Steroid profiling in male Wobbler mouse, a model of amyotrophic lateral sclerosis

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The Wobbler mouse is an animal model for human motoneuron diseases, especially amyotrophic lateral sclerosis (ALS), used in the investigation of both pathology and therapeutic treatment. ALS is a fatal neurodegenerative disease, characterized by the selective and progressive death of motoneurons, leading to progressive paralysis. Previous limited studies have reported steroidal hormone dysregulation in Wobbler mouse and in ALS patients, suggesting endocrine dysfunctions which may be involved in the pathogenesis of the disease. In this study, we established a steroid profiling in brain, spinal cord, plasma, adrenal glands and testes in two months-old male Wobbler mice and their littermates by gas chromatography coupled to mass spectrometry. Our results show in Wobbler mice i) a marked upregulation of corticosterone levels in adrenal glands, plasma, spinal cord regions (cervical, thoracic, lumbar) and brain, ii) a strong decrease in testosterone levels in testis, plasma, spinal cord and brain, and iii) increased levels of progesterone and especially of its reduced metabolites 5α-dihydroprogesterone, allopregnanolone and 20α-dihydroprogesterone in brain, spinal cord and adrenal glands. Furthermore, Wobbler mice showed a hypothalamic-pituitary-gonadal hypoactivity. Interestingly, plasma concentrations of corticosterone and testosterone correlate well with their respective levels in cervical spinal cord in both control and Wobbler mice. Testosterone downregulation is probably the consequence of adrenal hyperactivity and the upregulation of progesterone and its reduced metabolites may correspond to an endogenous protective mechanism in response to motoneuron degeneration. Our findings suggest that increased levels of corticosterone and decreased levels of testosterone in plasma could be a signature of motoneuron degeneration.

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Abbreviations: 3α-HSD: 3α-hydroxysteroid dehydrogenase, 3β-HSD: 3β-hydroxysteroid dehydrogenase, 5α-DHPROG: 5α-dihydroprogesterone, 5α-DHT: 5α-dihydrotestosterone, 17β-HSD2: 17β-hydroxysteroid dehydrogenase type 2, 17β-HSD3: 17β-hydroxysteroid dehydrogenase type 3, 20α-DHPROG: 20α-dihydroprogesterone, 20α-HSD: 20α-hydroxysteroid dehydrogenase, ADIONE: androstenedione, ALS: amyotrophic lateral sclerosis, AR: androgen receptor, ASAH1: acid ceramidase, CNS: central nervous system, DHEA: dehydroepiandrosterone, DOC: 11-deoxycorticosterone, GR: glucocorticoid receptor, GC/MS: gas chromatography/mass spectrometry, GnRH: gonadotropin-releasing hormone, HFB: heptafluorobutyrate derivative, HPA: hypothalamo-pituitary-adrenal axis, HPG: hypothalamo-pituitary-gonadal axis, HPLC: high performance liquid chromatography, LH: luteinizing hormone, P450c21: 21-hydroxylase, P450c11β: 11β-hydroxylase, P450c17: 17α-hydroxylase/17, 20 lyase, P450scc: cholesterol side-chain cleavage, PR: PROG receptor, PREG: pregnenolone, PROG: progesterone, SDR: short-chain dehydrogenases/reductases, SPE: solid phase extraction, TMS: trimethylsilyl derivative.

ALS is the most common motoneuron disease in adults affecting motor cortex and spinal cord (1). It is a progressive and fatal disease leading to death within 3–5 years after the onset of symptoms resulting from the failure of respiratory muscles. The main ALS symptoms are spasticity, hyperreflexia, weakness, paralysis and muscle atrophy. Men have a slightly more elevated risk (3:2) to develop ALS compared to women (2), and the sporadic form represents the large majority of cases. Understanding the pathological mechanisms and the cellular and molecular processes occurring in ALS is a prerequisite for developing treatments. This can be accomplished by using and investigating animal models that mimic the pathophysiology of the disease. The Wobbler mouse has been used as an animal model of human motoneuron diseases, especially the sporadic form of ALS, and has contributed to a better understanding of this complex disease (3, 4). The Wobbler phenotype is caused by a spontaneous autosomal recessive mutation in the gene encoding the vacuolar-vesicular protein sorting factor Vps54 that is a component of the Golgi-associated retrograde protein (GARP) complex (5). However, the Wobbler point mutation is not a complete loss-of-function and the Vps54-null mutation causes embryonic lethality. This partial mutation destabilizes the Vps54 protein and GARP and leads to retrograde vesicle transport impairments, but the link between GARP dysfunction and motoneuron degeneration is not clear. Recent data by Dennis and Citron greatly support the value of Wobblers for ALS research (6). These authors have demonstrated that Wobbler motoneurons suffer a relocation of TDP-43 (nuclear transactive DNA-binding protein) from the nuclear to the cytoplasmic compartment and changes of ubiquitination commonly found in motoneurons from patients with sporadic forms of ALS.

The disease may be already present in 4-days old Wobbler mice (difficulty in righting and astrogliosis in spinal cord) but it is generally admitted that the presymptomatic phase comprises the first 3 weeks of postnatal life during which the Wobbler mice do not present the clinical signs of the disease. The symptoms develop especially in the evolutionary phase between 3 weeks and 2–3 months including reduced body weight and size, motor defects, muscle atrophy and weakness. Degeneration of spinal cord and brainstem motoneurons, astrogliosis, microgliosis (7), decreased GABAergic inhibition (8), defects in spermiogenesis and male infertility (9) are the main features. The degeneration of motoneurons in Wobbler mice shows most of the characteristics of ALS, such as mitochondrial dysfunction, transport defects, protein aggregation and cortical hyperexcitability. After 3 months, there is a stabilization of the clinical symptoms.

The higher incidence of ALS in men compared to women raises the hypothesis of an involvement of sex steroid hormones in the etiopathogenesis of this disorder. Furthermore, lower plasma testosterone levels (10) and high levels of plasma cortisol were reported in ALS patients (11, 12) as well as hypercorticosteronemia in Wobbler (13) and in $SOD1^{G93A}$ transgenic mice (14), another experimental model of familial ALS. All these observations suggest that endocrine impairment and dysregulation of steroidogenesis could be involved in the pathogenesis of ALS. However, in these studies, steroid measurements were performed only in plasma by using indirect radioimmunoassays, which are limited by their specificity and by the analysis of a single steroid.

Gonads and adrenal glands are the major endocrine sites of steroidogenesis. Steroids are secreted in the blood and reach target tissues where they regulate different functions. Brain and spinal cord are both sources and targets of steroids. **Figure 1** gives an overview of the major steroidogenic pathways in mice involving a set of metabolizing enzymes giving arise to active steroid hormones. Some pathways are common to different steroidogenic tissues while others are tissue-specific (15). The first step in the synthesis of all steroids is the conversion of cholesterol to pregnenolone (PREG) by the P450scc enzyme. This step is rate-limiting and hormonally regulated. Steroidogenesis is continually adapted to needs. For example, we have measured increased levels of progesterone (PROG) and its metabolite 5α-dihydroprogesterone (5α-DHPROG) in nervous tissues following brain trauma (16), spinal cord injury (17) and cerebral ischemia (18).

In the central nervous system (CNS), steroids have neuroprotective and regenerative actions after injury and during neurodegenerative diseases (4, 19). Thus, the neuroprotective effects of PROG have been shown in models of injured nervous system and in some neurodegenerative diseases (4). An apparent dichotomy between neuroprotection vs exacerbation of damage was reported for estrogens and androgens depending on the context (20, 21). Chronic elevation of glucocorticoid levels in response to stress exerts damaging effects on the brain and results in neurotransmitter dysregulation (22). Hypercorticism also accompanies many diseases, with negative consequences for nervous functions (23). In the stressed brain, glucocorticoids can exacerbate inflammation (24). In stroke, high levels of corticosterone are associated with high morbidity, impaired functional recovery and ischemic injury to neurons (25).

Because of the important role steroids play in neuron viability, the aim of this study was to establish a comparative steroid profiling in control and Wobbler male mice. We measured PREG, PROG and its reduced metabolites,

11-deoxycorticosterone (DOC) and corticosterone, dehydroepiandrosterone (DHEA), androstenedione (ADIONE), testosterone and 5α-dihydrotestosterone (5α-DHT) and 17β-estradiol in spinal cord (cervical, thoracic and lumbar regions), brain, plasma, endocrine glands (testes and adrenal glands) in order to identify changes in steroidogenic pathways in symptomatic two months-old Wobbler mice. For this purpose, we used gas chromatography/mass spectrometry (GC/MS), which is the most reliable technology for identification and quantification of steroids present in low amounts within tissues and biological fluids (26, 27).

