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RESEARCH ARTICLE

TITLE

Chronic glucocorticoids consumption triggers and worsens experimental Alzheimer's disease-like pathology by detrimental immune-modulations.

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Short Title

Chronic glucocorticoids consumption and Alzheimer's pathology

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ABSTRACT

Introduction: Among the risk factors identified in the sporadic forms of Alzheimer's disease (AD), environmental and lifestyle elements are of growing interest. Clinical observations suggest that stressful events can anticipate AD onset, while stress-related disorders can promote AD. Here, we tested the hypothesis that a chronic treatment with glucocorticoids, is sufficient to trigger or exacerbate AD molecular hallmarks.

Methods: We first validated a rat model of experimental chronic glucocorticoids consumption (*corticosterone in drinking water for 4 weeks*). Then, to evaluate the consequences of chronic glucocorticoids consumption on the onset of amyloid- β ($A\beta$) toxicity, animals chronically treated with glucocorticoids were intracerebroventricularly injected with an oligomeric solution of $A\beta_{25-35}$ ($oA\beta$) (*acute model of AD*). We evaluated AD-related cognitive deficits and pathogenic mechanisms, with a special emphasis on neuroinflammatory markers.

Results: Chronic corticosterone consumption caused the inhibition of the non-amyloidogenic pathways, the impairment of $A\beta$ clearance processes and the induction of amyloidogenic pathways in the hippocampus. The principal enzymes involved in glucocorticoid receptor (GR) activation and Tau phosphorylation were upregulated. Importantly, the AD-like phenotype triggered by chronic corticosterone was analogous to the one caused by $oA\beta$. These molecular commonalities across models were independent from inflammation, as chronic corticosterone was immunosuppressive while $oA\beta$ was pro-inflammatory. When chronic corticosterone consumption anticipated the induction of the $oA\beta$ pathology, we found a potentiation of neuroinflammatory processes associated with an exacerbation of synaptic and memory deficits but also an aggravation of AD-related hallmarks.

Discussion/Conclusion: This study unravels new functional outcomes identifying chronic corticosterone consumption as a main risk factor for AD and suggests that glucocorticoid-based therapies should be prescribed with caution in populations with AD risk.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. This pathology is characterized by a progressive impairment of cognitive functions and the presence of senile plaques and neurofibrillary tangles (NFT) throughout the brain, including areas particularly involved in memory formation and emotional regulation. Plaques are composed of insoluble extracellular aggregates consisting mainly of amyloid- β ($A\beta$) peptides, while NFT result from the aggregation of hyper- and abnormal phosphorylation of the microtubule-stabilizing protein Tau [1,2]. Familial forms with known mutations of specific genes represent less than 5% of cases, while 95% of patients develop sporadic forms, for which several risk factors have been identified. Besides aging, there is growing evidence that environmental and lifestyle aspects may increase the probability to develop AD [3,4]. Clinical observations suggest that stressful life events can reduce the age of onset of AD [5], while stress-related disorders like depression or anxiety can promote AD symptoms and neuropathology [3,6]. This view is particularly supported by the fact that AD patients demonstrated an early deregulation of the hypothalamic-pituitary-adrenal (HPA) axis (*stress axis*), as well as elevated levels of glucocorticoids (GC; *stress hormone*) in plasma and CSF, and GC receptors (GR) impairment [7-9].

The HPA axis, pivotal for the stress response, triggers the adrenal cortex to release GC. These steroid hormones readily cross the BBB and bind to low affinity glucocorticoid receptors (GR) and high affinity mineralocorticoid receptors (MR) [10]. GC are necessary for normal cellular activity and fundamental for many CNS functions, including learning and memory [11]. While MR are localized mainly in the hippocampus, GR are more ubiquitous and highly expressed in the limbic system (prefrontal cortex (PFC), hippocampus and amygdala). These structures are strongly involved in cognitive and psychological functions and are also important components of the neural circuitry modulating HPA axis activity [12]. GC act synergistically with excitatory amino acids (like glutamate) and can induce neurotoxicity. Hence, a deregulation of the HPA axis activity and/or modification of GR functioning could be extremely toxic, especially in limbic structures [13], and thus could contribute to the cognitive decline and psychological symptoms that occur in AD. In transgenic (Tg) animal models of AD, chronic stress accelerates the onset of cognitive deficits, triggers amyloid precursor protein (APP) misprocessing, enhances plaque pathology, reduces $A\beta$ clearance, increases $A\beta$ levels, stimulates Tau hyperphosphorylation and its neuronal accumulation [14-16]. In an acute pathomimetic model of AD obtained after a single intracerebroventricular (icv) injection of an oligomeric solution of $A\beta_{25-35}$ (o $A\beta_{25-35}$)

[17-20], we demonstrated a strong, long-lasting activation of the HPA axis. This was associated with a modification of GR and MR expression in brain regions involved in the control of GC secretion (*i.e.* hippocampus, amygdala and hypothalamus) [21], supporting its involvement in the etiology of AD [14,22-25]. We also observed that an antagonist and two selective GR modulators (sGRm) could potently counteract the effects of $\alpha\text{A}\beta_{25-35}$ icv injection in the hippocampus and in the PFC, strongly arguing in favor of a therapeutic potential of modulating GR activity and a plausible pharmacological entry points to modify the progression of AD [4,19,20].

In the present study, we aimed to determine *in vivo* the physiological impact of a chronic GC supplementation in an AD context. For this purpose, we first established and validated a rat model of experimental chronic GC supplementation (*chronic corticosterone consumption in drinking water for 4 weeks*). Such long-term exogenous GC exposure in rodents has been widely described to induce anxiety/depressive-like behaviors [26-28] HPA axis impairment [29], memory and neurogenesis deficits, and hippocampal damages [27,30-32]. Then, to evaluate the consequences of chronic GC consumption and to decipher the impact of HPA axis dysregulation on the onset of $\text{A}\beta$ toxicity, animals chronically treated with GC were icv injected with $\alpha\text{A}\beta_{25-35}$ (CCo $\text{A}\beta$ group). We evaluated AD-related cognitive deficits and pathogenic mechanisms, with a special emphasis on neuroinflammatory markers.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (*Janvier Lab., Le Genest-Saint-Isle, France*) weighing 260-280g (8 weeks) at the beginning of the experiments were housed 1 week before experiments in a standard animal facility of the University of Montpellier (CECEMA, registration number D34-172-23) (12H/12H light/dark cycle with lights on at 07H00; $21 \pm 1^\circ\text{C}$, food and water *ad libitum*). All experiments, including sacrifices, were performed in conscious rats between 09H00 and 12H00, during the diurnal trough of the HPA axis circadian rhythm. Male has been preferred to female because the latter may present a confounding factor of the estrous cycle, a parameter that needs to be systematically controlled. Although unlikely, a possibility still exists that mixing males and females in each experimental group may introduce a variable difficult to account for. Furthermore, doubling the number of groups and rats goes beyond the 3R rule. We do recognize the prospective importance of using both genders, and results will need to be verified in female.

Amyloid- β peptide

In patients, soluble A β oligomers contains mainly the sequences A β ₁₋₄₀ and A β ₁₋₄₂ [2]. Nevertheless, they also contain peptides with shorter sequences such as A β ₂₅₋₃₅ or A β _{25-35/40} [33-35], with no difference between human and rodent [36]. These shorter peptides can be produced in AD patients by enzymatic cleavage of A β ₁₋₄₀ [33,35]. They contain extracellular and transmembrane residues that have been reported to be a biologically active region of A β [37-39] and to contain the highly hydrophobic region forming stable aggregates [38]. Interest in this undecapeptide, which itself shows a β -sheet structure [17,38], has grown over the last fifteen years, mainly because it induces neurite atrophy, neuronal cell death, synaptic loss, as well as synaptic plasticity and memory deficits in a similar way to A β ₁₋₄₀ and A β ₁₋₄₂ [40], but with better solubility and efficiency [41]. A β ₂₅₋₃₅ and scrambled A β ₂₅₋₃₅ peptides (*Eurogentec, France*) were dissolved in sterile water (1 μ g/ μ l) and stored at -20°C. Since soluble A β oligomers correlate better with the progression of the disease [42], A β ₂₅₋₃₅ and scrambled peptides were pre-aggregated by an *in vitro* incubation at 37°C (4 days) to obtain a solution mainly composed (more than 95%) of a mixture of soluble oligomer species (oA β ₂₅₋₃₅), as previously characterized [18].

Experimental procedures

First, to validate the chronic corticosterone consumption model, animals received drinking water containing 1, 5 or 10 mg/kg corticosterone (*Sigma-Aldrich, France*) dissolved in 0.45% β -cyclodextrin (*Sigma-Aldrich, France*) [27] for 4 weeks (SFig. 1A). A control cohort received the vehicle alone (0.45% β -cyclodextrin) in drinking water. All animals were weighed once per week. The general behavior or anxiety state of the different groups of rats was tested at the end of the treatment (after 4 weeks) in an open-field or in an elevated plus maze test (EPM), respectively. Animals were sacrificed by decapitation. Pituitary and adrenals were rapidly harvested to be weighed and blood samples were collected for corticosterone assay.

Then, a new cohort of rats received drinking water containing the effective dose of corticosterone (10 mg/kg) or the vehicle (β -cyclodextrin) for 5 weeks. At week 4, animals were divided into three groups. The first groups had no surgery (control groups), the second received an icv injection of incubated scrambled peptide (10 μ g/rat; scrambled groups) and the third received an icv injection of oA β ₂₅₋₃₅ (10 μ g/rat; oA β groups) (Fig. 1A). The animals were anesthetized with an intraperitoneal (ip) injection of 1 ml of a mixture of Ketamine and Xylazine (80 and 10 mg/kg b.w., respectively). As previously described [17,18], oA β ₂₅₋₃₅ was injected directly into the lateral ventricles using a David-Kopf stereotaxic apparatus (*Phymep, France*), (coordinates: AP -1 mm, L \pm 1.5 mm, DV -3.5 mm) [43]. All animals were

weighed before, 4 weeks after the treatments and 1 week after the icv injection of oA β (week 5) (Fig. 1A). Short-term memory was tested in a T-maze 1 week after the icv injection of oA β (Fig. 1A). Then, animals were sacrificed by decapitation. Blood samples and hippocampi were rapidly collected during the diurnal part of the HPA axis rhythm (*between 09H00 and 12H00*) for corticosterone assay and WB analysis. Naive rats received no treatment but were manipulated in the same manner as treated rats.

General behavior

The open-field test was used to characterize general behavior of rats, as previously reported [44]. The apparatus consists of a squared open-field (1 m² x 0.6 m high) made of white PVC with an infrared light emitting floor. An infrared sensitive CCD camera was placed above the field and connected to a videotracking system (*Noldus EthoVisonXT, France*). Animals were allowed to freely explore the open-field during 10 min. This test evaluates locomotion-related parameters, such as the total distance travelled or walking speed; exploratory-related parameters, including locomotion, immobility and number of rearings; anxiety-related indexes, such as latency to start and thigmotaxis (locomotion along the walls); and stereotyped responses, like rearing or grooming.

