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Sublethal exposure to emerging ciguatoxins alters behavior and brain markers of neurotoxicity in a sex-dependent manner in rats

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Abstract

Ciguatera is an emerging foodborne illness in Europe caused by ciguatoxins (CTXs). However, risk assessment remains limited by significant gaps in toxicological knowledge and by analytical challenges stemming from a scarcity of reference materials, which hinder the characterization of exposure and the toxicity of the various analogues. We administered a single intraperitoneal dose of CTX-3C (1.5, 5, or 15 ng/kg) to male and female rats, and of Caribbean ciguatoxin-1 (C-CTX-1; 0.15, 0.7, or 1.5 ng/kg) to females. These doses correspond to at most 1% and 0.04%, respectively, of the median lethal dose (LD50) in mice. We assessed motor and anxiety-like behavior, and measured acetylcholinesterase (AChE) activity and total antioxidant capacity (TAC) in the prefrontal cortex (PFC), hippocampus, and striatum. CTX-3C increased locomotor activity and motor incoordination and reduced open-arm entries in the elevated plus maze in both sexes, whereas rotarod performance decreased only in females, with effects persisting 24 h after injection. CTX-3C reduced AChE activity in all three regions and increased TAC. Dose responses differed between sexes and were non-monotonic: in males, the AChE reduction was absent at 15 ng/kg in the hippocampus and striatum. C-CTX-1 decreased AChE activity and TAC in females at doses up to 1.5 ng/kg. These results show that a single sublethal exposure to CTXs alters behavior and brain cholinesterase and antioxidant status in a sex-dependent manner and suggest that low-dose effects cannot be inferred from high-dose or lethality data. They support including low doses, both sexes and C-CTX-1 in toxicological evaluations of ciguatera.

Key words: Ciguatera; Ciguatoxins; Acetylcholinesterase; Antioxidant capacity; Sex differences; Risk assessment

1. Introduction

Ciguatera fish poisoning (CFP) is considered the most common non-bacterial disease of marine origin throughout the world, with an estimated annual incidence of between 50,000 and 500,000 cases. Historically, the CFP was mainly associated with tropical and subtropical coastal regions, particularly the South Pacific and the Caribbean (Soliño & Costa 2020; Traylor et al. 2024). However, in recent decades, climate-driven warming of coastal waters has been linked to poleward expansion of toxin-producing dinoflagellates, including *Gambierdiscus* and *Fukuyoa*, and to the emergence of autochthonous cases of ciguatera fish poisoning (CFP) in European regions previously considered disease-free (Canals et al. 2021; European Centre for Disease Prevention and Control 2021). Thus, confirmed outbreaks and detections of toxins have been recorded in the Canary Islands (Spain) and Madeira archipelago (Portugal) (Boada et al. 2010; Estevez et al. 2024; Nuñez et al. 2012), a trend that is explicitly attributed to climate change (Costa et al. 2023). The syndrome originates from the consumption of reef fish that have bioaccumulated ciguatoxins (CTX), a family of lipophilic and thermostable polyether compounds produced by these benthic dinoflagellates. From a clinical point of view, CFP manifests itself through a heterogeneous combination of gastrointestinal, cardiovascular, dermatological and neurological signs, including paresthesia, ataxia and cold allodynia, and in a considerable proportion of patients the symptoms persist for weeks or months after the acute episode (Traylor et al. 2024).

This geographical expansion has occurred against a backdrop of considerable regulatory uncertainty. The European Food Safety Authority (EFSA) has formally classified CTXs as an emerging group of marine biotoxins, noting that compounds within this group had recently been identified in European seafood products. However, no regulatory maximum levels had been established for CTX-group toxins in the EU (EFSA

CONTAM Panel 2010). Notably, the Panel deemed the toxicological database for these compounds (comprising primarily acute toxicity studies) to be limited and concluded that it was not possible to derive an acute oral reference dose from the available data on animal and human intoxications. Instead, a provisional guidance level (0.01 μg P-CTX-1 equivalents/kg fish) was proposed based on case reports rather than systematic dose-response studies (EFSA CONTAM Panel 2010) . More than a decade later, EU-funded initiatives, such as the EuroCigua I and II projects, continue to identify this same gap (insufficient toxicological and analytical data to underpin a formal risk assessment and regulation) as a priority for future research (EFSA 2026). This scarcity of controlled toxicological data, particularly on the sublethal behavioral and biochemical effects needed to establish dose-response relationships and support hazard characterization, motivates the present study.