Materials and Methods

Animals

A breeding colony of Wobbler mice is established in the animal facility at the Instituto de Biologia y Medicina Experimental. 10 breeding pairs of heterozygote male and female NFR/wr mice were used to generate wr/wr mice. For this study a total of 42 control and 35 Wobbler male mice were used. Each mouse from different litters was identified for its wobbler genotype using a validated protocol by employing an Alu I restriction polymorphism of a Cct4 amplification product for testing the allelic status at the wr locus (28) (**Supplemental Figure 1**). Cct4 diagnostic primers and restriction enzymes for genotyping were purchased from Promega. Depending on the litters, mice homozygotes for wobbler mutation represent, for example, 1/6, 2/8, 2/10, 2/12 over heterozygotes and controls. The average incidence of the wr/wr genotype in different litters represents 20%. After genotyping, mice with the desired genotype (homozygotes for Wobbler mutation and their wildtype littermates) were sacrificed and plasma and tissue were collected. Samples were frozen and stocked at −80°C. Analysis was performed when all samples were collected. Animals were housed in group cages containing two to three Wobblers and one control mouse. This social interaction plus supplementing the special nutrient prolongs the life span and improves the health status of the Wobbler mice. Mice were kept under conditions of controlled humidity and temperature (22°C), with lights on from 7:00 AM– 7:00 PM and fed standard mice chow supplemented with protein, mineral, and vitamin nutrients (Ensure, Abbott, Zwolle, Holland). Animal procedures followed the guide for the Care and Use of Laboratory Animals (NIH Guide, Insituto de Biologia y Medicina Experimental Assurance Certificate n° # A5072–01) and were approved by the Institute's Animal Care and Use Committee. All experiments were performed following the ARRIVE guidelines (www.nc3rs.org.uk).

Samples for steroid measurements

Two months-old genotyped symptomatic Wobbler male mice (n = 24) showing ambulatory difficulties, muscle atrophy and forelimb flexion, and control mice (n = 26) were killed by decapitation in the time range 10.00 AM - 12.00 AM, corresponding to the nadir levels of corticosterone in rodents and to avoid any potential circadian effects. For each mouse, blood sample was taken and the brain, spinal cord, testes and adrenal glands were dissected out on a bed of crushed ice. The blood was centrifuged at 3000g for 10min at 4°C, and the plasma was collected. Three segments (cervical, thoracic and lumbar) were collected from each spinal cord. Tissues samples were frozen on dry ice, weighed, and stored at −80°C until gas chromatography/mass spectrometry (GC/MS) analysis.

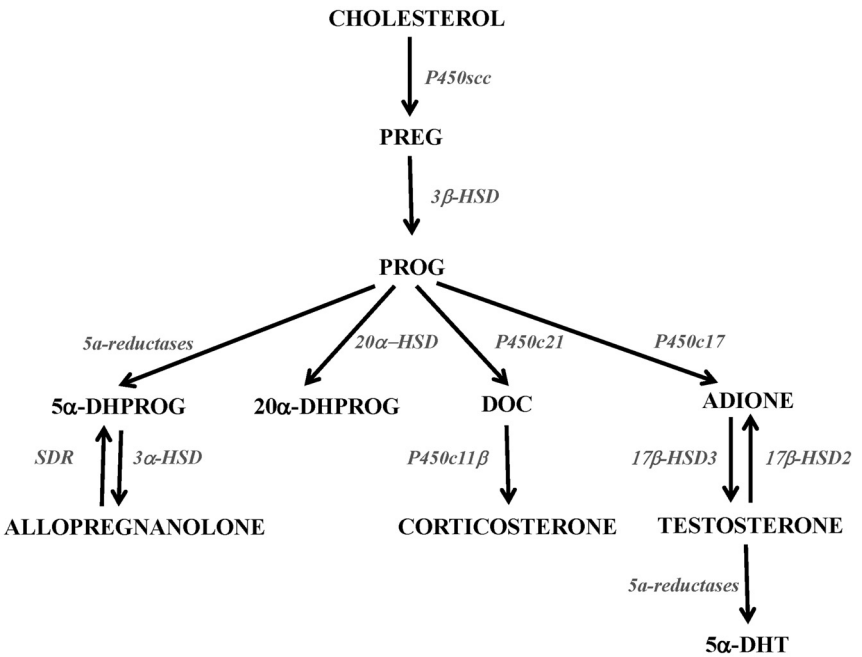

Figure 1. Main pathways of steroidogenesis in mice. Steroids are in capital letters with the following abbreviated names; PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Enzymes are indicated in italic with the following abbreviated names; P450scc: cholesterol side-chain cleavage, 3β-HSD: 3β-hydroxy-Δ5-steroid dehydrogenase, 20α-HSD: 20α-hydroxysteroid dehydrogenase, P450c21: 21-hydroxylase, P450c11β: 11β-hydroxylase; P450c17: 17α-hydroxylase/17,20 lyase, 17β-HSD3: 17β-hydroxysteroid dehydrogenase type 3, 17β-HSD2: 17β-hydroxysteroid dehydrogenase type 2, 3α-HSD: 3α-hydroxysteroid dehydrogenase, SDR: short-chain dehydrogenases/reductases.

Analysis of steroids by GC/MS

The cervical, thoracic and lumbar regions of spinal cord, brain, testes, adrenal glands and plasma from 24 Wobbler and 26 control mice were used. Samples from 2 different mice were randomly pooled to obtain sufficient amount of tissue, ie, at least 40 to 50 mg, and of plasma (at least 100 μl) to detect with high precision all the targeted steroids with a suitable signal-to-noise ratio. After combination, sample analysis was performed (n = 12 from Wobbler, n = 13 from control mice).

Steroids were extracted from tissues and plasma with 10 volumes of metha-

nol, and internal standards were added for steroid quantification: 2ng [$^2H_6$]5α-DHPROG (CDN Isotopes, Pointe Claire, Canada) for 5α-DHPROG, 2ng 19-norprogesterone (Steraloids, Newport, Rhode Island) for PROG, 20α-DHPROG and allopregnanolone, 2ng of epietiocholanolone for PREG, DHEA and 5α-DHT, 2ng [$^2H_5$]Testosterone (CDN Isotopes) for testosterone and ADIONE, 2ng [$^2H_5$]17β-estradiol (CDN Isotopes) for 17β-estradiol, 2ng [$^2H_8$]DOC for DOC (CDN Isotopes) and 10ng [$^2H_8$]corticosterone (CDN Isotopes) for corticosterone. The extraction protocol using methanol and methanol/$CHCl_3$ (1/1) has been described previously (29) and the supernatants were combined. The first purification step was performed by solid phase extraction (SPE) using C18 cartridges (500 mg, 6ml; International Sorbent Technology, Mid Glamorgan, UK). The simplified recycling/elution protocol was used to separate sulfated, unconjugated and fatty acid esters of steroids without cross-contamination (29). Briefly, sample extracts were dissolved in 1ml methanol and applied to the C18 cartridge followed by 5ml of methanol/water (85/15). The flow-through was collected and dried. After a previous reconditioning of the same cartridge with 5ml water, samples were dissolved in methanol/water (2/8) and reapplied. The cartridge was then washed with 5ml water and 5ml methanol/water (1/1) and unconjugated steroids were eluted with 5ml methanol/water (9/1). Then, the fraction containing unconjugated steroids was filtered and further purified and fractionated by high performance liquid chromatography (HPLC). The HPLC system consisted of a P1000XR Thermo Separation Product quaternary pump (Thermo Fisher Scientific, San Jose, CA), an AS 100XR Thermo Separation Product autoinjector and A 202 model Gilson fraction collector. Samples were dissolved in hexane/isopropanol (9/1). A Lichrosorb Diol column (25cm×4.6 mm, 5 μm) was used in a thermostated block at 30°C with a mobile phase flow of 1ml/min. The analytical column was first equilibrated in a solvent system of 90% hexane and 10% of a mixture hexane/isopropanol (85/15). The elution was first performed with 90% hexane and 10% hexane/isopropanol (85/15) for 8min and then with a linear gradient to 100% hexane/isopropanol (85/15) in 2min. This mobile phase was kept constant for 10min, after which a linear gradient to 100% methanol was applied. The column was rinsed with methanol for 15min. Three fractions were collected from the HPLC system and then derivatized: 1) 5α-DHPROG was eluted in the first fraction (3–13min) and then silylated with 50μl MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide)/NH_4I/DTE (1000/2/5), for 15min at 70°C, 2) the second fraction (13–25min) contained PROG, testosterone and their precursors and metabolites that were derivatized with 25μl heptafluorobutyric anhydride (HFBA) and 25μl anhydrous acetone for 1h at 20°C, 3) the third fraction containing corticosterone (25–33min) was derivatized with 25μl heptafluorobutyric anhydride (HFBA) and 25μl anhydrous acetone for 1h at 80°C. All the fractions were dried under a stream of N_2, and then resuspended in hexane for GC/MS analysis.