Spatial short-term memory

As previously reported, the T-maze test was used to rapidly assess cognitive ability in rats, especially the short-term memory deficits when performed in two successive sessions [17-19,21,45]. The T-maze consisted of two short arms (A and B), extending from a longer alley (C) and enclosed with high walls. The test involved two trials separated by 1 h. During the training session, one short arm (B) was closed. Rats were placed at the end of the long alley, allowed to visit the maze for 10 min and then returned into their home cage. During the test session, which was videotracked (*Noldus EthoVisonXT, France*), animals were placed in the maze for 2 min, with free access to all arms. The number of visits and time spent in each arm were measured. The results were expressed as ratio of the time spent in the initially closed novel arm, over the time spent in the previous arm and as a ratio of the number of entries into the novel arm over the familiar one. The apparatus was cleaned with diluted ethanol (50%) between animals.

Anxiety behavior

The anxiety state of rats was measured using their ability to explore open and enclosed arms of an elevated plus maze (EPM), as previously detailed [21]. The clear plexiglass apparatus consisted of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 45 cm high), extending from a central platform and placed 60 cm above the floor.

Each rat was placed at the center of the EPM facing the closed arm and its exploration behavior was recorded for 10 min (*Noldus EthoVisionXT, France*). The results were expressed as total time spent in the open arms and the total number of entries was counted to verify general motor activity. An entry into an arm was recorded if the animal crossed the line that connected that arm with the central platform with all four legs. The apparatus was cleaned with diluted ethanol (50%) between animals.

Corticosterone assay

Blood samples were collected at the time of sacrifice on 1 mg/ml EDTA (*Sigma-Aldrich, France*), centrifuged at 4°C, and plasma stored at -20°C until assayed for corticosterone [18]. Plasma corticosterone concentrations were assayed using a conventional ELISA kit (*Enzo-Life Sciences, USA*) in a 10 µl plasma sample diluted (1:40) with the assay buffer. The assay sensitivity was 27 pg/ml. The intra- and inter-assay coefficients were 6.6 and 7.8%, respectively.

WB analysis

WB were performed as previously described [18] in the whole hippocampus. All antibodies used are detailed in the [Supplementary Table 2](#). Briefly, after sacrifice, hippocampi were micro-dissected, weighed, immediately frozen in liquid nitrogen and stored at -20°C. Tissues were sonicated (*VibraCell; Sonics & Materials, USA*) in a lysis buffer [18] and centrifuged (4°C). Supernatants were collected and the protein concentration was measured using a BCA kit (*ThermoFisher Scientific, France*). Sixty µg from each sample were taken for WB analysis. Samples were separated in SDS-polyacrylamide gel (12%) and transferred to a PVDF membrane (*Merck-Millipore, France*). The membrane was incubated overnight (4°C) with the primary antibody, washed and then incubated for 2H with the appropriate horseradish peroxidase-conjugated secondary antibody. Peroxidase activity was revealed by using enhanced-chemiluminescence (ECL) reagents (*Luminata-Crescendo, Merck-Millipore*). The intensity of peroxidase activity was quantified using Image-J software (*NIH, Bethesda, MA, USA*). β -tubulin (β -Tub) was used as a loading control for all immunoblotting experiments.

Statistical analysis

Data are presented as mean \pm SEM and analyzed using one-way or two-way ANOVA followed by a Tukey's multiple comparison test (*GraphPad-Prism 9.0*). In addition, T-maze data are analyzed in comparison to chance level (CL), i.e., maximal deficit, using a one sample t-test (*GraphPad-Prism 9.0*). $P < 0.05$ was considered significant. The number of animals in each group is indicated within the columns and was determined by a statistical

power analysis based on our previous studies (*G*Power software*). Before each analysis of variance, the Gaussian distribution was evaluated and validated by a Kolmogorov-Smirnov test (*GraphPad-Prism 9.0*).

RESULTS

Validation of the chronic corticosterone consumption model

To validate this model (SFig. 1A), we assessed relevant parameters associated with chronic corticosterone treatment in drinking water [26,27,29]. The daily dose of 10 mg/kg of CORT was the sole dose that significantly increased the plasma concentrations of CORT, while the vehicle (0.45% β -cyclodextrin) and the doses of 1 and 5 mg/kg induced no modification in comparison to control rats (SFig. 1B). The dose of 10 mg/kg/day was also the most efficient to atrophy adrenal glands (STable 1) and to induce anxiety-like behavior. Indeed, in the open-field test (SFig. 1C), we identified several characteristic features of anxiety, such as the increase of the latency to start moving and of the total immobility time. We also observed a decrease of visits in the arena center and the number of grooming and rearing, while locomotion was not affected by the different doses. To confirm these general observations, we performed a well-defined anxiety test, the EPM. Animals treated with the two highest doses of corticosterone (5 and 10 mg/kg/day) displayed a decrease of the time spent in the open arms, characteristic of an anxious state (SFig. 1D).

Impact of chronic corticosterone on α A β toxicity

We next evaluated the impact of chronic corticosterone consumption (10 mg/kg/day) on the A β toxicity one week after the icv injection of oA β ₂₅₋₃₅ (CCoA β group) (Fig. 1A). Five weeks of chronic corticosterone consumption in drinking water induced short-term memory deficits (*T-maze test*) and potentiated significantly the oA β ₂₅₋₃₅-induced alterations of memory performances (Fig. 1B). No effects of scrambled peptide nor vehicle treatment were evidenced (Figs 1-4). One week after the icv injection of oA β ₂₅₋₃₅, an increase of plasma CORT concentrations was observed, which was partially blunted by the upstream chronic corticosterone consumption (Fig. 1C). In addition, an additive effect of CCoA β treatment was evidenced on body weight (Table 1) and on the hippocampal expression of cleaved-caspase 3, while the two treatments alone did not impact the later parameter (Fig. 1D).

Impact on GR signaling pathways

As previously reported in the PFC [20], the icv injection of oA β ₂₅₋₃₅ altered GR-associated signaling pathways in the hippocampus (Fig. 2A). Indeed, one week after oA β ₂₅₋₃₅, the expression of total and activated GR (*phosphorylated on Ser²¹¹*) was increased [44]

(Fig. 2B-D), and was associated with an increase of heat-shock protein 90/70 (*HSP90/70*) ratio (Fig. 2B,E), two GR chaperones reflecting the activity of GR and highly involved in AD pathophysiology [47-49] and an increase of Annexin A1 (*a potent endogenous anti-inflammatory effector functioning in synergy with GR*) [50,51] (Fig. 2B,F). We also observed the increased expression of the Src-kinase Fyn (Fig. 2B,G), the active form of GSK3 β (*phosphorylated on Tyr²¹⁶*) (Fig. 2H,I), the total form of Cdk5 (Fig. 2H,J) and the p25/p35 ratio (*associated with the activation of Cdk5*) [52] (Fig. 2H,K). These three enzymes are particularly relevant since they are involved in GR activation but also in the hyperphosphorylation of Tau [46,52-55]. Chronic corticosterone consumption induced a similar effect as oA β_{25-35} on all of these markers, except on Annexin A1 (Fig. 2B,F) and the p25/p35 ratio where we evidenced an additive effect of CCoA β treatments (Fig. 2H,K).

Impact on APP metabolism

Then, we characterized by Western blot the different pathways of APP maturation, through the assessment of the main cellular elements involved in the amyloidogenic and non-amyloidogenic pathways (Fig. 3A). One week after oA β_{25-35} injection and as previously reported [16,18], we evidenced the induction of the amyloidogenic pathway in the hippocampus (Fig. 3J), consistent with an A β_{1-42} increase, as previously reported by our group [20,56,57]. We observed an increased expression of both full-length APP (*precursor of amyloid proteins*) (Fig. 3B,C), C99 (*precursor of A β peptides*) (Fig. 3B,D), BACE1 (*β -APP cleaving enzyme, β -secretase*) (Fig. 3B,E) and PS1 (*presenilin-1, γ -secretase subunit*) (Fig. 3B,F). This induction is associated with a concomitant inhibition of non-amyloidogenic pathways (Fig. 3J), characterized by a decrease of sAPP α (*α -secretase-cleaved soluble APP ecto-domain*) (Fig. 3B,G), α -secretase ADAM10 (*A disintegrin and metalloprotease domain-containing protein 10*) (Fig. 3B,F) and IDE (*insulin-degrading enzyme, involved in the clearance of A β*) (Fig. 3B,G). Interestingly, chronic corticosterone consumption induced the same modifications as those observed 1 week after the icv injection of oA β_{25-35} (Fig. 3), and CCoA β treatment induced no supplementary effects, except for BACE1 where we showed a significant additive effect (Fig. 3B,E).

Impact on synaptic deficits and neuroinflammation

Decreased memory performances and GR signaling pathway alterations observed one week after the icv injection of oA β_{25-35} were associated in the hippocampus with pre-synaptic (SYN) and post-synaptic (PSD95) deficits (Fig. 4A-C). These deficits were potentiated by the prior overexposure to exogenous CORT in animals injected with oA β_{25-35} (Fig. 4A-C). Moreover, and as previously reported [19,20], synaptic deficits induced by the A β toxicity

were associated with important modifications of hippocampal inflammatory processes. Indeed, after 1 week, the icv injection of $\text{oA}\beta_{25-35}$ provoked both the induction of pro-inflammatory mechanisms (*activation of astrocytes (GFAP) and recruitment of microglia (Iba1)*) (Fig. 4A,D,E) and the induction of anti-inflammatory components, as interleukin-10 (IL-10) (Fig. 4A,E,F). By contrast, while chronic corticosterone favored anti-inflammatory pathways (Fig. 4G) by inhibiting astrocytes activation (Fig. 4A,D), microglia recruitment (Fig. 4A,E) and IL-10 induction (Fig. 4A,F), the CCoA β group displayed exacerbated neuroinflammation (Fig. 4G). Indeed, co-treatment increased the activation of astrocytes and the recruitment of microglia (Fig. 4A,D-E), and inhibited IL-10 (Fig. 4A,F). In addition, ADAM17, another α -secretase (*also known as tumor necrosis factor- α converting enzyme or TACE*), highly activated in neuroinflammatory processes and promoting pro-inflammatory cytokines maturation in turn (*for review, see [58]*), was up-regulated after the icv injection of $\text{oA}\beta_{25-35}$ (Fig. 4A,G). And although chronic corticosterone inhibited the expression of this metalloprotease, as previously observed with ADAM10 (Fig. 3B,F), the CCoA β treatment reestablished the hippocampal increase of ADAM17 (Fig. 4A,G).