At the molecular level, CTXs act primarily as potent activators of voltage-gated sodium channels (VGSCs), shifting their activation threshold toward more negative potentials and interfering with inactivation. This causes persistent sodium influx, membrane depolarization and repetitive firing in excitable tissues (Benoit et al. 1986; Yamaoka et al. 2004). CTXs constitute a family of structurally related congeners that differ according to their geographic origin and side-chain modifications. These structural differences translate into markedly different potencies. For instance, Pacific congeners such as P-CTX-1B have been reported to be more than an order of magnitude more toxic than the Caribbean congener C-CTX-1 in mouse bioassays (Nicholson & Lewis 2006). In contrast, recent *in vitro* assays on human Nav1.6 channels and Neuro-2a cells indicate that C-CTX-1 and CTX-3C have a similar potency (Raposo-García et al. 2023). In this context, CTX-3C, a Pacific-type congener widely used as an experimental reference toxin due to its relatively greater availability, and C-CTX-1 (CCTX1), the primary congener

implicated in Caribbean ciguatera outbreaks, represent pharmacologically related yet structurally distinct probes for the same molecular target. However, comparative *in vivo* data regarding their neurotoxic profiles remain scarce.

Beyond the activation of VGSCs, CTX exposure has been associated with altered cholinergic signaling and oxidative imbalance in nervous tissue, two mechanistically linked processes frequently used as convergent biomarkers of neurotoxicity. Acetylcholinesterase (AChE) activity reflects cholinergic tone and neuronal excitability, whereas antioxidant capacity reflects the tissue's ability to neutralize reactive oxygen species (ROS) generated by sustained depolarization and mitochondrial stress. Given that the central nervous system (CNS) does not respond uniformly to toxic and oxidative insults, reflecting regional differences in metabolic rate, antioxidant defenses, and receptor density, a single toxic insult can produce markedly different biochemical profiles across various brain areas (Pandya & Patani 2021). This regional heterogeneity justifies the separate assessment of AChE activity and antioxidant capacity across multiple CNS regions, the approach adopted in the present study.

From a behavioral perspective, evidence that CTXs alter CNS function remains limited. Chronic exposure to low doses of P-CTX-1B has been shown to induce anxiety-like behaviors, spatial memory deficits, and decision-making impairments in rats (Wang et al. 2017), indicating that CTXs can cause lasting neurobehavioral changes beyond the period of acute intoxication. However, to our knowledge, no study has combined a broader behavioral battery, including measures of spontaneous locomotor activity, motor performance, and anxiety-like behavior, with a region-specific biochemical profile in the same CTX-exposed animals.

The inclusion of sex as a biological variable is particularly relevant in this context. Sex-dependent differences regarding susceptibility, toxicokinetics, and behavioral effects

have been documented for other marine biotoxins. For example, in a mouse model of acute domoic acid intoxication, females (especially older ones) exhibited more severe neurotoxic signs and higher tissue toxin concentrations than males (Hendrix et al. 2023). *In vivo* studies of CTXs have been conducted almost exclusively in male animals and, to our knowledge, sex differences have not been investigated for this toxin family, despite the growing recognition that toxicological study designs failing to account for sex may mask clinically relevant differences in exposure risk.

A further limitation is the scarcity of certified reference material for Caribbean CTXs. Even recent efforts to establish calibration solutions and reference matrices for C-CTX-1 identify this as a major obstacle to research and regulatory analysis of this congener (Mudge et al. 2026). Because of the limited quantity of C-CTX-1 available, it was tested only in female rats and only for AChE activity and total antioxidant capacity (TAC). We present these data as an exploratory complement to the more extensive CTX-3C dataset, not as a parallel experiment.

Taken together, the present study aims to (i) characterize the effects of low doses of CTX-3C on free locomotion, motor coordination and performance on the rotarod, and anxiety-like behavior in the elevated plus maze (EPM), together with AChE activity and TAC across discrete brain regions, in male and female rats, and to determine whether the dose response differs between sexes; and (ii) explore whether C-CTX-1, at lower doses, also alters AChE activity and TAC in female rats.