Calibration was carried out from standards and biologic samples were analyzed by GC/MS with an AS 3000 autosampler (Thermo Fisher Scientific, Waltham, MA). The Focus GC gas chromatograph was coupled with a DSQII mass spectrometer (Thermo Fisher Scientific). Injection was performed in the splitless mode at 250°C (1min splitless time), and the oven temperature of the gas chromatograph was initially maintained at 50°C for 1min, and then ramped to 200°C at 20°C/min, then to 285°C at 10°C/min, and finally ramped to 350°C at 30°C/min. The helium carrier gas flow was maintained constant at 1ml/min during the analysis. The transfer line and ionization chamber temperatures were 300°C and 220°C, respectively. Electron impact ionization was used for mass spectrometry with ionization energy of 70 eV. Steroids detection was performed in single ion monitoring mode and identification was supported by the retention time and two diagnostic ions for each steroid (**Supplemental Table 1**). Steroid quantification was based on the major diagnostic ion, known as the quantification ion. The GC/MS analytical procedure was fully validated in terms of identification, reproducibility, linearity and precision in the nervous tissue and plasma of rats (16, 17, 26), mice (18) and humans (30).

Gonadotropin-releasing hormone (GnRH) protein content determination

For hypothalamic GnRH protein content, the hypothalamus of control (n = 12) and Wobbler (n = 7) mice was excised and rapidly frozen. Tissues were processed as previously described. Briefly, tissues were homogenized in 200μl ice-cold 0.1N HCl, the homogenate was centrifuged at 13000g at 4°C for 30min, and the supernatant was recovered. Samples were stored at −80°C until assayed for GnRH by RIA. GnRH assay sensitivity was 9.8pg. Intra- and interassay coefficients of variation were 7.1 and 11.6%, respectively (31).

Luteinizing hormone (LH) determination

Trunk blood of control (n = 12) and Wobbler (n = 7) mice was collected and sera were obtained and frozen for hormone determinations. LH protein levels were determined by RIA with kits from NHPP, NIDDK & Dr. Parlow. Results were expressed in terms of RP3 rat LH standards, as these systems recognize mouse samples. Assay sensitivity: 0.11ng/ml. Intra- and interassay coefficients of variation were 7.2 and 11.4% (31).

Statistics

All data are presented as means ± SEM. Student's *t* test were used for steroid levels comparison in brain, plasma, adrenal glands and testes, and for hypothalamic GnRH contents, serum LH levels and weight measurements in Wobbler and control mice. Two-way ANOVA (genotype x region) followed by Bonferroni post hoc tests were used for the comparison of steroid levels between spinal cord regions in Wobbler and their littermates. Pearson's correlation was used to analyze associations between steroid concentrations in plasma, and cervical spinal cord and brain in Wobbler and control mice. In all cases, P values < 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 4 (GraphPad Software, Inc., La Jolla, USA).

Results

Two months-old Wobbler male mice exhibited a significant decrease in body weight (62% vs controls; $P < .0001$). The weights of different regions of the spinal cord, brain and testes of Wobbler mice were significantly lower in comparison to control mice. Only the weight of the

adrenal glands was similar between the two genotypes (**Table 1**).

1. Changes in steroid levels in the cervical, thoracic and lumbar spinal cord of male Wobbler mice

Steroid profiling in spinal cord regions of Wobbler and littermate male mice is summarized in **Figure 2**. Two-way ANOVA (genotype x region) revealed a significant effect of the genotype on levels of PREG ($F_{1,68}$ = 19.33; $P < .0001$), PROG ($F_{1,68}$ = 5.477; $P < .05$), 5α-DHPROG ($F_{1,68}$ = 82.2; $P < .0001$), allopregnanolone ($F_{1,51}$ = 20.02; $P < .0001$), 20α-DHPROG ($F_{1,68}$ =14.17; $P < .001$), DOC ($F_{1,69}$ = 11.05; $P < .01$), corticosterone ($F_{1,69}$ = 32.57; $P < .0001$), ADIONE ($F_{1,54}$ = 16.59; $P < .001$), testosterone ($F_{1,69}$ = 21.06; $P < .0001$) and 5α-DHT ($F_{1,68}$ = 16.58; $P < .001$) in the three regions of the spinal cord.

In the cervical region of the spinal cord, Bonferroni post hoc tests indicated higher levels of 5α-DHPROG (x2.9, $P < .001$) and corticosterone (x4.3, $P < .05$) and lower levels of testosterone (x6.6, $P < .05$) in Wobbler mice compared to controls. The concentrations of PREG (x1.5), PROG (x3.9), 20α-DHPROG (x3.4) and DOC (x3) tended to be higher and that of ADIONE (x3.7) lower in Wobbler mice but without reaching statistical significance.

In the thoracic region of spinal cord, Bonferroni post hoc tests showed a marked increase of the levels of 5α-DHPROG (x3.2, $P < .0001$), allopregnanolone (x6, $P < .01$), 20α-DHPROG (x4, $P < .01$), DOC (x2.1, $P < .05$) and corticosterone (x4.3, $P < .01$) and decreased levels of ADIONE (x2.5, $P < .01$), testosterone (x5.6, $P < .05$) and 5α-DHT (x12, $P < .05$) in Wobbler relative to control mice. Levels of PREG (x1.5) and PROG (x2) were moderately higher in Wobbler mice compared to control mice without reaching statistical significance.

Table 1. Weights of mice, brain, spinal cord regions, testis and adrenal glands of control (n = 13) and Wobbler (n = 12) male mice. Data are expressed as means ± SEM Statistical analysis: Student t test, $^{*}P < 0.05$ $^{****}P < 0.0001$

	Control	*Wobbler*
	Weight g ± SEM	
Mice	28.52 ± 0.21	10.85 ± 0.66****
	mg ± SEM	
Brain	342.4 ± 2.98	236.3 ± 3.83****
Cervical	36.38 ± 1.53	26.58 ± 3.19*
Thoracic	28.78 ± 1.33	22.18 ± 2.35*
Lumbar	41.11 ± 1.08	26.51 ± 2.15****
Testis	165.4 ± 2.53	83.44 ± 7.82****
Adrenal glands	9.15 ± 0.76	8.31 ± 0.43

In the lumbar region of spinal cord, higher levels of PREG (x1.7, $P < .01$), 5α-DHPROG (x4.8, $P < .0001$), allopregnanolone (x6, $P < .0001$) and corticosterone (x4.5, $P < .01$) and lower levels of 5α-DHT (x15, $P < .01$) were measured in Wobbler mice comparatively to control mice. As for the cervical and thoracic regions of spinal cord, increased levels of PROG (x2.1), 20α-DHPROG (x3.4) and DOC (x1.6) and decreased levels of ADIONE (x2.1) and testosterone (x11) were measured in Wobbler mice but the differences were not statistically significant.

In addition to the genotype effect, two-way ANOVA also indicated a significant region effect on the levels of PREG ($F_{2,68}$ = 5.83; $P < .01$), allopregnanolone ($F_{2,51}$ = 3.96; $P < .05$) and DOC ($F_{2,69}$ = 3.58; $P < .05$). Bonferroni post hoc tests indicated higher levels of PREG ($P < .05$) and allopregnanolone ($P < .01$) in the lumbar region comparatively to the cervical region of the spinal cord of Wobbler mice.

2. Changes in steroid levels in the brain of male Wobbler mice

Steroid profiling was also performed in the brain of Wobbler mice, characterized by motoneuron degeneration in motor cortex and brainstem.

Data reported in **Figure 3** show similar patterns of changes in steroid levels in the brain of Wobbler mice as in spinal cord regions. Student's t-tests indicated that brain concentrations of PREG (x1.6, $P < .05$), 5α-DHPROG (x4.4, $P < .0001$), 20α-DHPROG (x6.2, $P < .01$), DOC (x3.6, $P < .01$) and corticosterone (x2, $P < .01$) were higher, whereas those of androgens such as ADIONE (x6.5, $P < .001$), testosterone (x7, $P < .05$) and 5α-DHT (x5.5, $P < .05$) were lower in brain of Wobbler male mice compared to controls. Brain levels of allopregnanolone were higher in Wobbler mice (x6) albeit no significant statistical difference with controls was found due to a large variability in Wobbler mice. It is noteworthy that steroid levels were in general higher in spinal cord regions than in brain, especially in Wobbler mice.

3. Changes in steroid levels in plasma of male Wobbler mice

As for the nervous structures, higher levels 5α-DHPROG (x3.8, $P < .001$), allopregnanolone (x4, $P < .01$), 20α-DHPROG (x5.4, $P < .01$), DOC (x4, $P < .01$) and corticosterone (x3.5, $P < .01$) were measured in plasma of Wobbler mice comparatively to controls. Levels of PROG (x2.8, $P = .055$) tended to be higher in Wobbler mice. Conversely, lower levels of ADIONE (x5.4, $P < .01$) and testosterone (x14, $P < .05$) were measured in plasma of Wobbler mice. Levels of 5α-DHT (x2) also tended to be

lower but the difference did not reach statistical significance (**Figure 4**).