DISCUSSION

In previous studies, we provided evidence for a vicious cycle between AD and the HPA axis. We showed that the pathology, and especially the A β toxicity, rapidly increases GC secretion which, in turn, modulates APP processing, A β_{1-42} synthesis, and Tau phosphorylation [19-21]. Here, in order to characterize the repercussion of chronic GC consumption on AD pathophysiology, we treated animals with corticosterone in drinking water upstream of the icv injection of $\text{oA}\beta_{25-35}$. We reported that chronic corticosterone consumption in the $\text{oA}\beta_{25-35}$ model exacerbated cognitive decline, amyloid pathology (Fig. 3J) and neuroinflammation (Fig. 4H), notably in close association with an enhanced activity of GR signaling pathways. Many biochemical AD hallmarks impaired by $\text{oA}\beta_{25-35}$ injection were equivalently impaired by chronic GC treatment. We could hypothesize that in AD, the deregulation of these intracellular pathways could be due to GC overexposure and HPA axis impairment induced by A β toxicity, more than A β toxicity itself. This not only implies a specific susceptibility to stress in AD, but also brings new information on how chronic GC consumption could be an important risk factor for AD.

Model of chronic GC consumption

Firstly, we aimed to validate our chronic GC consumption model, well known to be a model of chronic anxiety/depression [26,27] associated with memory deficits and

hippocampal alterations [31,59]. According to the literature, in our study we focused on the anxious state displayed in animals treated for 4 weeks with corticosterone in drinking water. At the optimal dose determined (*i.e.* 10 mg/kg/day), animals developed many anxiety-like behaviors in the open-field and EPM tasks. At this dose, we also evidenced an increase of GC plasma levels associated with an atrophy of adrenal glands. These results are in accordance with studies showing that chronic corticosterone administration suppressed the inhibitory feedback of GC on the HPA axis activity [29,60], characteristic of mood disorders such as generalized anxiety or major depression [61,62]. In accordance to previous works conducted by others groups, we validated this model as a chronic model of GC overexposure, displaying HPA axis impairment and anxiety-like behaviors.

Synergistic effects

Secondly, we compared the effects of chronic GC consumption alone or in combination with the icv injection of $\alpha\text{A}\beta_{25-35}$. CORT treatment caused short-term memory and synaptic deficits in the hippocampus, with no modification on caspase 3 levels. These alterations following GC overexposure are widely described in the literature. It was shown that chronic stress or GC administration altered synaptic terminal structure, neurogenesis and induced neuronal atrophy, more particularly in the hippocampus [13,29,63-65]. Since CORT treatment also induced anxiety-like behaviors in the EPM, we could not totally exclude this emotional aspect in the T-maze performance. However, as reported by others, CORT treatment induced many hippocampal alterations (dendritic retraction, decreased cell proliferation and global volume) [31,58] sufficient to induce such short-term memory deficits. In addition, Han et al., [16] also reported an aggravation of cognitive impairment in APP/PS1 mice submitted to chronic unpredictable stress. By contrast, it is interesting to note that CCoA β group displayed exacerbated memory and synaptic deficits. Furthermore, we also observed a synergistic effect of this co-treatment, that induced caspase 3 expression in the hippocampus, whereas the two treatments alone did not impact it. This suggested that the potentiation of synaptic and memory deficits could be due to the induction of apoptosis processes.

Additive effects

Moreover, we evidenced some additive effects of the exogenous GC treatment and the icv injection of $\alpha\text{A}\beta_{25-35}$. Indeed, we showed that the induction of BACE1 was potentiated. This enzyme was identified as the major β -secretase-like protein, highly involved in the amyloidogenic cleavage of APP, and thus in A β peptides production [66]. These findings are consistent with the study conducted by Green et al., [14]. The authors have shown that

one-week treatment of dexamethasone in 4 months-old 3xTg mice significantly increased BACE1 expression and higher levels of A β ₁₋₄₀ and A β ₁₋₄₂. Our result was in accordance with the literature since BACE1 production could be triggered by chronic stress as well as by A β . In fact, BACE1 transcription is under a direct control of GC since a glucocorticoid response element (GRE) has been described in the promoter region of BACE1 [67]. In addition, BACE1 production could be also indirectly regulated by stress and A β through the activation of others intracellular mediators. (i) Both chronic GC overexposure [68,69] and A β toxicity [2,70] induce the c-jun N-terminal kinase/activator protein 1 (JNK) pathway (or stress-activated kinase (SAPK)), one of the main pathways favoring BACE1 production [71]. (ii) The p25/Cdk5 pathway, upregulated in our study, was also known to trigger BACE1 transcription [66]. This complex of enzymes is highly involved in AD pathology, particularly for its action on Tau hyperphosphorylation [2,52], but also on GR activation via phosphorylation on Ser211 in rodent (*Ser224 in human*) [72]. Some studies demonstrated that chronic unpredictable stress [73,74], as well as A β pathology [52,75] increased p25/Cdk5 expression and activity. (iii) These mechanisms could be due to an increased activity of Calpain1, a member of cysteine proteases family showing aberrant activity in AD [76]. This enzyme is particularly involved in the activation of GSK-3 β [77] and the maturation of p35 to p25 [78]. Interestingly, we demonstrated recently in the oA β ₂₅₋₃₅ model that all these enzymes (*i.e.* GSK3- β , p25/Cdk5 and calpain1) were directly or indirectly regulated by GC and GR in the prefrontal cortex [20]. Our results suggest that Calpain1 activity may also be potentiated by chronic GC overexposure since the hippocampal expression of two of its principal substrates was increased. So, by these different mechanisms, it was not surprising that combined toxicity of chronic corticosterone consumption and oA β ₂₅₋₃₅ injection had additive effects on the expressions and/or activities of BACE1 and p25/Cdk5. Since these two enzymes are highly involved in amyloid and Tau pathologies, it brings new data on how chronic GC consumption is detrimental in AD development. However, further specific investigations are needed to characterize precisely tau system, in particular to assess the levels of Tau hyperphosphorylation epitopes, its eventual aggregation, its localization and its staining pattern.

Effects on APP metabolism and GR signaling pathways

On the principal proteins associated with GR signaling pathways (*i.e.* GR, p(Ser211)GR, HSP90/HSP70, GSK3- β , Cdk5 and Fyn) and APP metabolism (*i.e.* APP, C99, IDE, sAPP α , ADAM10 and PS1) assessed in our study, there was no additive effect of chronic corticosterone on the A β toxicity. Interestingly, in 3xTg mice, one-week treatment with dexamethasone was able to induce an increase of APP and C99 levels [14]. However,

the authors used 4 months-old 3xTg animals before the establishment of important AD-like symptoms. Thus, dexamethasone could potentiate the expression of these markers. In our study, the modifications induced by each treatment (*chronic corticosterone* or $\text{oA}\beta_{25-35}$) were closely similar. This suggests common regulatory mechanisms and supports the important role played by GR in the pathophysiology of AD (*for review, see [3,4,6,24]*). Indeed, we recently demonstrated in this acute model of AD ($\text{oA}\beta_{25-35}$), that these pathways were impaired [20] and associated with a strong and long-lasting disruption of the HPA axis activity and functionality [21]. Then, to evaluate the contribution of GR in the $\text{A}\beta$ toxicity, we tried to restore their functionality using new selective GR modulators (sGRm) [19,20]. This family of compounds has the advantage to selectively abrogate pathological effects of GR overactivation, while retaining their physiological function [79,80]. By restoring their functionality with sGRm, we counteracted all parameters measured and induced by the $\text{A}\beta$ toxicity, highlighting the central role played by GR in the development of AD [20]. Taken together, all of these results highly suggest that a part of $\text{A}\beta$ toxicity could be a consequence of the GC overexposure and GR signaling impairment.

However, the two α -secretases ADAM10 and ADAM17 are differentially regulated in the hippocampus by chronic corticosterone consumption and $\text{oA}\beta_{25-35}$. We found that $\text{A}\beta$ toxicity increased ADAM17 levels while inhibiting ADAM10 expression. By contrast chronic corticosterone decreased the two α -secretases. These proteases are neuroprotective by triggering the non-amyloidogenic pathway and the generation of sAPP α . But the precise role of ADAMs in AD pathogenesis is not fully understood. ADAM10 seems to constitutively cleave APP while fine regulation is mediated by ADAM17 [81]. In the CSF of AD patients, ADAM10 is decreased [82], which is correlated with the inhibition of the non-amyloidogenic pathway. Inversely, ADAM17 activity is increased both in early and advanced phases of AD [83], that evidences more complex regulatory mechanisms. In fact, besides its neuroprotective role, ADAM17 is also highly activated by neuroinflammatory processes and promotes pro-inflammatory cytokines maturation in turn (*for review, see [58]*). These findings are relevant with our results in $\text{oA}\beta$ and CCo $\text{A}\beta$ groups. In these animals, we found decreased ADAM10 and sAPP α expressions and increased ADAM17 expression concomitantly with enhanced neuroinflammatory processes (Fig. 4H). So, we could imagine that ADAM10 participated to APP non-amyloidogenic cleavage more than ADAM17, that could be regulated by pro-inflammatory processes. To summarize, we found that both $\text{oA}\beta$ and chronic corticosterone consumption altered APP processing balance by increasing the amyloidogenic (*C99, BACE1, PS1*) and inhibiting the non-amyloidogenic (*ADAM10, IDE*) pathways (Fig. 3J). It is interesting to note that the main difference observed between

chronic corticosterone and oA β groups is their inflammatory phenotypes. Such difference could participate to explain the exacerbation of AD-related markers in CCoA β animals (Fig. 4H).

Effects on inflammatory processes

Neuroinflammation is a key feature of AD pathology. Activated microglia and astrocytes trigger the overproduction of pro-inflammatory cytokines, which promotes A β accumulation and thus neurodegeneration [2]. As we already observed [17-20], we confirmed in this study that oA β ₂₅₋₃₅ induced neuroinflammation, since it increased GFAP and Iba1, but also ADAM17, which is highly activated by neuroinflammatory processes and, as discussed above, promotes pro-inflammatory cytokines maturation in turn (*for review*, see [58]) (Fig. 4H). Inversely, chronic corticosterone consumption favored the anti-inflammatory pathways (Fig. 4H). It was well known that stress and GC influence substantially the level and the quality of the immune response. The activation of GR-AnnexinA1 pathway inhibits the pro-inflammatory response, thus mediating the major immunosuppressive effects of GC [51,84]. Here, we observed the decrease of GFAP and Iba1 levels in the hippocampus, associated with the increase of GR, p(S211)GR, Annexin A1 and the anti-inflammatory cytokine IL-10 expressions. Same results were obtained in recent research that investigated the effects of chronic stress on astroglial cells. Both chronic variable stress and chronic restraint stress increased GC plasma levels while decreasing GFAP expression by around 20% in astrocytes [85-87]. Such variation in astrocytes was sufficient to trigger depressive and anxiety-like behaviors [85-87]. The authors found that this modification is not due to glial cell loss, but could be due to an important remodeling of astrocytes morphology and network. This decrease of astrocytes plasticity led to a loss of their trophic functions on neurons, particularly on synaptic formation, LTP induction and growth factor expression [85,87,88]. Based on our results, these alterations could be linked to synaptic and memory deficits induced by the chronic corticosterone consumption. Moreover, a negative glucocorticoid-responsive element (nGRE) on the promoter of GFAP was identified, indicating that GC inhibits directly the transcription of GFAP [89]. So, it is not surprising that in these conditions, chronic corticosterone seemed to be highly immunosuppressive (Fig. 4H). To reinforce this view, these results were also correlated with ADAM17 decreased expression. As mentioned, ADAM17 expression and activity are under the control of pro-inflammatory processes [58].