2. Methodology

2.1. Animals

Adult male and female Sprague-Dawley rats (*Rattus norvegicus*; 250-400 g] at the start of the experiments) were obtained from the breeding facilities of CINBIO

(Biomedical Research Center), University of Vigo, Spain. Animals were acclimatized for at least 1 week before the study and housed in groups of two per cage in a temperature- and humidity-controlled room (22 ± 2 °C, 50-60%) on a 14-h light/10-h dark cycle, with standard commercial chow and tap water available *ad libitum*. Experiments were performed during the light phase between 09:00 and 13:00.

All procedures complied with European Union Directive 2010/63/EU and Spanish legislation (Real Decreto 53/2013) on the protection of animals used for scientific purposes. The experimental protocol was reviewed and approved by the Animal Welfare and Ethics Committee of the University of Vigo (approval code ES360570215601/24/FUN01/BIOL.AN.08/LFF/01). The study is reported in accordance with the ARRIVE 2.0 guidelines (du Sert et al. 2020), and all efforts were made to minimize animal discomfort and distress.

2.2. Ciguatoxins

CTX-3C. CTX-3C standard (purity > 99%) was purchased from Wako (Fujifilm Wako Pure Chemical Corporation, Japan) and dissolved in methanol. Immediately before administration, the stock solution was diluted in 0.9% saline (pH 7.4) to obtain the doses tested, with a final methanol concentration never higher than 5%. Control animals received the same vehicle (0.9% saline with the same final methanol concentration).

C-CTX-1. The C-CTX-1 reference material (5.5 mg of purified toxin) was prepared from fish from the Canary Islands (Spain) and the Madeira Archipelago (Portugal) (Castro et al. 2022) and kindly provided by Dr. Ana Gago-Martínez (Department of Analytical and Food Chemistry, University of Vigo) within the EuroCigua project. The material was

dissolved in methanol and, immediately before administration, diluted in 0.9% saline (pH 7.4) to obtain the doses tested.

Administration. All solutions were administered intraperitoneally (i.p.) in a volume of 3 mL/kg. CTX-3C was given at 1.5, 5, and 15 ng/kg to males and females, and C-CTX-1 at 0.15, 0.7, and 1.5 ng/kg to females only.

Dose selection. Doses were selected well below the reported lethal and sublethal range. To our knowledge, no LD_{50} values have been reported in rats, the species used here, so the reference available is the intraperitoneal LD_{50} in mice: 1.3-2 µg/kg for CTX3C and 3.6 µg/kg for C-CTX-1 (EFSA CONTAM Panel 2010). Relative to these values, the highest doses used (15 ng/kg of CTX3C and 1.5 ng/kg of C-CTX-1) correspond to approximately 1% and 0.04% of the respective LD_{50}. In rats, P-CTX-1 has been given as a single sublethal dose of 0.26 ng/g (260 ng/kg) (Wang et al. 2017; Zhang et al. 2013), the same dose used in mice (Kumar et al. 2017), and repeated low doses (0.065 ng/g every 72 h) induced anxiety-like behavior and cognitive deficits (Wang et al. 2017). CTX3C, which is commercially available, was used as the base compound, and its doses (1.5, 5 and 15 ng/kg) were chosen at the low end of the range tested by Raposo-García et al. (2022), who gave 10, 32 and 102 ng/kg daily for 28 days by oral gavage without observable symptoms or lethality and extending below it (1.5 and 5 ng/kg). The study began with C-CTX-1 at 0.7 ng/kg, the first dose tested, and the range was then extended to 0.15 and 1.5 ng/kg.

2.3. Treatment and experimental groups

A total of 75 rats were initially used. Prior to treatment, five animals were excluded for failing to meet the minimum performance threshold during rotarod training (see below), resulting in a final cohort of 70 animals (25 males and 45 females). The animals were randomly assigned to 12 experimental groups. For CTX-3C, both males and females were assigned to a control group (n = 7) and three dosage groups (1.5, 5, and 15 ng/kg; n = 6 per group). For C-CTX-1, only females were used; they were assigned to three dosage groups (0.15, 0.7, and 1.5 ng/kg; n = 5, 5, and 5, respectively). The intermediate dose for each toxin was evaluated first, followed by the addition of a lower and a higher dose to establish a dose-response relationship. For AChE, the C-CTX-1 groups were compared against the female control group from the CTX-3C experiment; for TAC, they were compared against an independent control group (n = 6), as this marker was measured using a different kit. No formal sample size calculation was performed; group size was based on previous studies and on the minimum number of animals needed to compare doses and sexes (Festing & Altman 2002). Some brain samples were lost during processing, so the number of animals analyzed in the biochemical assays ranged from 4 to 7 per group (exact values in the figure legends). The study followed the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020).