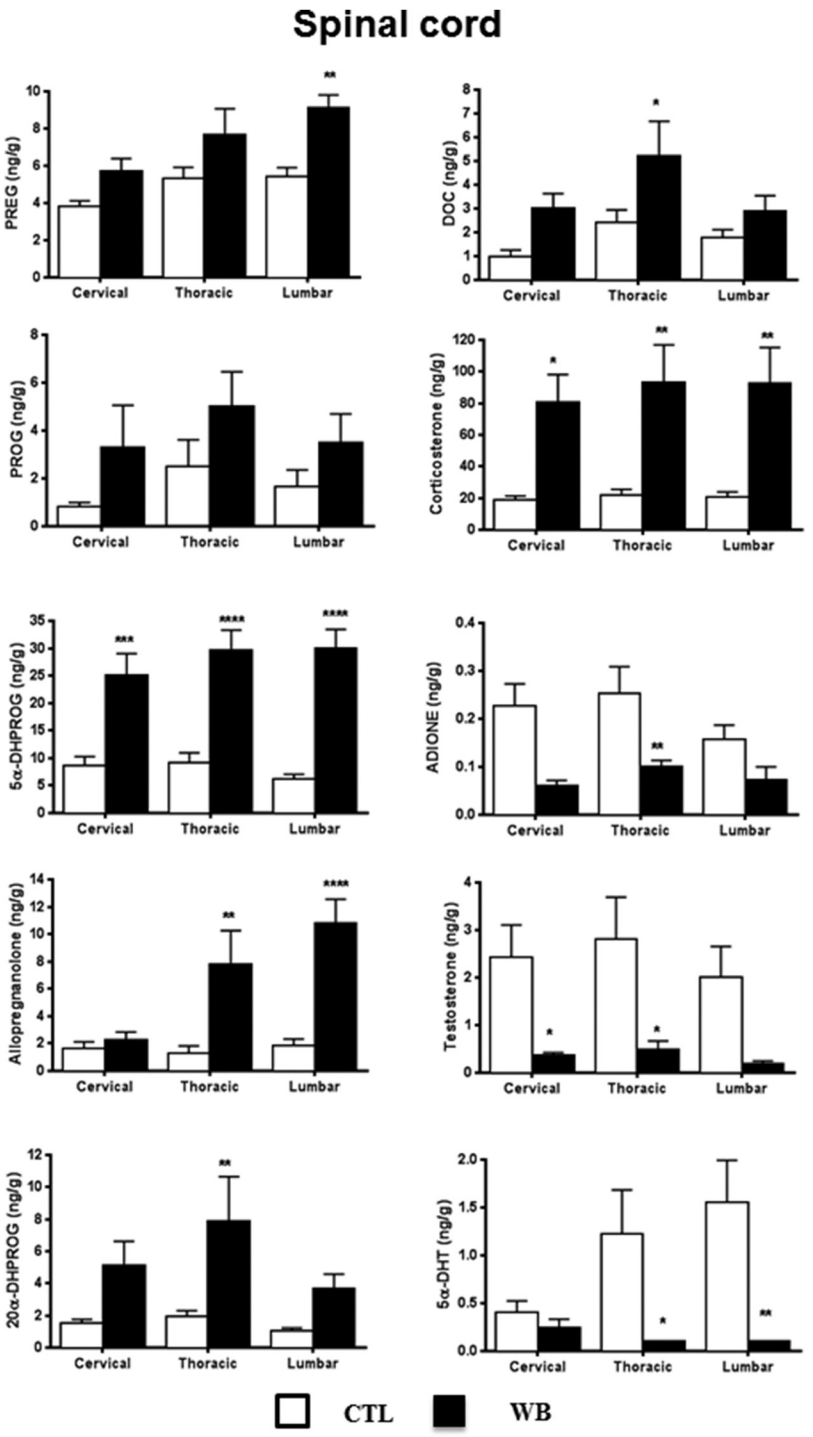

Figure 2. Steroid profiling in cervical, thoracic and lumbar regions of spinal cord of control and Wobbler male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Steroid concentrations are expressed as ng/g ± SEM. *White bars*: control mice (CTL, n = 13), *black bars*: Wobbler mice (WB, n = 12). Statistical analysis: two-way ANOVA (genotype x spinal cord region) followed by Bonferroni post hoc tests. *, $P < .05$; **, $P < .01$, ***, $P < .001$, ****, $P < .0001$.

4. Dysregulation of the hypothalamo-pituitary-gonadal (HPG) axis in male Wobbler mice

The steroid status was evaluated in testis to assess a possible impairment of testosterone biosynthesis in Leydig cells in Wobbler mice. **Figure 5** shows clearly that testosterone synthesis is impaired in Wobbler mice. Testosterone concentrations were 11-fold lower in Wobbler testis ($P < .05$) as well as its precursor ADIONE (x8, $P < .01$) and metabolite 5α-DHT (x7, $P < .05$) comparatively to control mice. Conversely, corticosterone levels were higher in testis of Wobbler ($P < .01$) as compared to control mice. PROG, 5α-DHPROG and 20α-DHPROG levels were also increased in testis of Wobbler mice (x5, x6 and x3-fold, respectively), without however reaching statistical significance.

In order to go further in the analysis of testosterone synthesis deficiency, the levels of GnRH in hypothalamus and of the LH gonadotropin in serum were also measured. Our data show a reduction in levels of GnRH (54.6 ± 7.5pg/mg in Wobblers (n = 7) vs 107.6 ± 7.57pg/mg in controls (n = 12); $P < .01$) and LH (0.44 ± 0.12ng/ml in Wobblers (n = 11) vs 1.14 ± 0.23ng/ml in controls (n = 18); $P < .05$) in Wobbler mice. The histological analysis of testis showed deficits in spermiogenesis, gaps between Sertoli cells and germ cells, and reduction in size of Leydig cells (**Supplemental Figure 2**).

5. Changes in steroid levels in the adrenal glands of male Wobbler mice

Steroid measurements were also performed in adrenal glands to investigate if hypercorticosteronemia observed in CNS and plasma came from adrenal stimulation due to a dysfunction of the hypothalamic-pituitary-adrenals (HPA) axis in Wobbler mice. Steroid analysis showed a very strong stimulation of corticoste-

rone biosynthesis in adrenal glands of Wobbler mice (**Figure 6**). Levels of corticosterone rose from 12.13 μg/g in control to 60.67 μg/g (x5, $P < .01$) in Wobbler adrenal glands. These results were supported by the significantly increased levels of adrenal corticosterone precursors, PREG (x3.5, $P < .05$), PROG (x5.5, $P < .0001$) and DOC (x3, $P < .01$) as well as the PROG metabolite, 20α-DHPROG (x20, $P < .05$), in Wobbler adrenal glands comparatively to controls. Allopregnanolone levels were slightly higher (x2) in Wobbler, but this difference was not statistically significant. As for the other tissues, testosterone level was significantly diminished in Wobbler adrenals ($P < .01$). Finally, levels of 5α-DHPROG, ADIONE and 5α-DHT were similar in Wobbler relatively to control mice.

