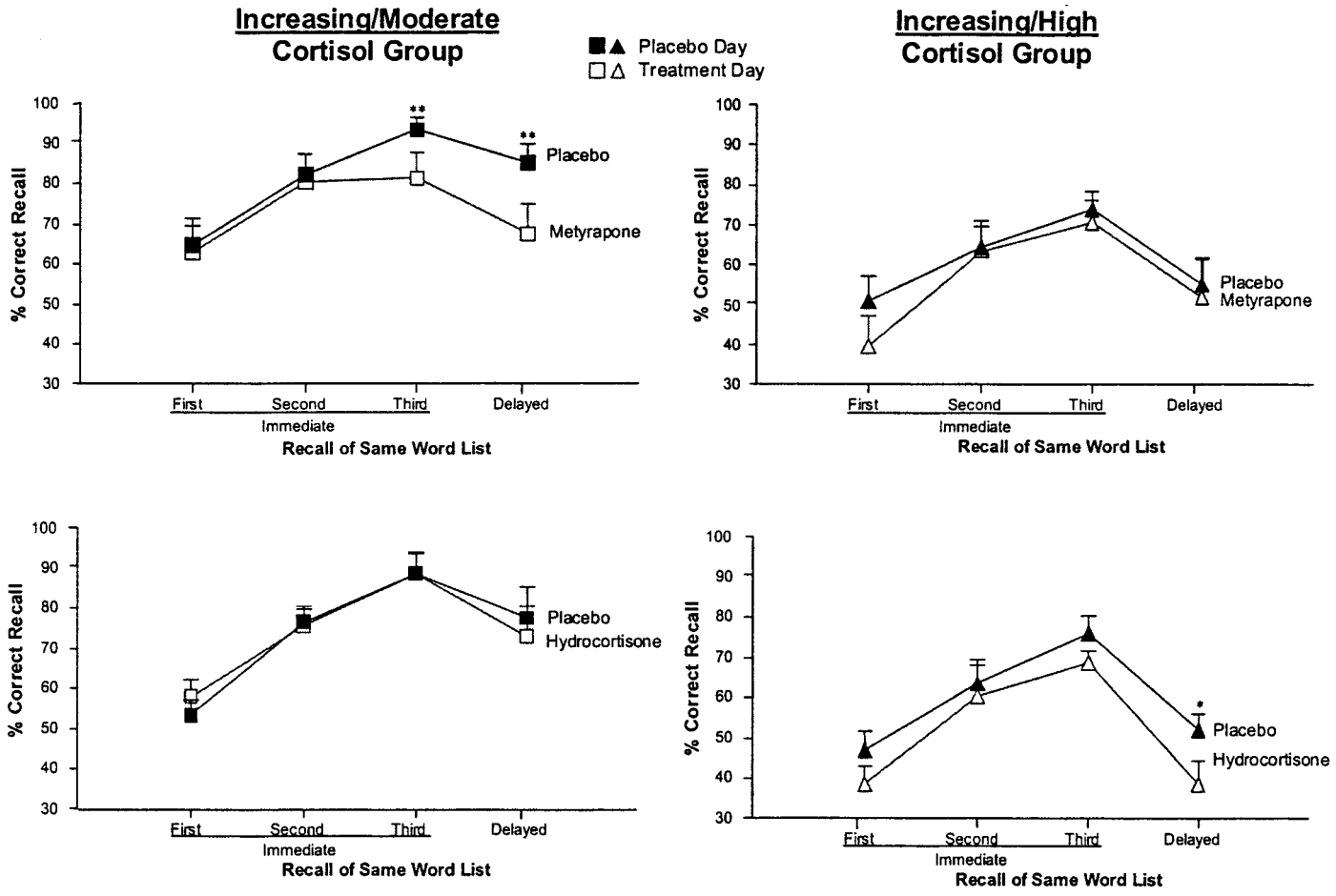

FIG. 3. Percent correct recall of the same word list over three immediate recall trials (first, second, and third recall) and after a 20-min delay (delayed recall) in the increasing/moderate cortisol group (*left*) and the increasing/high cortisol group (*right*) during conditions of metyrapone and hydrocortisone treatment, compared with paired placebo conditions. Percent correct recall during treatment significantly different from percent correct recall during placebo at *, $P < 0.05$; **, $P < 0.005$.