Otherwise, we observed attractive results in CCoA β animals. We found in this group that GC plasma levels were higher than in control group, but not as well as chronic

corticosterone consumption or oA β groups. Such result could be explained by the atrophy of adrenals and the impairment of HPA axis, indicating that this endocrine system was exhausted in CCoA β animals. In accordance with our results, other groups demonstrated that chronic GC overexposure might blunt stress-induced corticosterone secretion [29,90]. These findings were obtained with animals pretreated with GC and then submitted to behavioral stressors. This observation could be extended to cellular stressors such as oA β ₂₅₋₃₅ in our study. These results in CCoA β animals could also explain the incapacity of GC to restrain inflammatory processes induced by oA β . Indeed, in these animals we observed a potentiation of pro-inflammatory (GFAP, Iba1, ADAM17) and an inhibition of anti-inflammatory (IL-10) markers (Fig. 4H). This incapacity of GC to regulate neuroinflammatory processes could partly explain the exacerbation of AD hallmarks evidenced here. Such phenomenon was already described in chronically stressed animals injected with lipopolysaccharide (LPS) [91-94]. Thus, in accordance with our results, it appears that chronic stress, as well as chronic corticosterone consumption, primes the neuroinflammatory response to a subsequent pro-inflammatory stimulus as oA β ₂₅₋₃₅ or LPS. Therefore, the neuroimmune context is more responsive to inflammation, also favoring GC insensitivity or reducing the HPA response [94-96].

Furthermore, additional effects of chronic corticosterone consumption and oA β ₂₅₋₃₅ injection were evidenced in the hippocampus on Annexin A1. As previously mentioned this protein is activated by pro-inflammatory mediators and is an important anti-inflammatory actor mediating GC and GR effects [50,51]. However, no study identified a GRE on the promoter region of Annexin A1 gene, and its transcription seems to be mediated by pro-inflammatory cytokines and oxidative stress [97,98]. Here, surprisingly, even if GR signaling pathways were impaired, Annexin A1 levels were highly increased in CCoA β animals. Taken together, we can envisage that this increase of Annexin A1 could be a compensatory mechanism to counteract exacerbated pro-inflammatory processes. Further investigations are needed to assess if the GR-Annexin A1 axis is still functional, even in a context of GR overactivation.

We focused on GR as we previously observed and established its central role in AD pathophysiology [3,19,20]. In stress conditions, and a fortiori in pathological conditions, GR seem to be more involved than MR [10]. However, a recent work demonstrated that hippocampal MR are overexpressed and overactivated by oxidative stress. This leads to microglia activation, pro-inflammatory mediators secretion, and down-regulation of anti-inflammatory factors [99]. Thus, we cannot totally exclude the involvement of MR in our

study. Further investigations are needed to decipher the precise role of MR between chronic stress, high levels of GC and the pathophysiology of AD.

CONCLUSIONS

All these results evidenced the crucial role played by GC and GR, and brought new information on how chronic corticosterone consumption could accelerate the development of AD by generating a deleterious inflammatory environment. It also reinforces the idea making chronic GC overexposure, as well as anxiety, as main risk factors in AD. Despite physiological anti-inflammatory properties of GC, prior chronic exposure to corticosterone markedly potentiates neuroinflammation associated with a subsequent innate immune system challenge induced by $\text{oA}\beta$ injection. The direct consequence of this exacerbated neuroinflammation is the aggravation of synaptic and memory deficits (Fig. 5).

It also highlights the therapeutic potential of new GR modulators, called sGRm, which are in capacity to reestablish GR functioning and signaling pathways and subsequent HPA axis physiological activity. These compounds represent a really attractive therapeutic approach in all stress-related disorders, pathologies displaying GC overproduction, or pathology needing an anti-inflammatory treatment [4,19,100-103].

Otherwise, the present study strongly argues in favor of the hypothesis suggesting that AD, as well as depression, could be a stress-related disorder [4]. Indeed, for years, clinical evidences established a strong correlation between elevated GC and higher risk to develop AD [104]. Thus, it was not surprising that recent clinical trials showed no difference in cognitive decline in AD patients prescribed with the GC receptor agonist, prednisone [105]. That's why this work also aims to alert about the risk to prescribe GC-based therapies in the elderly or in early AD patients.

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STATEMENT OF ETHICS

Animal procedures were conducted in strict adherence to the European Union Directive of 2010 (2010/63/EU). The National French Animal Welfare Committee and the local committee at the University of Montpellier approved all protocols (*authorization: CEEA-LR-12160*). All efforts were made to minimize the number of animals used, potential pain, suffering and distress.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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AUTHOR’S CONTRIBUTIONS

GC, CZ, CH and AB performed experiments. LG designed the study, wrote the protocol, performed part of experiments and analyzed the data (with the help of GC, CH, CZ and AB). LG and GC wrote the manuscript. CZ, CH, NC, NM and CD corrected the manuscript. All authors read and approved the final manuscript. All funders had no role in data collection, analysis or in the writing of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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LEGENDS

Fig. 1

Impact of chronic corticosterone consumption on $\text{oA}\beta_{25-35}$ -induced toxicity. **Panel A:** Experimental protocol - At T0, adult male rats (Sprague Dawley) were treated with corticosterone in drinking water (10 mg/kg/day, dissolved in 0,45% β -cyclodextrine) for 5 weeks (CORT animals, red columns) and compared to a second group treated only with β -cyclodextrine (vehicle animals, blue columns). At week 4, animals received any icv injection (control (C) group), an icv injection of $\text{oA}\beta_{25-35}$ (10 $\mu\text{g}/\text{rat}$, “ $\text{oA}\beta$ ” group) or an icv injection of Scrambled control (“S” group). The last day before sacrifice (day 34) the spatial short-term memory of each rat was tested in a T-maze. The following day (day 35), the animals were sacrificed between 9H00 and 12H00 (*during the diurnal trough of the HPA axis circadian rhythm*), blood samples and hippocampi were rapidly collected for corticosterone assay and Western blot analysis, respectively. **Panel B:** Spatial short-term memory performance was determined in a T-maze test and was expressed as the ratio of the time spent in the initially closed arm (B) over the time spent in the previous arm (A). Two-way ANOVA: $F_{2,39} = 44.7$ for group, $p < 0.0001$; $F_{1,39} = 36.4$ for treatment, $p < 0.0001$; and $F_{2,39} = 1.09$ for interaction, ns. In addition, all data were analyzed in comparison to chance level (CL), i.e., maximal deficit, using a one sample t-test. All data are different to the CL, except rats co-treated with CORT and $\text{oA}\beta$. **Panel C:** Plasma concentrations of corticosterone (CORT) were determined by Elisa and expressed as ng/ml. Two-way ANOVA: $F_{2,30} = 9.48$ for group, $p < 0.001$; $F_{1,30} = 0.15$ for treatment, ns; and $F_{2,30} = 15.3$ for interaction, $p < 0.0001$. **Panel D:** Variations of apoptosis hallmark (caspase-3, 19 kDa) were evaluated in each group and normalized with the variations of β -tubulin (β -tub, 55 kDa) and compared with non-injected rats (control group: C). Two-way ANOVA: $F_{2,30} = 1.99$ for group, ns; $F_{1,30} = 8.45$ for treatment, $p < 0.01$; and $F_{2,30} = 11.0$ for interaction, $p < 0.001$.

Data are expressed as means \pm SEM in percent of naive rats. * $p < 0.05$ and ** $p < 0.01$ vs. respective group treated with vehicle. ++ $p < 0.01$ vs. respective control (C) group. \emptyset $p < 0.05$ and $\emptyset\emptyset$ $p < 0.01$ vs. respective scrambled (S) group, \blacklozenge $p < 0.05$ and $\blacklozenge\blacklozenge$ $p < 0.01$ vs. selected group, ** $p < 0.01$ vs. chance level (CL).

Fig. 2

The cumulative effects in the hippocampus of chronic corticosterone consumption and $\text{oA}\beta_{25-35}$ injection on GR signaling pathways (**Panels A**), were evaluated by Western blot. Variations of the expression of GR (95 kDa) (**Panels B,C**), the phosphorylation of GR (p[Ser211]GR, 95 kDa) (**Panels B,D**), the expression ratio of HSP90/HSP70 (90 kDa / 70

kDa) (**Panels B,E**), ANNEXIN A1 (35 kDa) (**Panels B,F**), Fyn (59 kDa) (**Panels B,G**), the activation of GSK-3 β (ratio of p[Tyr216]GSK-3 β /GSK-3 β total, 46kDa) (**Panels H,I**), the Cdk5 (30 kDa) (**Panels H,J**) pathways, the expression ratio of p25/p35 (25 & 35 kDa) (**Panels H,K**) were evaluated in each group and normalized with the variations of β -tubulin (β -tub, 55 kDa). For experimental protocol see **Fig.1A**. Two-way ANOVA: **GR**: $F_{2,30}= 3.71$ for group, $p < 0.05$; $F_{1,30}= 20.8$ for treatment, $p < 0.001$; and $F_{2,30}= 2.63$ for interaction, ns; **pGR**: $F_{2,30}= 4.48$ for group, $p < 0.05$; $F_{1,30}= 21.5$ for treatment, $p < 0.0001$; and $F_{2,30}= 3.98$ for interaction, $p < 0.05$; **HSP90/HSP70**: $F_{2,30}= 10.7$ for group, $p < 0.001$; $F_{1,30}= 7.23$ for treatment, $p < 0.001$; and $F_{2,30}= 0.53$ for interaction, ns; **Annexin A1**: $F_{2,30}= 17.4$ for group, $p < 0.0001$; $F_{1,30}= 43.8$ for treatment, $p < 0.0001$; and $F_{2,30}= 1.06$ for interaction, ns; **Fyn**: $F_{2,30}= 11.1$ for group, $p < 0.001$; $F_{1,30}= 15.9$ for treatment, $p < 0.001$; and $F_{2,30}= 0.83$ for interaction, ns; **p[Tyr216]GSK-3 β /GSK-3 β** : $F_{2,30}= 18.4$ for group, $p < 0.0001$; $F_{1,30}= 13.8$ for treatment, $p < 0.001$; and $F_{2,30}= 1.95$ for interaction, ns; **Cdk5**: $F_{2,30}= 3.38$ for group, $p < 0.05$; $F_{1,30}= 40.3$ for treatment, $p < 0.0001$; and $F_{2,30}= 2.75$ for interaction, ns; **p25/p35**: $F_{2,30}= 19.5$ for group, $p < 0.0001$; $F_{1,30}= 56.5$ for treatment, $p < 0.0001$; and $F_{2,30}= 1.21$ for interaction, ns.

Data are expressed as means \pm SEM in % of naive rats. * $p < 0.05$ and ** $p < 0.01$ vs. respective group treated with vehicle. + $p < 0.05$ and ++ $p < 0.01$ vs. respective control (C) group. \emptyset $p < 0.05$ and $\emptyset\emptyset$ $p < 0.01$ vs. respective scrambled (S) group, $\blacklozenge\blacklozenge$ $p < 0.01$ vs. selected group.