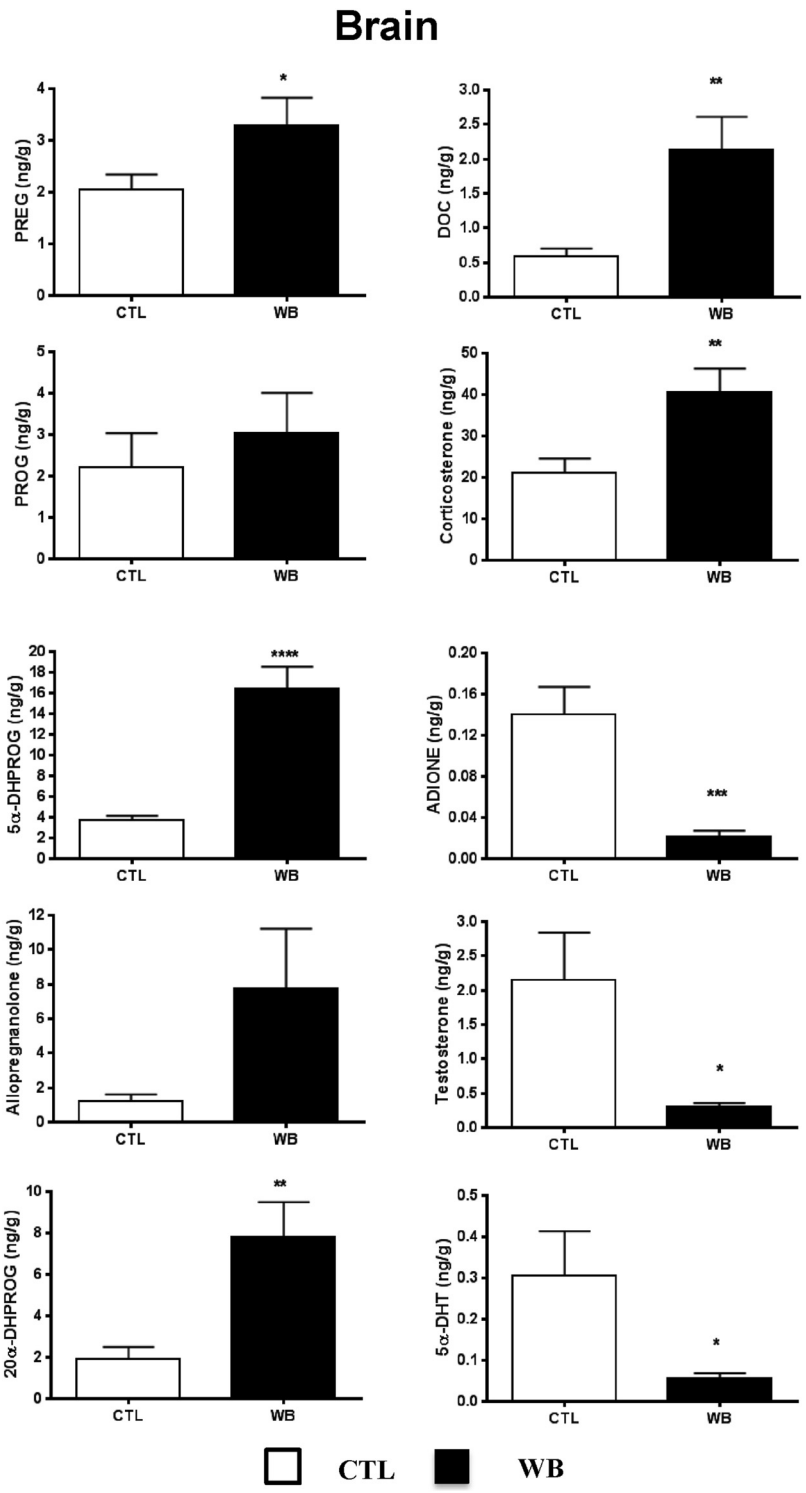

Figure 3. Steroid profiling in brain of control and Wobbler male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Steroid concentrations are expressed as ng/g ± SEM. *White bars*: control mice (CTL, n = 13), *black bars*: Wobbler mice (WB, n = 12). Statistical analysis: Student's *t* test. *, $P < .05$; **, $P < .01$, ***, $P < .001$.

The adrenal gland of Wobbler mice showed different signs of hypertrophy. Indeed, the ratio of adrenal gland weight/body weight was higher in Wobbler mice comparatively to controls $P < .001$) (**Supplemental Figure 3A**). The histological analysis of adrenal cortex revealed some foci of cells with increased size in zona Fasciculata of Wobbler mice (**Supplementary Figure 3B4 and 3C**) as compared to control mice (**Supplementary Figure 3B3 and 3C**).

DHEA and 17β-estradiol were systematically searched for, but were not detected in all the tissues investigated. The limits of detection of DHEA and 17β-estradiol were 20pg/g in tissues and 20pg/ml in plasma.

6. Correlations between steroids levels in plasma and the cervical region of the spinal cord and brain

Pearson's correlations between steroids levels in plasma and the cervical region of the spinal cord and brain were performed in order to assess if plasmatic steroids could reflect levels in the CNS, where motoneuron degeneration occurs. Strong positive correlations were found for corticosterone and testosterone between their levels in plasma and in the cervical region of the spi-

nal cord in both control and Wobbler mice (**Table 2**). The slopes of the lines were similar in controls and Wobbler mice. In addition, positive correlations were also found for PREG and 5α-DHPROG levels in plasma and cervical region of spinal cord of Wobbler mice only (**Table 2**). Pearson's correlation between steroid concentrations in plasma and brain indicated strong positive associations for 5α-DHPROG and testosterone levels in control mice and for corticosterone specifically in Wobbler mice.

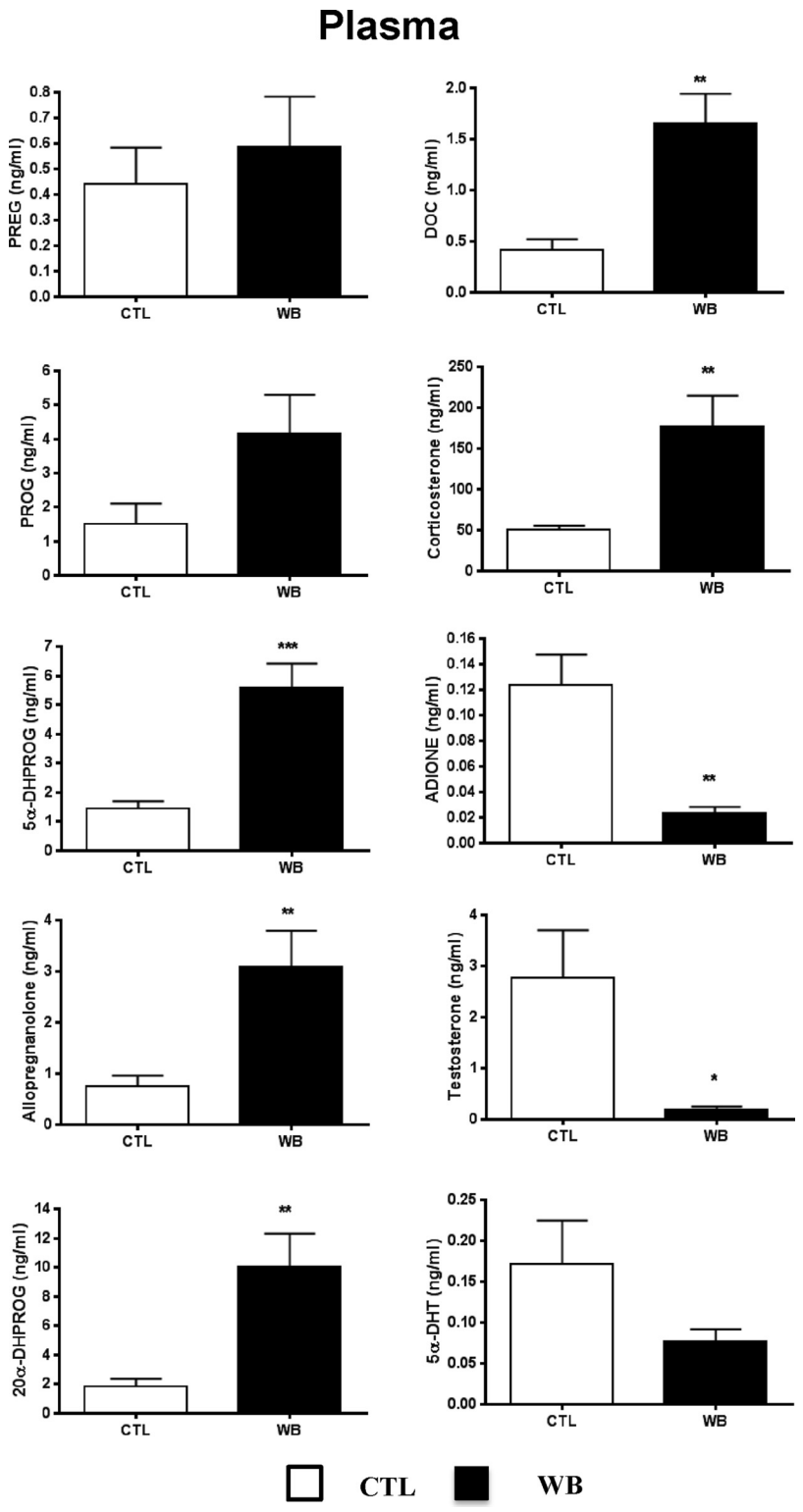

Figure 4. Steroid profiling in plasma of control and Wobbler male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Steroid concentrations are expressed as ng/ml ± SEM. *White bars*: control mice (CTL, n = 13), *black bars*: Wobbler mice (WB, n = 12). Statistical analysis: Student's *t* test. *, $P < .05$; **, $P < .01$, ***, $P < .001$.

Discussion

In this study, we have demonstrated marked changes in steroid levels in male Wobbler mice, an experimental model of ALS. Steroid profiling performed by GC/MS in brain and spinal cord regions, plasma and endocrine glands, revealed particular steroid patterns in symptomatic Wobbler mice as compared to their control littermates: upregulation of progesterone and its reduced metabolites and of corticosteroids, and downregulation of androgens. In addition, we have shown positive correlations between plasma and cervical spinal cord levels of corticosterone and testosterone both in control and Wobbler mice. Taken together, these findings suggest that increased levels of corticosterone and decreased levels of testosterone in plasma could be a signature of the motoneuron disease process.