TABLE 2. Correlation coefficients between changes in hormone levels after metyrapone and hydrocortisone treatment, and changes in immediate (mean of the three recall trials) and delayed memory in the increasing/moderate cortisol group and the increasing/high cortisol group

	Increasing/moderate cortisol group	Increasing/high cortisol group	Total group
Changes in memory after metyrapone			
Immediate memory & cortisol	−0.04	−0.13	−0.11
Delayed memory & cortisol	0.11	−0.06	−0.01
Immediate memory & ACTH	−0.19	0.04	−0.08
Delayed memory & ACTH	−0.27	−0.19	−0.12
Changes in memory after hydrocortisone			
Immediate memory & cortisol	0.12	−0.14	0.09
Delayed memory & cortisol	−0.01	0.12	0.07
Immediate memory & ACTH	−0.06	−0.13	−0.11
Delayed memory & ACTH	−0.19	−0.03	−0.17

Correlation coefficients are presented separately for each group, as well as for the entire sample of participants.

levels that are in the subclinical range of dysfunction of the HPA axis. Indeed, the cortisol levels reported by our group (5, 8) and others (6) are well below the range observed in disorders of the HPA axis such as Cushing's disease. Still, what most of these reports show is that a cumulative exposure to moderately high cortisol levels in the elderly human individual may be a risk factor for the development of memory impairments.

This suggestion is strengthened by the results of the hormone replacement study, which show that memory function in elderly human individuals can be acutely modulated by pharmacological manipulation of glucocorticoids, although the extent and direction of these changes depend on the cortisol history of each individual. In the increasing/moderate cortisol group, metyrapone treatment significantly impaired memory, but hydrocortisone replacement restored

memory performance to the baseline level observed on the placebo day. In contrast, metyrapone treatment did not have any significant effect in the increasing/high cortisol group, although hydrocortisone replacement further impaired delayed memory. These results cannot be explained by glucocorticoid-induced changes in glucose levels because glucose levels were not influenced by our pharmacological manipulation.

Also, the endocrine response to metyrapone and hydrocortisone replacement was similar in both groups of subjects. Thus, the metyrapone-induced suppression of glucocorticoid synthesis resulted in comparable elevations in plasma ACTH levels in the increasing/high cortisol and increasing/moderate cortisol groups. Likewise, subsequent infusion of hydrocortisone suppressed plasma ACTH levels to the same extent in both groups of participants. These results suggest that sensitivity to either the removal or replacement of circulating glucocorticoids in the increasing/high cortisol group is comparable with that observed in the increasing/moderate cortisol group. Decreased HPA axis sensitivity to cortisol feedback inhibition has recently been reported in aged human populations, compared with younger populations (23, 26) but may not be associated with variations in HPA activity among elderly subjects.

The lack of group difference in the endocrine response to metyrapone and hydrocortisone replacement could explain the absence of significant correlations between the changes in cortisol and ACTH levels induced by the metyrapone/hydrocortisone protocol and the changes in memory performance after each treatment. This would suggest that the magnitude of changes in cortisol and ACTH levels following metyrapone and hydrocortisone treatment is not the factor explaining the changes in memory performance observed after each treatment. Rather, and as suggested by previous hormone replacement studies performed in animals (17–22, 31), the presence or absence of circulating cortisol levels seems to be the important variable explaining the memory performance observed after each treatment.

Indeed, the results obtained in the increasing/moderate cortisol subjects are strikingly similar to those reported in rodents with adrenalectomy followed by low replacement doses of glucocorticoids (17–22, 31) and point to a modulatory influence of cortisol on human memory. This modulatory influence of glucocorticoids on memory function is not confined to the elderly population because recent results obtained in young normal controls using the same endocrine protocol also revealed impairing effects of metyrapone on memory function and restoring effects of hydrocortisone (32).

The absence of metyrapone effects on memory function in the increasing/high cortisol group suggests that the baseline memory deficit observed in this group of elderly individuals is not because of the currently high cortisol levels observed at the time of testing. Although we still find that elderly individuals with a significant increase of cortisol levels over years (the increasing/high cortisol group) are impaired in memory function, the absence of metyrapone effect in this group does not exclude the impact of factors other than glucocorticoids in explaining the presence of memory impairments. It is known that a consequence of glucocorticoid hypersecretion is accelerated neuron loss in the aging hippocampus (1, 2), but we also know that a consequence of hippocampal damage (because of age or degenerative disease) is adrenocortical negative-feedback insensitivity and glucocorticoid hypersecretion (33, 34). The interaction of these abnormalities occurs with aging in the rat and is potentiated by acute conditions, which further elevate glucocorticoid levels. Greater sensitivity to acute increases of glucocorticoids because of age- or disease-related hippocampal dysfunction could explain the impaired memory performance of the increasing/high cortisol group after hydrocortisone replacement.