2.4. Behavioral evaluation

Behavior was evaluated starting 60 minutes after administration of the toxin, in the following order: rotarod at 60 minutes, open field → rotarod → EPM (at 120 minutes), and rotarod at 24 hours. Animals were habituated to the testing room for 2 hours before each test, and the apparatus was cleaned with 10% ethanol between animals. Scoring was done live/from video by an observer blind to treatment.

2.4.1. Spontaneous locomotor activity (open field).

The animals were individually placed in the center of a wooden arena (60 x 60 x 35 cm) divided into nine quadrants (20 x 20 cm) to evaluate the number of sections crossed by the animal during 5 min. Immediately after the animal was placed in the center of the arena, its movements were scored. Locomotor activity was considered only when the animal placed its four paws into one square (Assis et al., 2018; Leite et al., 2013).

2.4.2. Motor performance and incoordination (rotarod).

Motor impairment was measured on rotarod apparatus following the protocol used by (Costas-Ferreira et al., 2023). Briefly, the animals were trained under continuous acceleration (1 rpm/s) in 1-min sessions on a rotarod apparatus (Ugo Basile, Italy). The rotational velocity at which the animal dropped off the rotating bar was taken as the performance score. Animals that did not reach a stable baseline (at least 15 rpm) in 12 trials were discarded. The animals that presented performances between 15 and 40 rpm were selected for the experiment. After selection, experimental and control groups were matched based on both their body weight and mean performance during the last training sessions on the rotarod. Motor incoordination was expressed as the maximal motor impairment index (MMI): MMI (%) = (baseline score − lowest score) × 100 / baseline score, where the lowest score is the minimum value recorded after administration.

2.4.3. Elevated plus maze.

The elevated plus maze was used based on its documented ability to detect both anxiolytic- and anxiogenic-like drug effects in rats (Pellow et al. 1985). Briefly, the apparatus was made of wood covered with impermeable Formica and was placed 50 cm

above the floor. The four arms were 50 cm long and 10 cm wide. Two opposite arms were surrounded by walls (40 cm high, closed arms), while the other two were devoid of enclosing walls (open arms) surrounded by a 1-cm high Plexiglas edge. The four arms were connected by a central platform (10 x 10 cm2). Each animal was placed in the center of the maze facing a closed arm. Whenever a rat placed all four paws onto an arm, one entry was recorded. The animals were observed for a 5-min test period and the anxiogenic-like effects were defined as a decrease in the proportion of open arm entries divided by the total number of arm entries, and a decrease in the time spent on open arms relative to the total time spent on both arms. The total number of closed arm entries was utilized as a measure of locomotor activity.

The number of entries (all four paws) and the time spent in open arms (OAE, TOA) and closed arms entries (CAE) were recorded. The percentage of open-arm entries (%OAE = OAE / [OAE + CAE] × 100) and of time in open arms (%TOA = OAT / [TOA + CAT] × 100) were calculated, and CAE was analyzed as an index of general activity.

2.5. Tissue collection

At the end of the behavioral tests (24 hours after administration), animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and euthanized by rapid decapitation. Brains were removed immediately and the PFC, striatum, hippocampus, cerebellum and brainstem were dissected on ice and weighed. Tissues were frozen at -80 °C until biochemical analysis.

2.6. Biochemical assays

2.6.1. Acetylcholinesterase activity

AChE activity was quantified using a cholinesterase activity assay kit according to manufacturer's protocol (MAK119, Sigma, St. Louis, MO, United States) with minor modifications. Briefly, rat striatum was homogenized (10 µL/mg) in 0.1 M phosphate buffer (pH 7.5) at 4°C followed by centrifugation at 15000 × g for 10 min (Centrifuge 5418R, Eppendorf, Hamburg, Germany). Aliquots of the cleared supernatants were added to the kit reaction mixture and the reaction proceeded at room temperature. Absorbance at 405 nm was quantified using a microplate reader (EZ Read 400 ELISA, Cambridge, UK) at 2-min intervals from time 0 (tissue sample addition) up to 10 min. AChE activity was expressed as units/L. One unit of AChE is the amount of enzyme that catalyzes the production of 1.0 µmol of thiocholine per minute at room temperature at pH 7.5.