As expected, the size and weight of Wobbler male mice were lower than their control littermates. These reductions starts at the third to the fourth week and persists to the end of the life (3, 5, 32). It has been proposed that it may partially result from nutritional deprivation, probably linked to muscle atrophy in the face and forelimbs (33). Our results suggest that the elevated endogenous levels of glucocorticosteroids may also contribute to the reduced body weight. Indeed, chronic high levels of corticoids have been shown to result

in decreased body weight (34) by decreasing food intake via inhibition of ghrelin synthesis and an increase in leptin levels (35, 36). In addition, the low levels of androgens in Wobblers may also contribute to body weight loss and a decrease in muscle strength in Wobbler mice. The high levels of corticosterone and low levels of androgens may also explain in part the decreased weights of brain and spinal cord, where motoneuron degeneration occurs, and of the testes of Wobblers characterized by impaired spermatogenesis and infertility (9). Interestingly, although not as marked as in Wobbler mice, brain volume loss has also been reported for patients with ALS, suggesting that ALS may be a degenerative disease not confined to motor systems (37).

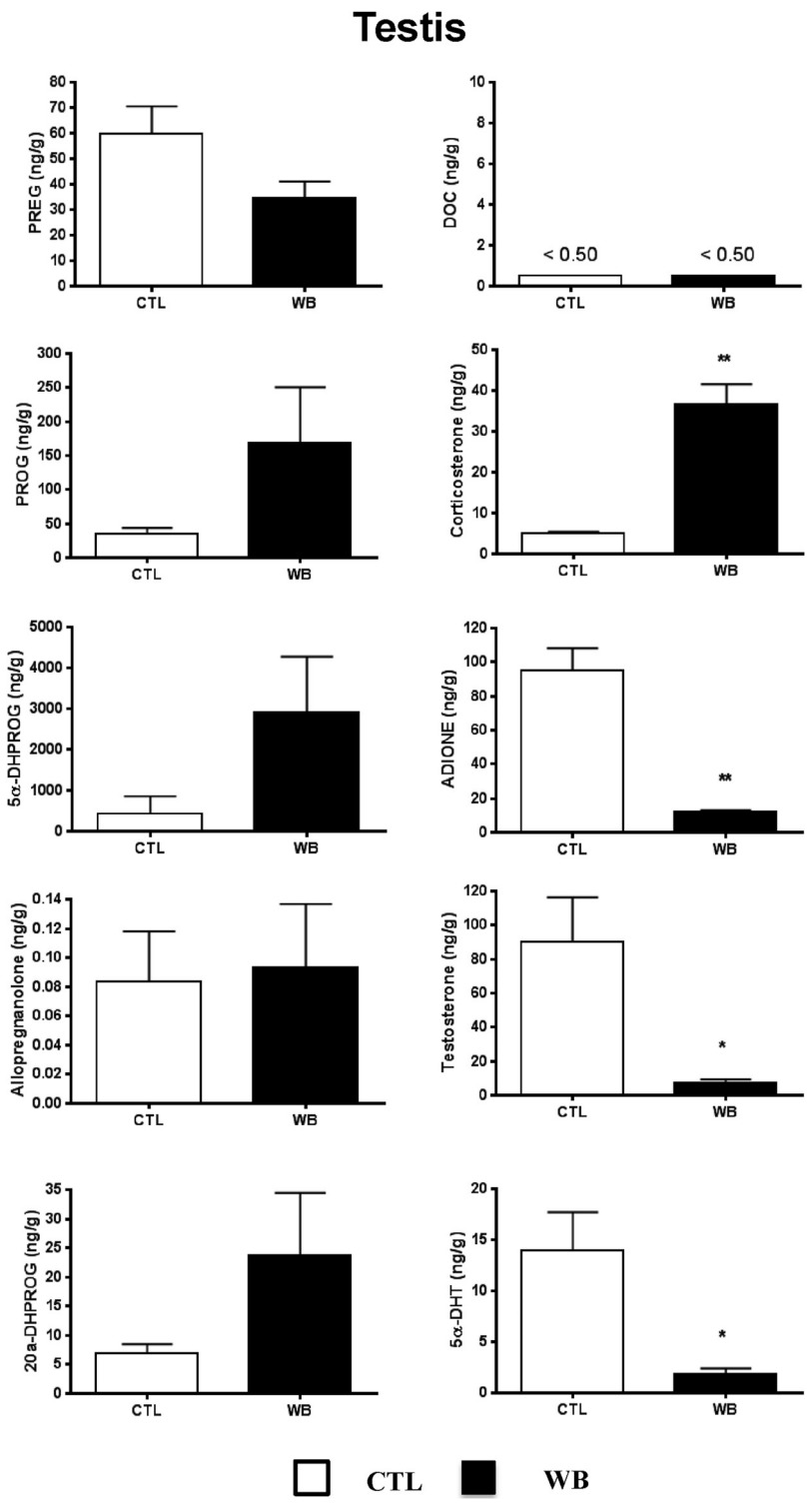

Figure 5. Steroid profiling in testis of control and Wobbler male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Steroid concentrations are expressed as ng/g ± SEM. *White bars*: control mice (CTL, n = 13), *black bars*: Wobbler mice (WBL, n = 12). Statistical analysis: Student's *t* test. *, $P < .05$; **, $P < .01$.

Upregulation of PREG, PROG and its metabolites in the Wobbler CNS: a possible endogenous neuroprotective response to neurodegeneration

The CNS is both a site of synthesis and a target for steroids. Thus, steroid amounts present in the nervous system result from both the endocrine glands and local synthesis. The higher amounts of PREG measured in the Wobbler spinal cord and brain when compared to plasma levels may reflect an increase in local synthesis. PROG levels were significantly enhanced only in the spinal cord and not in brain of Wobbler mice as compared to control. A series of arguments indicate that the adrenal glands may significantly contribute to the increase of PROG in spinal cord. Indeed, i) PROG levels in spinal cord and plasma were in equilibrium, ii) PROG levels were higher in plasma of Wobbler relative to control mice, and iii) PROG levels were also strongly higher in adrenal glands of Wobbler mice. High levels of 5α-DHPROG, about 5-times higher than in plasma, were observed in the Wobbler spinal cord. Consistently, the accumulation of 5α-reduced metabolites (5α-DHPROG and allopregnanolone) was also markedly increased in the Wobbler brain, pointing to a strong 5α-

reductase activity in the CNS. Interestingly, levels of allopregnanolone, a metabolite which also exerts neuroprotective effects, were only increased in the brain and the thoracic and lumbar regions of the spinal cord, but not in the cervical part where motoneurons degenerate.

Upregulation of PREG, PROG and especially its reduced metabolites in CNS may be a part of protective, rescue or even regenerative processes in response to neurodegeneration or injury (19). Indeed, brain and spinal cord levels of PREG, PROG and 5α-DHPROG, are markedly upregulated in response to traumatic brain injury (TBI) (16), cerebral ischemia (18), and spinal cord transection (17). Neuroprotective effects of PROG and allopregnanolone have been demonstrated in many injury models, including stroke (38), excitotoxic damage of hippocampal neurons (39), traumatic spinal cord injury (4), and in mouse models of spontaneous spinal motoneuron degeneration, Niemann-Pick type C disease and Alzheimer's disease (40–42). According to a widely accepted concept, the neuroprotective effects of PROG may be mediated by its metabolite allopregnanolone, but recent studies have shown that the intracellular PROG receptors (PR) play a crucial role in both the neuroprotective and remyelinating actions of PROG (18, 43, 44).

Importantly, previous reports have shown that PROG could partially restore morphological, molecular and functional abnormalities in Wobbler mice. PROG was able to rescue motoneurons from degeneration by recovering histopathological abnormalities and mRNA expression of the sodium pump (45), blocking NO synthesis and oxidative damage (46), improving neuromuscular function (47), modulating BDNF and choline acetyltransferase (48, 49), improving motoneuron and glial cell abnormalities (50) and preventing mitochondrial dysfunction (51). Nestorone, a PR agonist with high-affinity and high-specificity for PR, decreased astrogliosis, microgliosis, and motoneuron degenera-