Fig. 3

The cumulative effects in the hippocampus of chronic corticosterone consumption and oA β_{25-35} injection on APP processing (**Panels A**), were evaluated by Western blot. Variations of the expression of APP (120 kDa) (**Panels B,C**), C99 (13 kDa) (**Panels B,D**), BACE1 (β -secretase, 70 kDa) (**Panels B,E**), PS1 (γ -secretase, 20 kDa) (**Panels B,F**), sAPP α (100 kDa) (**Panels B,G**), Adam10 (α -secretase, 70 kDa) (**Panels B-H**), IDE (110 kDa) (**Panels B,I**) were evaluated in each group and normalized with the variations of β -tubulin (β -tub, 55 kDa). For experimental protocol see Fig.1A. Two-way ANOVA: **APP**: $F_{2,30}= 5.14$ for group, $p < 0.01$; $F_{1,30}= 19.5$ for treatment, $p < 0.0001$; and $F_{2,30}= 5.98$ for interaction, $p < 0.01$; **C99**: $F_{2,30}= 11.6$ for group, $p < 0.001$; $F_{1,30}= 5.34$ for treatment, $p < 0.05$; and $F_{2,30}= 5.48$ for interaction, $p < 0.01$; **BACE1**: $F_{2,30}= 16.7$ for group, $p < 0.0001$; $F_{1,30}= 41.3$ for treatment, $p < 0.0001$; and $F_{2,30}= 0.34$ for interaction, ns; **PS1**: $F_{2,30}= 7.85$ for group, $p < 0.01$; $F_{1,30}= 8.75$ for treatment, $p < 0.01$; and $F_{2,30}= 2.63$ for interaction, ns; **sAPP α** : $F_{2,27}= 9.56$ for group, $p < 0.001$; $F_{1,27}= 35.8$ for treatment, $p < 0.0001$; and $F_{2,27}= 18.5$ for interaction, $p < 0.0001$; **ADAM10**: $F_{2,30}= 9.52$ for group, $p < 0.001$; $F_{1,30}= 5.81$ for treatment, $p < 0.05$; and

$F_{2,30}= 1.38$ for interaction, ns; **IDE**: $F_{2,30}= 3.14$ for group, $p < 0.05$; $F_{1,30}= 14.3$ for treatment, $p < 0.001$; and $F_{2,30}= 4.54$ for interaction, $p < 0.05$. **Panel J**: Radar chart displaying the profile of APP processing (*non-amyloidogenic pathway; sAPP α , ADAM10 & IDE, vs. amyloidogenic pathway; C99, BACE1 & PS1*) occurring in each experimental condition (*control, treated with CORT, treated with oA β or treated with CORT and oA β*).

Data are expressed as means \pm SEM in % of naive rats. * $p < 0.05$ and ** $p < 0.01$ vs. respective group treated with vehicle. + $p < 0.05$ and ++ $p < 0.01$ vs. respective control (C) group. \emptyset $p < 0.05$ and $\emptyset\emptyset$ $p < 0.01$ vs. respective scrambled (S) group, \blacklozenge $p < 0.05$ and $\blacklozenge\blacklozenge$ $p < 0.01$ vs. selected group.

Fig. 4

The cumulative effects in the hippocampus of chronic corticosterone consumption and oA β_{25-35} injection on synaptic deficits and neuroinflammation were evaluated by Western blot. Variations of the expression of PSD95 (95 kDa) (**Panels A,B**), Synaptotagmine (SYN) (65 kDa) (**Panels A,C**), GFAP (50 kDa) (**Panels A,D**), Iba1 (21 kDa) (**Panels A,E**), IL-10 (25 kDa) (**Panels A,F**) and ADAM17 (55 kDa) (**Panel A,G**) were evaluated in each group and normalized with the variations of β -tubulin (β -tub, 55 kDa). For experimental protocol see **Fig.1A**. Two-way ANOVA: **PSD95**: $F_{2,30}= 18.8$ for group, $p < 0.0001$; $F_{1,30}= 66.7$ for treatment, $p < 0.0001$; and $F_{2,30}= 0.60$ for interaction, ns; **SYN**: $F_{2,30}= 37.8$ for group, $p < 0.0001$; $F_{1,30}= 16.7$ for treatment, $p < 0.001$; and $F_{2,30}= 0.37$ for interaction, ns; **GFAP**: $F_{2,30}= 69.5$ for group, $p < 0.0001$; $F_{1,30}= 1.99$ for treatment, ns; and $F_{2,30}= 19.0$ for interaction, $p < 0.0001$; **Iba1**: $F_{2,30}= 55.9$ for group, $p < 0.0001$; $F_{1,30}= 22.9$ for treatment, $p < 0.0001$; and $F_{2,30}= 7.80$ for interaction, $p < 0.01$; **IL-10**: $F_{2,30}= 5.84$ for group, $p < 0.01$; $F_{1,30}= 1.20$ for treatment, ns; and $F_{2,30}= 100$ for interaction, $p < 0.0001$; **ADAM17**: $F_{2,30}= 67.4$ for group, $p < 0.0001$; $F_{1,30}= 6.49$ for treatment, $p < 0.05$; and $F_{2,30}= 5.43$ for interaction, $p < 0.01$. **Panel H**: Radar chart displaying the inflammatory profile (*anti-inflammatory mediators; IL-10 & ADAM-17, vs. pro-inflammatory mediators; GFAP, Iba1*) occurring in each experimental conditions (*control, treated with CORT, treated with oA β or treated with CORT and oA β*) and in relation with plasma CORT levels.

Data are expressed as means \pm SEM in % of naive rats. * $p < 0.05$ and ** $p < 0.01$ vs. respective group treated with vehicle. + $p < 0.05$ and ++ $p < 0.01$ vs. respective control (C) group. \emptyset $p < 0.05$ and $\emptyset\emptyset$ $p < 0.01$ vs. respective scrambled (S) group, $\blacklozenge\blacklozenge$ $p < 0.01$ vs. selected group.

Table 1

Effects of icv injection of oA β_{25-35} on body weights (g) of corticosterone-treated rats.

	Control groups			Scrambled groups			oA β_{25-35} groups		
	C	CV	CC	S	SV	SC	oA β	oA β V	oA β C
Before treatments	162 ± 4	163 ± 5	166 ± 3	171 ± 3	172 ± 8	167 ± 3	168 ± 4	168 ± 3	165 ± 4
After CORT treatment (4 weeks)	370 ± 10	362 ± 14	336 ± 9	371 ± 11	358 ± 13	329 ± 5	352 ± 6	343 ± 7	319 ± 15
1 week after oA β_{25-35} icv injection (5 weeks)	392 ± 12	371 ± 15	332 ± 10 **/+	372 ± 12	362 ± 10	313 ± 5 **/++	325 ± 4	324 ± 6	292 ± 11 **/+◇
N	8	7	8	8	8	8	8	9	9

The data are shown mean ± SEM and analyzed using a two-way ANOVA: Before treatment, $F_{2,64} = 0.09$; $p > 0.05$ for CORT treatment, $F_{2,64} = 1.09$, $p > 0.05$ for groups and $F_{4,64} = 0.28$, $p > 0.05$ for interaction. After CORT treatment, $F_{2,64} = 2.76$; $p > 0.05$ for CORT treatment, $F_{2,64} = 2.53$, $p > 0.05$ for groups and $F_{4,64} = 0.06$, $p > 0.05$ for interaction. After oA β_{25-35} icv injection, $F_{2,64} = 22.6$; $p < 0.01$ for CORT treatment, $F_{2,64} = 21.5$, $p < 0.01$ for groups and $F_{4,64} = 0.87$, $p > 0.05$ for interaction. ** $p < 0.01$ vs. respective control rats. + $p < 0.05$ and ++ $p < 0.01$ vs. respective vehicle injected rats. ◇ $p < 0.05$ vs. control and scrambled rats treated with CORT. **N**: number of rats. **C**: control; **CV**: control + vehicle (β -CD 0.45%); **CC**: control + CORT (10 mg/kg/day); **S**: A β_{25-35} scrambled (10 μ g/rat icv); **SV**: scrambled + vehicle; **SC**: scrambled + CORT; **oA β** : oA β_{25-35} (10 μ g/rat icv); **oA β V**: oA β_{25-35} + vehicle and **oA β C**: oA β_{25-35} + CORT.