It is important to note that the absence of a metyrapone effect in the increasing/high cortisol group can be interpreted only in relation to the acute variation of cortisol levels that was induced by the pharmacological treatment. Although both groups presented different memory performance based on their long-term cortisol history, it might still be possible that long-term treatment with metyrapone could lead to beneficial effects on the memory performance observed in the increasing/high cortisol group. Such positive effects of long-term treatment with substances that decrease cortisol levels have been obtained in depressed patients showing hypercortisolism, although these effects were found on depression ratings and not on memory performance (35–38). Moreover, a recent study of Cushing's patients revealed that surgical treatment of hypercortisolism in these patients leads to a reversal of the glucocorticoid-induced hippocampal atrophy reported to occur in this population, with an average volume increase of 3.2% and variations up to 10% in some patients (39, 40). Altogether, these results tend to suggest that the long-term effect of a pharmacological decrease of cortisol levels could potentially have a positive impact on memory performance in the increasing/high cortisol group.

Interestingly, the modulatory effects of glucocorticoids on memory performance that we report in the present study have recently been explained in terms of the hippocampal glucocorticoid receptor system (41). Circulating glucocorticoids bind with high affinity to two receptor subtypes; the MR and GR. These receptors exhibit a subtle but important difference in their affinity for glucocorticoids. MRs bind glucocorticoids with an affinity that is about 2–5 times higher than that of the GRs. The significance of this difference in affinity is apparent under basal glucocorticoid levels. Low, basal glucocorticoid levels serve to activate mainly MRs, whereas the elevated glucocorticoid levels characteristic of periods of stress activate both MRs and GRs.

In animals, acute MR activation serves to maintain hippocampal neuronal integrity, facilitate long-term potentiation, and improve learning and memory, but GR activation has precisely the opposite effects (41). The long-term effects of elevated glucocorticoid levels on hippocampal degeneration have been clearly linked to GR activation (3). However, recent studies have shown that MRs rather than GRs are significantly reduced during long-term elevations of glucocorticoid levels (42–46). Thus, both acute and chronic variations in circulating glucocorticoid levels can regulate hippocampal function through differential changes in GR-mediated actions (41).

Based on this mechanistic model, the absence of any

metyrapone-induced memory impairments in the increasing/high cortisol group could be explained by decreased MR expression, such that acute reduction in circulating cortisol levels are without effect on memory. It may also be that the presence of lower baseline memory performance in the increasing/high cortisol group is related to a down-regulation of hippocampal MR and the loss of the memory-enhancing effects of basal glucocorticoid levels. In contrast, the hydrocortisone-induced impairment in the increasing/high cortisol group suggests a GR effect. Indeed, an intact GR sensitivity, coupled with a down-regulation of MR, could explain the heightened sensitivity of this population to physiological increases in glucocorticoids.

Although the MR/GR balance theory of glucocorticoid action on the brain (41, 47) could help explain the positive and negative effects of glucocorticoids under various conditions, it is important to note that the short-term effects of glucocorticoids we observed in both groups might be too rapid to be explained by a genomic action, given that the time course of the experiment left very little time for the hormone to activate the cellular and network machinery underlying complex information processing. In this case, acute effects of glucocorticoids other than via changes in MR/GR activation have to be considered. For example, glucocorticoids increase Ca2+ influx and the Ca2+-dependent afterhyperpolarization, thereby substantially altering neuronal function (48). MR activation in the hippocampus is associated with reduced calcium currents and stable synaptic responses to excitatory or inhibitory amino acid neurotransmitters (49, 50). In contrast, higher levels of glucocorticoids activate GRs, which result in increased calcium currents and exaggerated afterhyperpolarization, dampening synaptic responses to excitatory inputs (51). These results could help shed new light on the acute impact of glucocorticoids on human cognitive function.

Another mechanism by which metyrapone treatment might have led to memory impairments in the increasing/moderate cortisol group is through a metyrapone-induced increase in bioactive 11-deoxycortisol metabolites such as tetrahydro-11-deoxycortisol, hexahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone (THDOC). These steroids are metabolites of early products in the glucocorticoid biosynthetic pathway, and studies have shown that both 11-deoxycortisol (52) and THDOC (53) are neurally active steroids. For example, 11-deoxycortisol has been shown to induce changes in electrical activity in the hippocampus and amygdala of cats *in vivo* (52), and THDOC has been shown to potentiate the actions of γ-aminobutyric acid in rat brain synaptosomal preparations (53). It is thus possible that some of the memory-impairing effects of metyrapone in the increasing/moderate cortisol group may be mediated by increasing the 11-deoxysteroids and their metabolites. This would go along with studies suggesting that the antidepressant actions of metyrapone may be mediated by these active neurosteroids (54–56).

Although the exact mechanism underlying the long-term effects of elevated levels of glucocorticoids on aged human memory is still unknown, the present study suggests that individual differences in long-term exposure to this steroid is a determinant of each individual's response to acute variations in this hormone. Further studies assessing the threshold for glucocorticoid-induced memory impairments during human aging as well as the subsequent modulatory effects of drugs acting on MRs or GRs should yield valuable data as to the potential pharmaceutical interventions that could be designed to prevent further cognitive decline in aged human populations at risk for glucocorticoid hypersecretion.

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