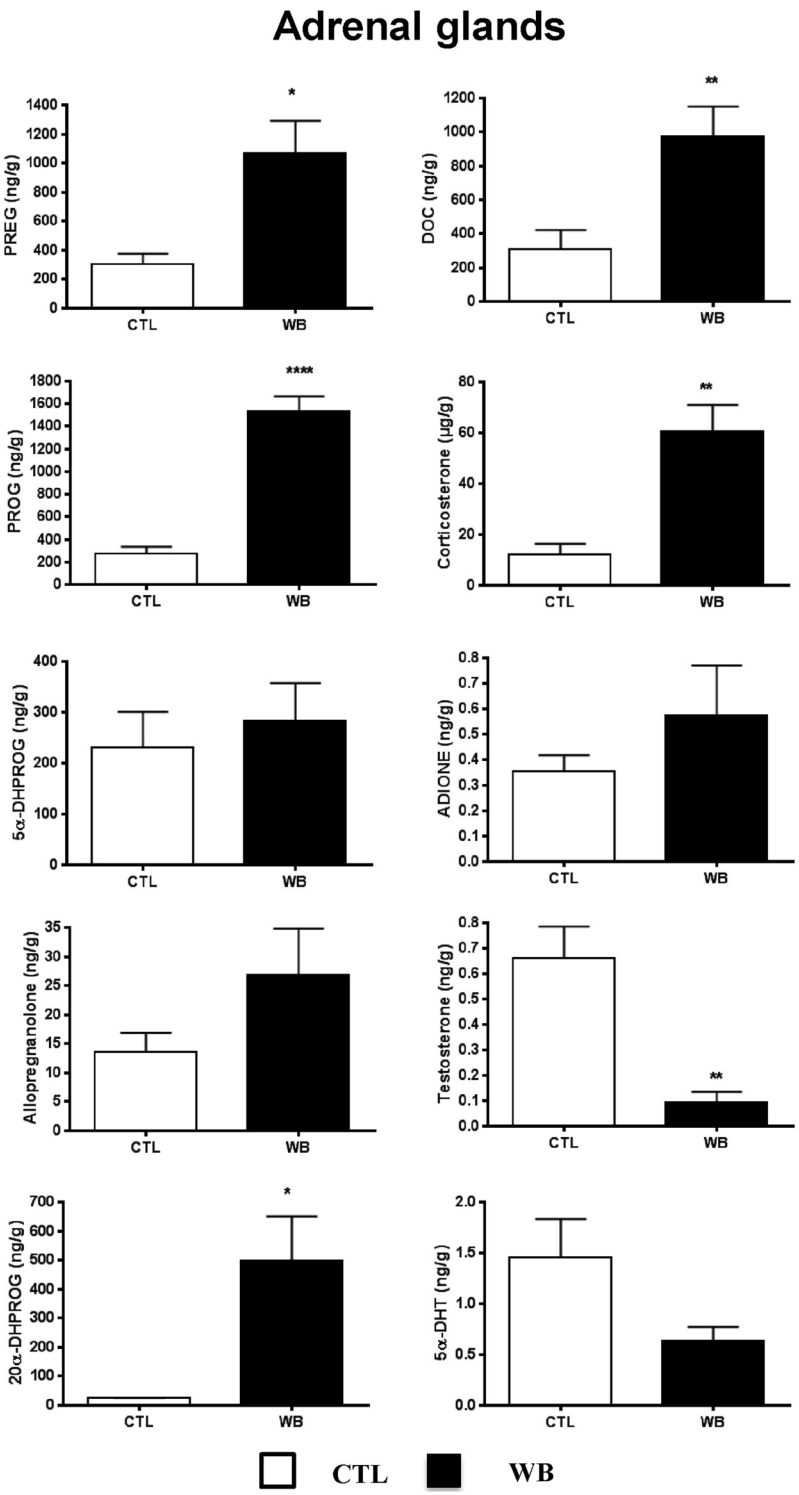

Figure 6. Steroid profiling in adrenal glands of control and Wobbler male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone, 20α-DHPROG: 20α-dihydroprogesterone, DOC: 11-deoxycorticosterone, ADIONE: androstenedione, 5α-DHT: 5α-dihydrotestosterone. Steroid concentrations are expressed as ng/g ± SEM. *White bars*: control mice (CTL, n = 13), *black bars*: Wobbler mice (WB, n = 12). Statistical analysis: Student's *t* test. *, $P < .05$; **, $P < .01$, ****, $P < .0001$.

Table 2. Pearson's correlation (r value) and slope of the line of steroid levels in plasma *vs.* cervical spinal cord and brain in control ($n = 13$) and Wobbler ($n = 12$) male mice. PREG: pregnenolone, PROG: progesterone, 5α-DHPROG: 5α-dihydroprogesterone. ns: not significant. $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ $^{****}P < 0.0001$

	Cervical		*Brain*	
	Control	***Wobbler***	***Control***	***Wobbler***
	r value (slope)			
PREG	-0.23^{ns} (−0.50)	0.67^{*} (2.07)	0.43^{ns} (0.85)	0.02^{ns} (0.05)
PROG	-0.32^{ns} (-0.83)	-0.09^{ns} (-2.11)	0.27^{ns} (4.64)	0.55^{ns} (0.98)
5α-DHPROG	0.51^{ns} (3.35)	0.66^{*} (3.03)	0.87^{***} (1.32)	0.42^{ns} (0.97)
Allopregnanolone	-0.18^{ns} (-0.32)	-0.22^{ns} (-0.16)	0.42^{ns} (-0.02)	0.50^{ns} (0.27)
Corticosterone	0.65^{*} (0.34)	0.66^{*} (0.31)	0.46^{ns} (0.30)	0.82^{**} (0.11)
Testosterone	0.99^{****} (0.75)	0.85^{*} (0.94)	0.96^{****} (0.72)	-0.38^{ns} (-0.42)

tion in Wobbler mice (52). PROG also delayed neurodegenerative processes and prolonged lifespan in transgenic mice expressing the human G93-SOD1 mutant, a genetic ALS model, via activation of an autophagy pathway (53). Interestingly, endogenous levels of PROG were also found to correlate positively with factors predicting better prognosis and survival in ALS patients (54).

PROG can also be converted to 20α-DHPROG. 20α-reduction represents a competitive metabolic pathway relatively to the 5α-reduction (55). 20α-DHPROG levels were increased in brain, spinal cord, plasma, testis, and adrenal glands of Wobbler mice. 20α-DHPROG can be formed in several tissues such as adrenal glands and liver and can also be synthesized in the brain of rats (56) and mice (57). Notably in mice, the expression of the 20α-hydroxysteroid dehydrogenase (20α-HSD) was observed in cerebral cortex and hippocampus, and it has been suggested that 20α-HSD might regulate the availability of PROG for PR and for the formation of neuroactive metabolites by the 5α-reductases (57). Levels of 20α-DHPROG were increased by 20-fold in the adrenal glands of Wobbler mice, probably as a consequence of a chronic stress as suggested by Higashi et al (58).

Upregulation of corticosterone: a possible contribution to motoneuron degeneration in Wobbler mice

We have shown a marked increase in corticosterone levels, as well as in its precursor DOC, in brain, spinal cord, plasma, and adrenal glands of Wobbler relative to control mice. These results confirm and extend the results of previous studies showing higher corticosterone levels in plasma of Wobbler (13), and of $SOD1^{G93A}$ mice (14). Furthermore, Fidler and al. showed that corticosterone levels correlated negatively with the onset of paralysis and survival in $SOD1^{G93A}$ mice. Importantly, these mice exhibited acceleration of the disease progression and of several neuropathological features such as astrogliosis, microglial activation and motor function decline when submitted to a repeated restraint-induced stress condition (14). Here, we show that in addition to plasma, all the regions of the spinal cord and brain contain higher amounts of corticosterone in Wobbler mice as compared with their littermates, most likely resulting from adrenal gland hyperactivity and hypertrophy of zona fasciculata.

Several reports have demonstrated a marked dysfunction of the HPA axis in ALS patients that may aggravate motoneuron degeneration. Thus, a loss of cortisol circadian rhythm, an increase in saliva cortisol in the morning and evening and decreased response to stress have been reported (11). Other studies have reported higher morning levels of cortisol in patients with the sporadic form of ALS (54) and Roozendaal et al have shown a blunted cortisol awakening response correlating with ALS severity (59). Furthermore, there is an increased risk for developing ALS in individuals exposed to psychological and physical stress (60) and an accelerated functional decline and shorter lifespan in individuals displaying high levels of psychological distress (61). Thus, differences in the exposure to stressful events may account for the variability observed between the ALS patients in terms of disease time course and survival.

The link between corticoids and neurodegeneration is corroborated by several reports showing HPA hyperactivity in other neurological disorders such as Alzheimer's disease, vascular dementia (62) and Huntington's disease (63). Therefore, it is not surprising that in addition to spinal cord and brain stem pathology, hippocampal abnormalities are found in ALS patients (64), triggered by the

hyperactivity of the glucocorticoids receptor (GR). This hypothesis is based on central glucocorticoid action on hippocampal morphology and function, where a severe and/or prolonged stress can mediate deleterious effects (65). Similarly, Wobbler mice also present a dysfunctional hippocampus (66). By using a selective and high affinity GR antagonist, a recent study has shown that blocking the GR increased neuronal progenitors, decreased astrogliosis and modified microglial phenotype in the hippocampus of Wobbler mice treated with corticosterone (13).