FIGURE 1

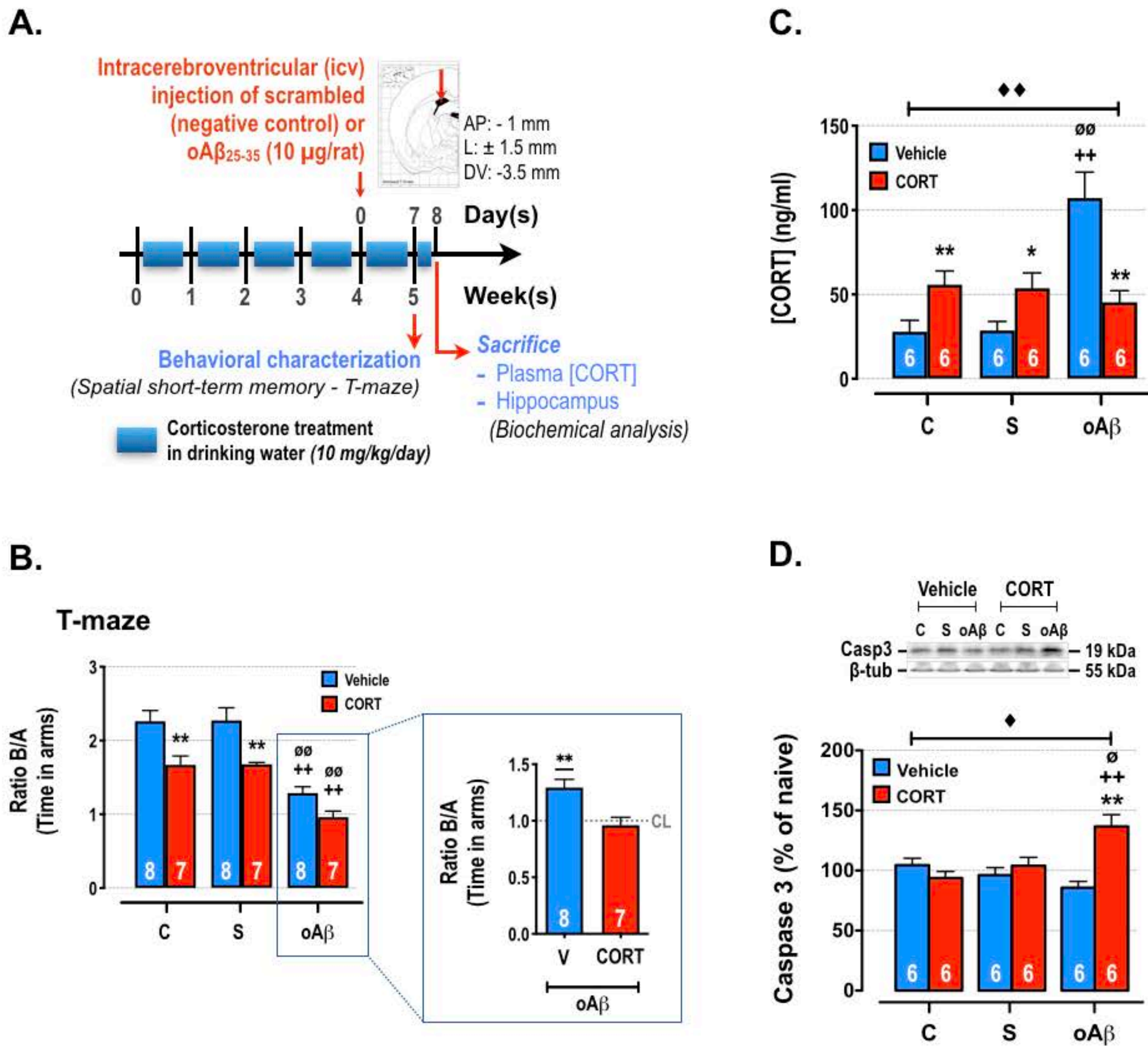


FIGURE 2

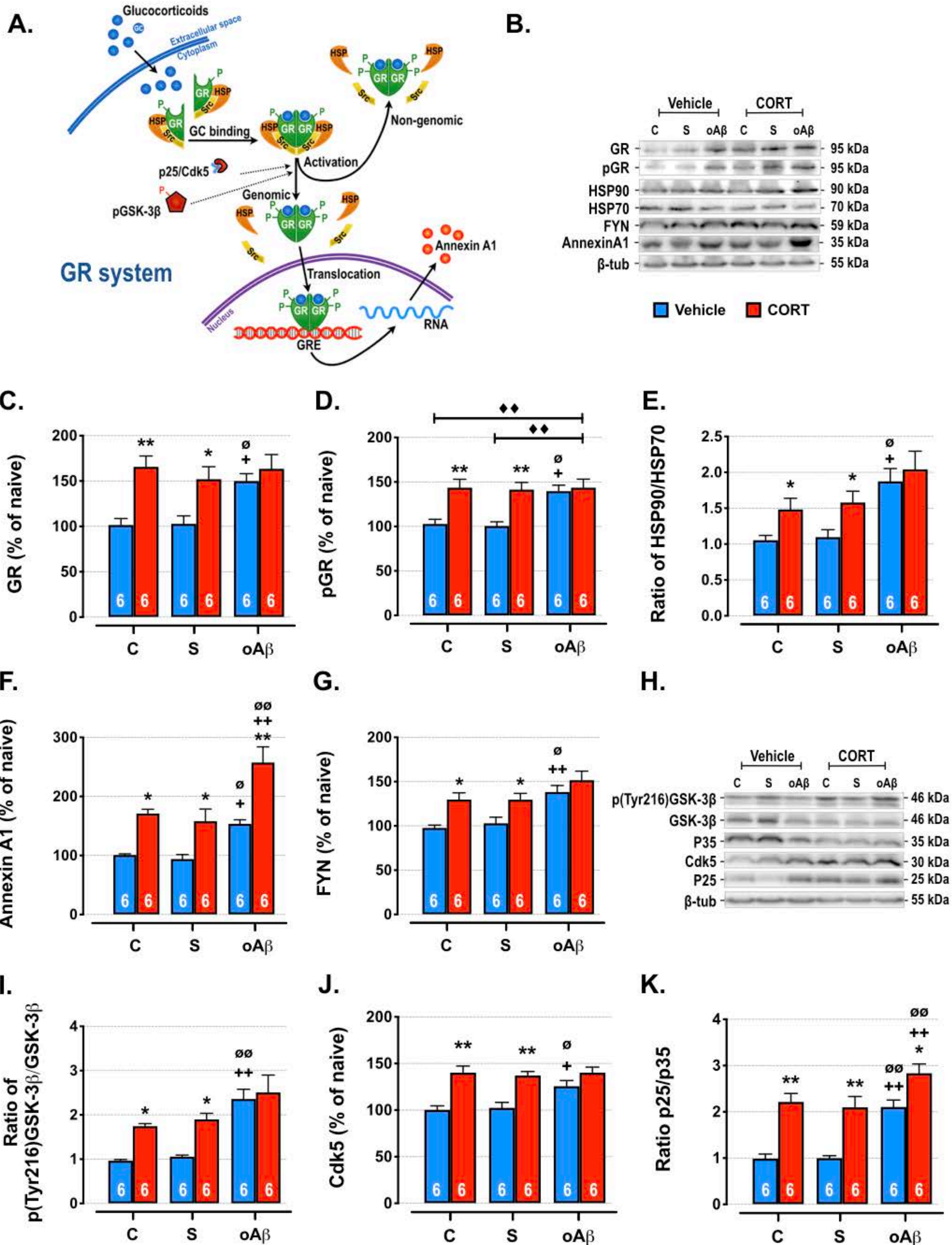


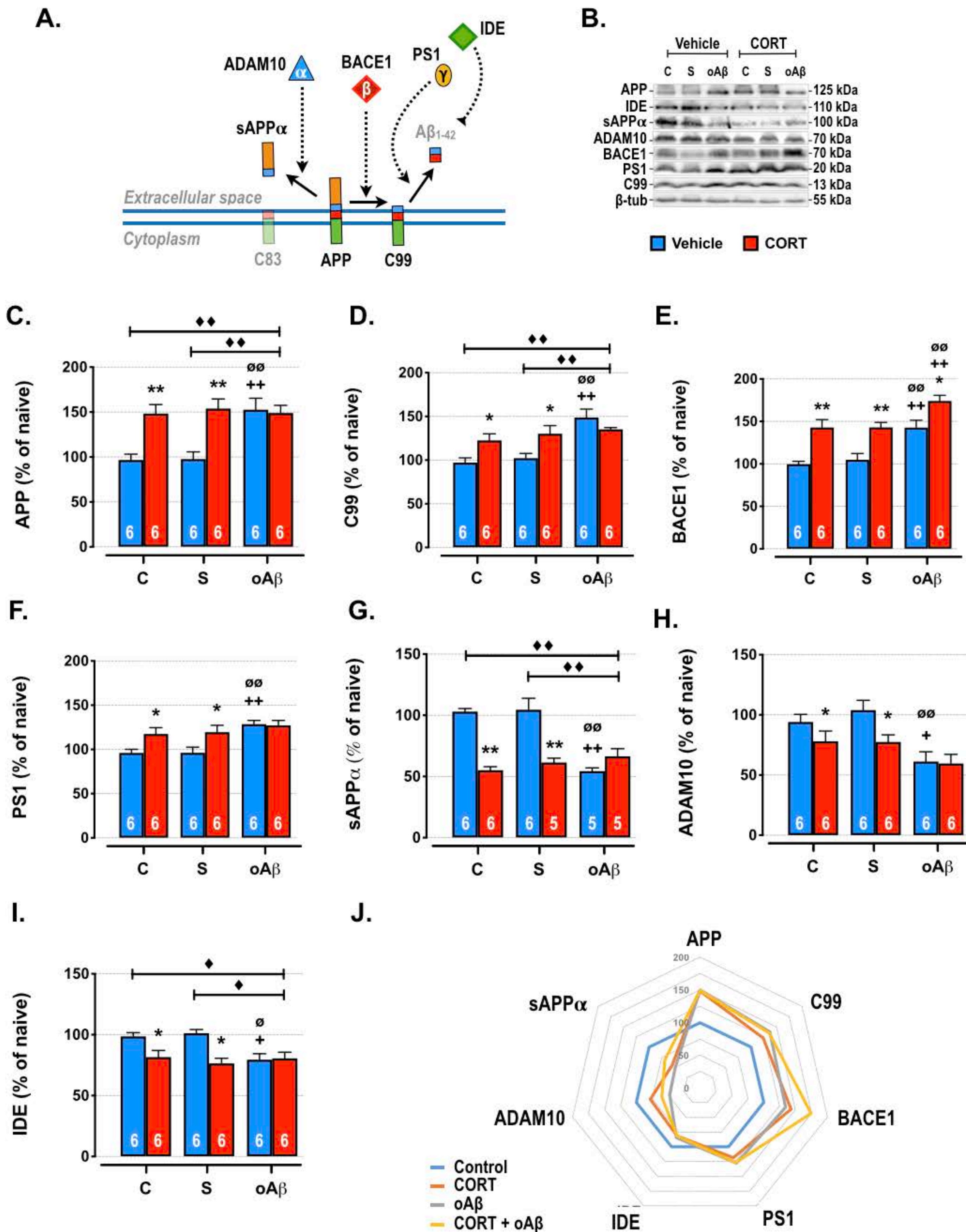
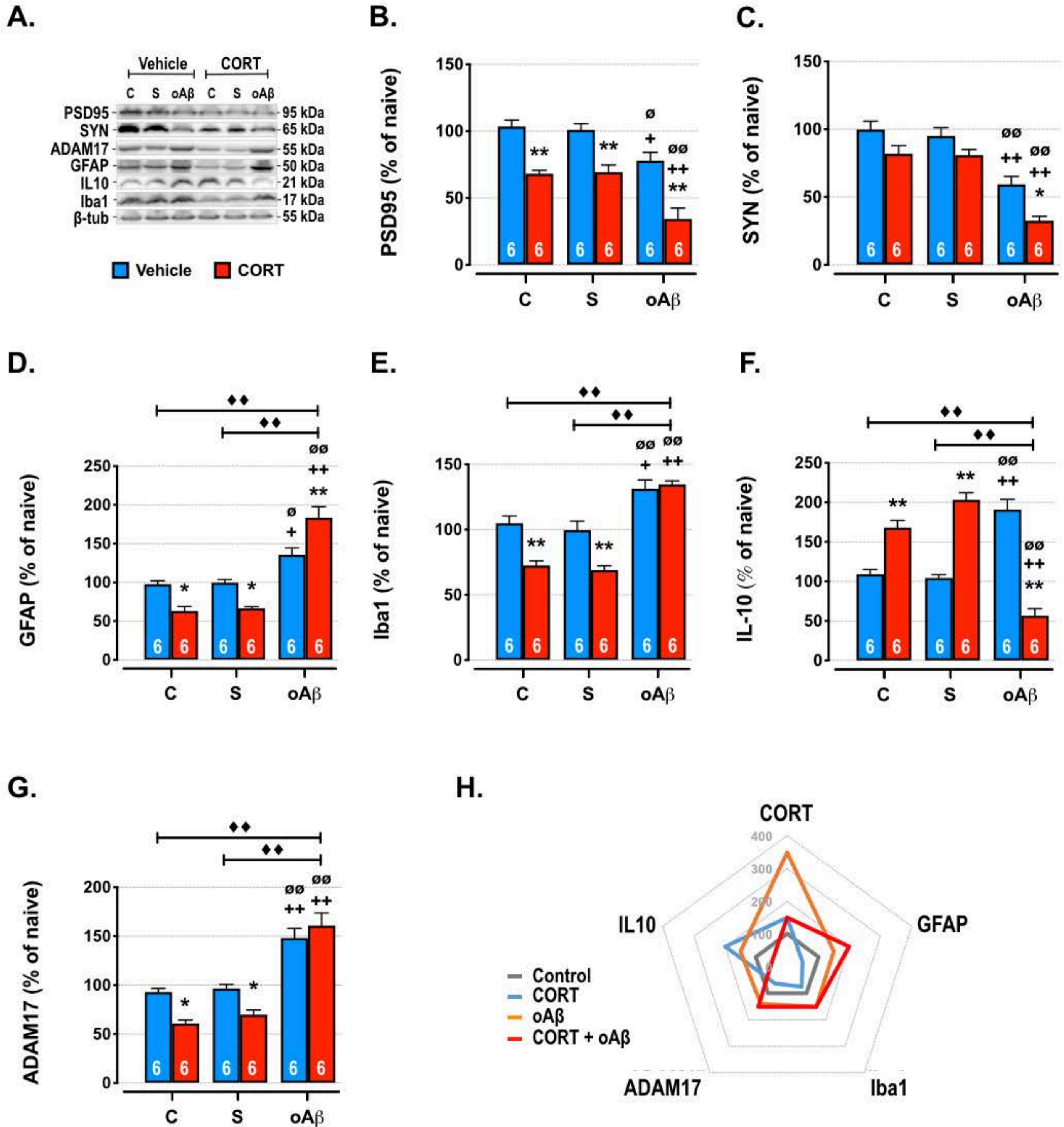
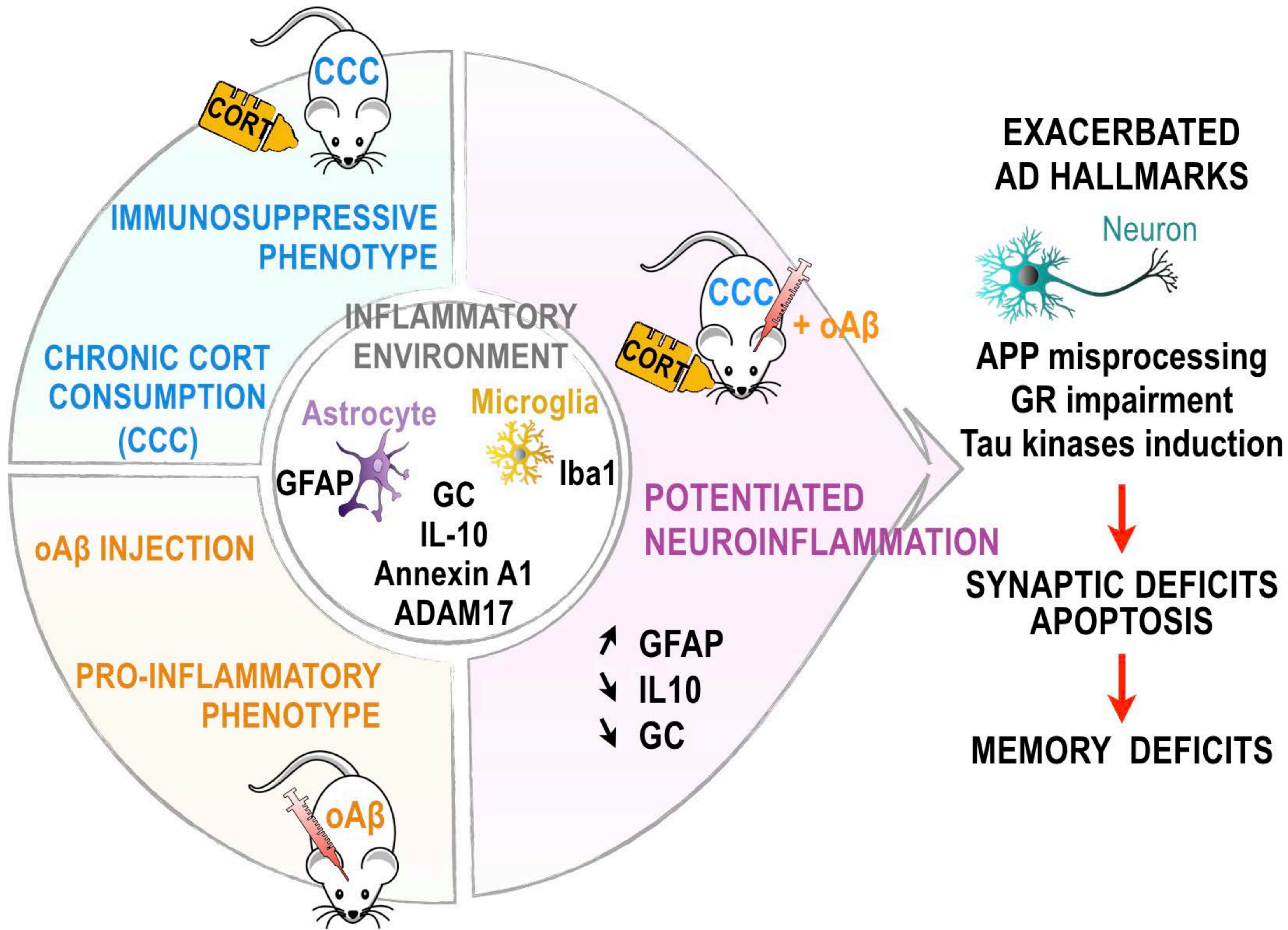
FIGURE 3

FIGURE 4



GRAPHICAL ABSTRACT



SUPPLEMENTARY LEGENDS

Sup-Fig. 1

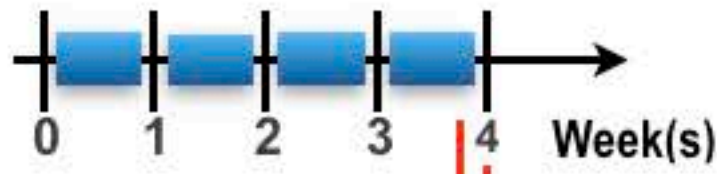
Validation of the chronic corticosterone consumption model. **Panel A:** Experimental protocol - At T0, adult male rats (Sprague Dawley) were chronically treated with corticosterone in drinking water (at 1, 5 or 10 mg/kg/day, dissolved in 0,45% β -cyclodextrine) for 4 weeks). One group did not receive any treatment (control rats, white column), a second group were treated only with β -cyclodextrine (vehicle control - blue column). Two days before sacrifice, the locomotor activity and the anxious-like behavior of each rat were tested in an Open-field (day 26) and in elevated plus maze (EPM) (day 27). The following day (day 28), the animals were sacrificed, blood samples and hippocampi were rapidly collected for corticosterone assay and Western blot analysis, respectively. **Panel B:** Plasma concentrations of corticosterone (CORT) were determined by Elisa and expressed as ng/ml. One-way ANOVA: $F_{4,20} = 17.7$ $p < 0.0001$. **Panel C:** Anxious-like behavior was tested in the open-field. Each animal was placed in the center of the arena and allowed to freely explore for 10 min. Parameters analyzed: latency to start moving (sec), total immobility time (sec), number of entries in the center zone of the open-field, numbers of grooming, number of rearing, and total distance moved (m). One-way ANOVA: $F_{4,20} = 9.57$ ($p < 0.001$) for latency to start moving; $F_{4,20} = 8.33$ ($p < 0.001$) for total immobility time; $F_{4,20} = 7.44$ ($p < 0.001$) for number of entries in the center zone of the open-field; $F_{4,20} = 13.0$ ($p < 0.0001$) for numbers of grooming; $F_{4,20} = 6.48$ ($p < 0.01$) for numbers of rearing; $F_{4,20} = 0.85$ (ns) for total distance moved. **Panel D:** Anxious-like behavior was determined in the EPM paradigm. Each animal was placed at the center of the EPM and allowed to freely explore for 10 min. Data were expressed as time spent in the open arms (OA) in % of total time. One-way ANOVA: $F_{4,25} = 8.77$ ($p < 0.001$).

Data are expressed as means \pm SEM. * $p < 0.05$ and ** $p < 0.01$ vs. control (C) group, + $p < 0.05$ and ++ $p < 0.01$ vs. vehicle (V) group, \emptyset $p < 0.05$ and $\emptyset\emptyset$ $p < 0.01$ vs. selected group.

SUPPLEMENTARY FIGURE 1

A.

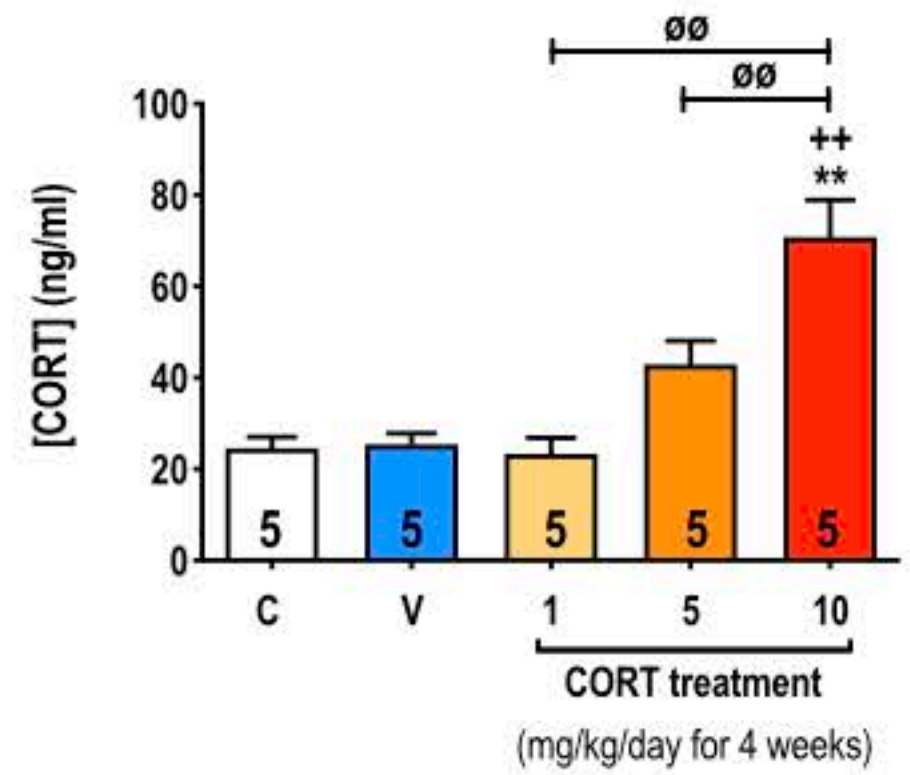
Corticosterone treatment
in drinking water (1, 5 or 10 mg/kg/day)



Behavioral characterization
(Open-field / Elevated Plus Maze)