A potential mechanism of the adrenal dysfunction in Wobbler mice and ALS patients might be linked to an impairment of the acid ceramidase (ASAH1). Indeed, ASAH1 suppression increased the transcription of adrenal genes involved in steroidogenesis, and ASAH1 is a main inhibitor of steroidogenic capacity in human adrenal cortex (67). Furthermore, mutations in ASAH1 have been associated with spinal cord atrophy-progressive myoclonic epilepsy (SMA-PME), an inherited neuromuscular disorder characterized by degeneration of motoneurons in the spinal cord and leading to progressive atrophy of skeletal muscles and paralysis (68).

Dysregulation of HPG axis in male Wobbler mice

We also showed an important deficiency of androgen synthesis in the testes of Wobbler mice. Indeed, testosterone levels, as well as its precursor ADIONE and its metabolite 5α-DHT were markedly decreased in testis. A 10-fold drop in testosterone levels was also observed in plasma and CNS tissues. The size of Leydig cells as well as GnRH and LH levels were also significantly decreased in Wobbler mice, demonstrating dysregulations at all the levels of HPG axis. These results are consistent with the defects of spermiogenesis and sterility observed in Wobbler mice (9). A decrease in testosterone levels, measured by RIA, was reported in serum of ALS patients of both sexes (10), suggesting a possible involvement of androgens in the pathophysiology of the motoneuron disease. However, a recent study using immunoradiometric kits did not show a decline in testosterone levels with increasing age in ALS men (69). Thus, analysis of steroids in ALS patients using mass spectrometric techniques is needed.

The cross-talk between the HPA and HPG axes could contribute to the important changes in steroid levels observed in Wobbler mice. Several studies have focused on the mechanisms by which stress disrupts reproductive functions (70, 71). The adrenal axis, stress and high doses of glucocorticoids disrupt all aspects of the HPG axis, including reproductive behavior, LH secretion and sex steroid synthesis and release (70, 71). Thus, the high levels of corticosterone secreted by the adrenals of the Wobbler mice may explain, at least partially, the marked decrease in GnRH, LH and androgens. It is noteworthy that several components of the gonadal axis can also affect the HPA axis. Indeed, it is well known that androgens act negatively at several levels of the HPA axis, including hypothalamic CRH, pituitary ACTH and adrenal glucocorticoids, This effect is mainly mediated by the AR in multiple brain regions (72).

In addition to the cross-talk between HPG and HPA axes, a common dysregulation at the hypothalamus-pituitary levels and/or at the steroidogenic gland level could also be considered in Wobbler mice. Indeed, given the reduced levels of both LH and testosterone in Wobbler mice, it could be expected that GnRH levels will markedly increase. The observed decreased level of GnRH in our study suggest a probable dysregulation at the central level. It is conceivable that the Golgi defects in Wobbler mice may cause altered synthesis, trafficking or release of hypothalamic CRH and GnRH and of gonadotropins as the Golgi apparatus is important for the intracellular vesicular transport and is localized at the center of the secretary pathway. Besides, steroid hormone production in endocrine glands is a multistep process including the regulation of cholesterol uptake and transport as well as the transport of steroid substrates between the endoplasmic reticulum and the mitochondria. The cytoskeleton such as microfilaments and microtubules as well as a protein complex called “transducesome” (19) play a key role in assuring an efficient processing and transport of cholesterol in steroidogenic cells. Thus, defects in Golgi apparatus and mitochondria, as reported in Wobbler mice, could easily cause steroidogenesis dysfunctions in this context. Importantly, a recent publication by Xu et al (73) showed that a Golgi-localized membrane protein progestin and adipoQ receptors 3 (PAQR3) regulates cholesterol homeostasis by anchoring specific transcription factors to the Golgi apparatus. Thus, the Wobbler mutation, by means of partial loss of function of Vps54 and GARP, may cause a dysregulation of GnRH and LH synthesis/release and of cholesterol homeostasis and steroidogenesis in steroidogenic cells.

There is male predominance in ALS with a gender ratio of 3:2, but this sex differences is age-dependent (74). A hormonal factor has been suspected to play a role in the higher incidence of the disease in males. Several lines of evidence indeed point to an association between dysfunctions in androgen signaling and motoneuron degeneration. For example, the spinal and bulbar muscular atrophy (SBMA) is a genetic pathology due to an AR mutation with signs of androgen insensitivity, and is characterized by motoneuron degeneration and endocrine abnormalities (75). In a mouse model of SBMA, castration of males reduced muscular atrophy and weakness and testosterone

administration in females markedly increased the symptoms (76). The use of synthetic anabolic substances has been suspected to contribute to an increased risk of ALS in soccer players (60). The use of nandrolone decanoate, an anabolic androgenic steroid, in $SOD1^{G93A}$ mice led to muscle hypertrophy but also to motoneurons death and shorter survival of the treated mice (77). Treatment with nandrolone induced the recruitment of AR into insoluble complexes, pointing to an important role of AR homeostasis in the pathogenesis of motoneurons. However, two recent studies using the $SOD1^{G93A}$ mice have shown myotrophic and neuroprotective effects of 5α-DHT (78) and preventive effects of nandrolone on the structural alterations of the neuromuscular junctions (79).

The question of sex specificity needs to be addressed in Wobbler mice. Indeed, Wobbler females are infertile (3). Surprisingly, unlike for males, no reviews (except the one cited above) mention this fact and no studies have been undertaken to explain this infertility. Indeed, there are no studies concerning Wobbler female mice fertility, gametogenesis, and regularity of cycles or steroidogenesis. Given our present results in males, it is likely that gametogenesis, steroidogenesis and the HPG axis are also affected in Wobbler females.

As expected, the levels of DHEA were below the level of detection of GCMS in all tissue and plasma samples. Indeed, rodents utilize 17alpha-hydroxyprogesterone as substrate yielding androstenedione as product in the contrary of human P450C17 enzyme which uses 17alpha-hydroxypregnenolone as substrate yielding DHEA as product. This is also consistent with recent results by Sondhi et al who showed that levels of DHEA in mice were less than 20pg/ml (80). However, some reports detected measurable low amounts of DHEA in rat brain (81). The discrepancies might relate to strain or technical procedures. Cholesterol indeed can be oxidized readily during the entire sample workup, and can give rise, under particular conditions to DHEA. Thus, the different steps of purification and fractionation upstream of steroid measurements are very important to consider as we have already discussed in previous papers (27, 29).

Increased levels of corticosterone and decreased levels of testosterone in plasma: a signature of motoneuron degeneration?

The search for plasma biomarkers is important for monitoring disease progression and assessing treatment efficacy in animal models as well as in ALS patients. Changes in circulating glucocorticoids and androgens may influence the time course of the disease by preventing and/or accelerating motoneuron degeneration in Wobbler mice. In wildtype and Wobbler mice, no correlation exists for PROG and allopregnanolone between their plasma and spinal cord and brain levels. Conversely, plasma and cervical spinal cord corticosterone and testosterone contents are well correlated. Our findings show that corticosterone levels are increased and testosterone levels decreased in Wobbler mice compared to controls, and their levels in plasma reflect their amounts in spinal cord. It will be important to conduct longitudinal studies for assessing the evolution of plasma levels of steroids in experimental ALS models and ALS patients. These data will be useful to identify the onset of endocrine dysfunctions and to adapt treatment strategies to ameliorate the pathological features of motoneuron diseases.

Conclusion

Our study highlights marked changes in steroid levels in the symptomatic male Wobbler mouse, a model of ALS, with an upregulation of adrenal glucocorticoids and a downregulation of testicular androgens. HPA hyperactivity in Wobbler mice, also observed in other models of neurodegenerative disorders, possibly results from chronic stress exposure resulting from the disease process and disturbed central glucocorticoid feedback pathways. We suggest that hypercorticosteronemia may accelerate several pathological features such as motoneuron degeneration in cortex, brainstem and spinal cord, as well as astrogliosis and microglial hyperactivity. Thus, regulators of glucocorticoid signaling pathways could be useful for ameliorating the pathological features of ALS. On the other hand, the upregulation of PROG and its reduced metabolites in CNS of Wobbler may participate in endogenous neuroprotective and rescue mechanisms. Our results demonstrate a reduction in androgen levels in Wobbler mice. The precise role of androgen deficiency in the degeneration of motoneurons in Wobbler mice and ALS patients warrants further investigations; a dysregulation of androgen signaling has been shown to be associated with motoneuron diseases.

To conclude, our findings 1) underline the interdependence between the nervous system and endocrine system, 2) suggest that a combined rather than a single steroid therapy will be required to treat the consequences of the steroid imbalance, and 3) provide some keys for developing novel therapeutic approaches based on progesterone, glucocorticoid modulators and androgens to ameliorate the ALS pathogenesis. In addition, increased levels of corticosterone and decreased levels of androgens in plasma could be a signature of motoneuron degeneration. Steroid profiling may thus be used as a potential biomarker for degenerative diseases of motoneurons.

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Full-length report

Disclosure Summary: The authors have nothing to disclose

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