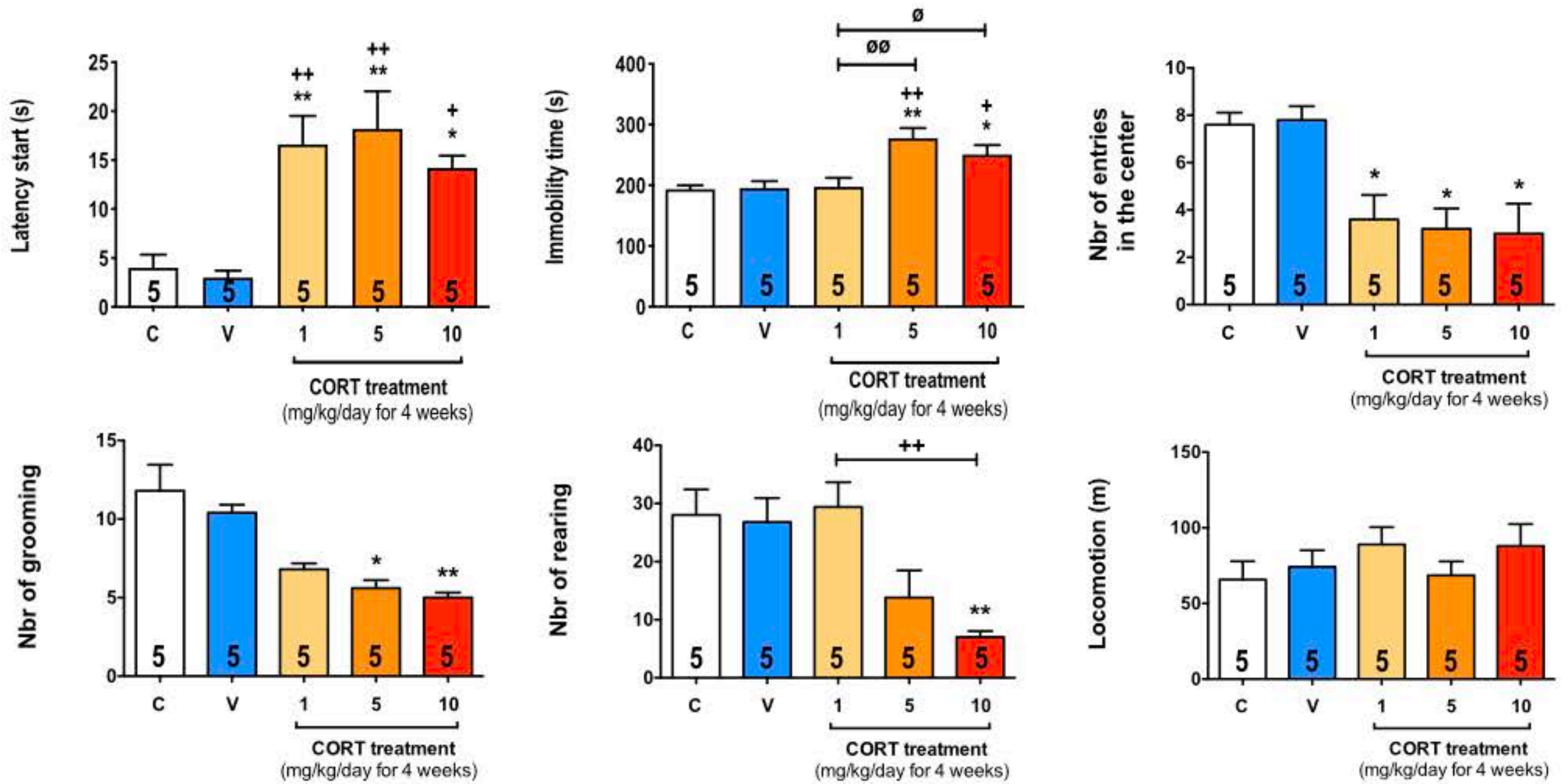
Sacrifice
Plasma [CORT]

B.



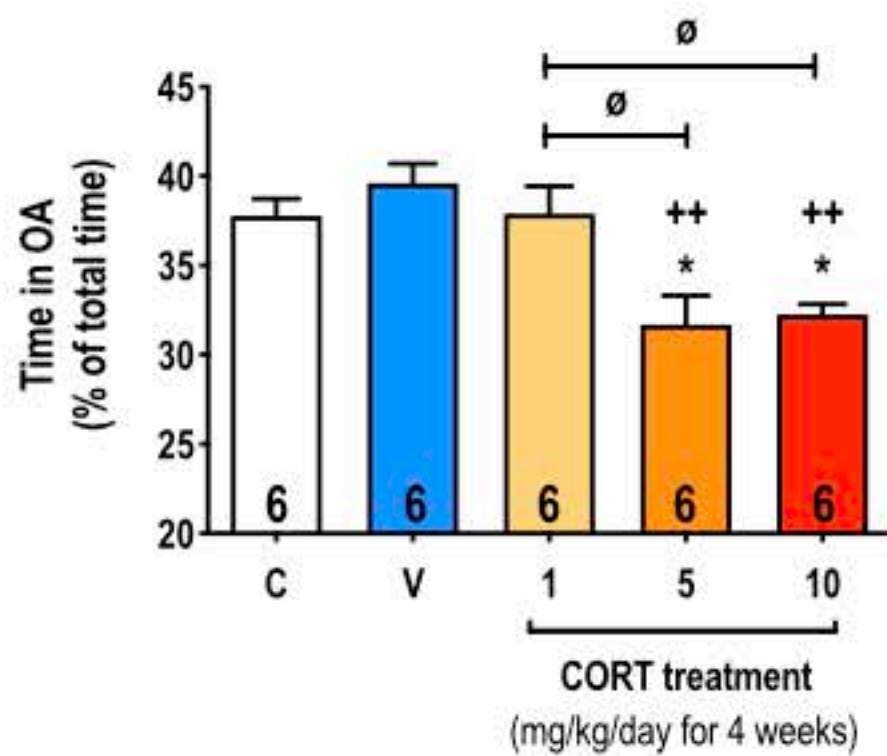
C.

Open-field



D.

Elevated plus maze



Supplementary Table 1

Body, adrenal and pituitary weights of corticosterone-treated rats.

	Control	Vehicle (β -CD)	CORT (mg/kg/day – 4 weeks)			Anova (one way)
			1	5	10	
Body weight (g)						
<i>Before treatment</i>	130 \pm 2	132 \pm 1	131 \pm 2	129 \pm 2	130 \pm 2	$F_{4,20} = 0.14$; $p > 0.05$
<i>After 1 week</i>	188 \pm 4	190 \pm 1	194 \pm 6	193 \pm 5	186 \pm 6	$F_{4,20} = 0.47$; $p > 0.05$
<i>After 2 weeks</i>	237 \pm 5	235 \pm 6	246 \pm 5	240 \pm 7	233 \pm 5	$F_{4,20} = 0.77$; $p > 0.05$
<i>After 3 weeks</i>	284 \pm 4	279 \pm 5	296 \pm 5	286 \pm 10	283 \pm 8	$F_{4,20} = 0.88$; $p > 0.05$
<i>After 4 weeks</i>	332 \pm 4	323 \pm 5	348 \pm 5	332 \pm 10	318 \pm 8	$F_{4,20} = 2.66$; $p > 0.05$
Adrenal weight (mg) (after 4 weeks)	43.2 \pm 3.6	43.6 \pm 4.0	45.2 \pm 1.7	35.8 \pm 2.1 */+	34.0 \pm 2.4 **/++	$F_{4,20} = 3.14$; $p < 0.05$
Pituitary weight (mg) (after 4 weeks)	11.5 \pm 0.7	10.6 \pm 0.6	11.0 \pm 0.6	10.4 \pm 0.3	10.2 \pm 0.6	$F_{4,20} = 0.69$; $p > 0.05$
N	5	5	5	5	5	-

The data are shown mean \pm SEM. N: number of rats. * $p < 0.05$ and ** $p < 0.01$ vs. control group. + $p < 0.05$ and ++ $p < 0.01$ vs. vehicle injected group. β -CD: β -cyclodextrin; CORT: corticosterone

Supplementary Table 2

Antibodies used in Western blot experiments

Protein	Mol. weight	Antibody	Dilution	Ref.	Supplier
Primary antibodies					
ADAM10	72 kDa	Rabbit anti-ADAM10	1/1000	AB19026	Merck-Millipore, France
APP/C99	125/13 kDa	Rabbit anti-APP/C99	1/750	PA1-84165	Thermo-Fisher Scientific, France
BACE1	70 kDa	Rabbit anti-BACE	1/1000	#5606	Cell Signaling/Ozyme, St Cyr-l'Ecole, France
Caspase 3	19 kDa	Rabbit anti-caspase 3	1/500	#9665	Cell Signaling/Ozyme, France
Cdk5	30 kDa	Rabbit anti-Cdk5	1/500	#2506	Cell Signaling/Ozyme, France
Fyn	59 kDa	Rabbit anti-Fyn	1/500	#4023	Cell Signaling/Ozyme, France
GFAP	55 kDa	Mouse anti-GFAP	1/2000	G3893	Sigma-Aldrich, France
GR	95 kDa	Rabbit anti-GR	1/1000	#3660	Cell Signaling/Ozyme, France
GSK-3 β	46 kDa	Mouse anti-GSK-3 β	1/2000	610202	BD-Biosciences, Rungis, France
HSP70	70 kDa	Rabbit anti-HSP70	1/500	#4872	Cell Signaling/Ozyme, France
HSP90	90 kDa	Rabbit anti-HSP90	1/1000	#4877	Cell Signaling/Ozyme, France
Iba1	17 kDa	Rabbit anti-Iba1	1/750	013-19741	Wako Chem., Osaka, Japan
IDE	110 kDa	Rabbit anti-IDE	1/3000	AB9210	Merck-Millipore, France
IL-10	21 kDa	Rabbit anti-IL10	1/500	AB192271	Abcam, Cambridge, United-Kingdom
p[Tyr216]GSK-3 β	46 kDa	Mouse anti-p[Tyr216]GSK-3 β	1/2000	612313	BD-Biosciences, France
p35/p25	35/25 kDa	Rabbit anti-p35/p25	1/500	#2680	Cell Signaling/Ozyme, France
pGR	95 kDa	Rabbit anti-p[Ser211]GR	1/1000	#4161	Cell Signaling/Ozyme, France
PS1	22 kDa	Rabbit anti-PS1	1/1000	#5643	Cell Signaling/Ozyme, France
PSD95	95 kDa	Rabbit anti-PSD95	1/2000	#3450	Cell Signaling/Ozyme, France
sAPP α	100 kDa	Mouse anti-sAPP α	1/50	11098	IBL, Hamburg, Germany
SYN	65 kDa	Mouse anti-synaptotagmine	1/1000	MAB5200	Merck-Millipore, France
β -Tub	50 kDa	Mouse anti- β -Tubulin	1/7500	T4026	Sigma-Aldrich, France
Secondary antibodies					
IgG	Goat anti-rabbit IgG peroxidase conjugate		1/2000	A61-54	Sigma-Aldrich, France
IgG	Goat anti-mouse IgG peroxidase conjugate		1/2000	A67-82	Sigma-Aldrich, France