2.6.2. Total antioxidant capacity (TAC)

TAC was measured using a commercial kit and expressed as Trolox equivalents using a total antioxidant capacity kit according to manufacturer's protocol (MAK 187 and MAK 334, Sigma, St. Louis, MO, USA). The assay is based on the reduction of Cu^{2+} to Cu^{+} by protein and non-protein antioxidants present in the tissue; the reduced Cu^{+} forms a colored complex whose absorbance is proportional to the total antioxidant capacity. PFC, striatum, and hippocampus were homogenized in 1 mL of distilled water using a pestle. In the next step, 100 µL of each sample and 100 µL of the Cu^{2+} working solution were poured into wells. After the solution was incubated at room temperature (20 °C) for 90 min, samples were centrifuged (Centrifuge 5418R, Eppendorf, Hamburg, Germany) at 250 ×g for 5 min at 4 °C to separate solid tissue components. The supernatant was diluted 1:5 to bring values within range of kit standards. Each brain region was weighed and homogenized using a constant tissue-to-buffer ratio (1:4, w/v). For each brain region, biochemical assays were performed in technical duplicates, and the mean of the two

measurements was used for statistical analysis. Subsequently, absorbance was measured at 570 nm using a microplate reader (EZ Read 400 ELISA, Cambridge, UK). Higher values indicate greater TAC, expressed as mmol Trolox equivalents.

2.7. Statistical analysis

Data are presented as mean ± SEM, with individual values shown in the Figures. Single-measure behavioral variables (line crossings, MMI, %OAE, %OAT, CAE) and biochemical variables (AChE and TAC, separately for each brain region) were analyzed by two-way ANOVA (sex x dose, type III sums of squares) after square-root (counts), logarithmic (MMI, AChE, TAC) or arcsine-square-root (percentages) transformation. Residual normality and homogeneity of variance were checked with Shapiro-Wilk and Levene tests. Rotarod speed (RPM) was analyzed by repeated-measures ANOVA (sex x dose x time; baseline and 60 min, 120 min and 24 h after administration), with Greenhouse-Geisser correction when Mauchly's test indicated a violation of sphericity.

Treated groups were compared with the control of the same sex using Dunnett's test (at each time point for the rotarod). Sex differences within each dose were tested with the pooled error term of the ANOVA and Holm's correction across the four dose levels. No correction was applied across behavioral tests or across brain regions. Holm-adjusted p values across the five regions are given in the supplementary material. C-CTX-1 (females only) was analyzed by one-way ANOVA on log-transformed values followed by Dunnett's test against its own control. Because TAC for C-CTX-1 was measured with a different kit and its own control (MAK 187), the two toxins were compared only as a percentage of the respective control. Statistical significance was set at $p < 0.05$.

Analyses were performed in Python 3.13 with NumPy 2.5, pandas 3.0, SciPy 1.18 and statsmodels 0.15 (ANOVA models with statsmodels; Dunnett’s, Shapiro-Wilk,

Levene and Welch tests with SciPy; Holm correction with statsmodels), and Figures were drawn with Matplotlib 3.11. Repeated-measures ANOVA with Greenhouse-Geisser correction was implemented in Python and validated against the statsmodels AnovaRM function.

3. Results

3.1. Effects of CTX-3C on motor behavior

3.1.1. Open field

CTX-3C increased locomotor activity in the open field with dose ($F_{(3,42)} = 21.11$, $p < 0.001$), without a main effect of sex ($F_{(1,42)} = 1.05$, $p = 0.310$) or a sex x dose interaction ($F_{(3,42)} = 1.71$, $p = 0.180$). In females, crossings increased only at 15 ng/kg (+31%, $p < 0.01$ vs. control); 1.5 and 5 ng/kg did not differ from control. In males, crossings increased at 5 (+20%, $p < 0.05$) and 15 (+48%, $p < 0.001$) ng/kg, whereas 1.5 ng/kg had no effect (Figure 1).

3.1.2. Rotarod performance

Figure 2 show that baseline rotarod speed was higher in females than in males ($F_{(1,42)} = 8.96$, $p < 0.01$) and did not differ among dose groups ($F_{(3,42)} = 2.07$, $p = 0.118$). Repeated-measures ANOVA of rotarod speed showed a significant sex x dose x time interaction ($F_{(9,126)} = 3.88$, $p < 0.001$, Greenhouse-Geisser) and a sex x dose interaction ($F_{(3,42)} = 3.45$, $p < 0.05$). Analyzed by sex, dose affected rotarod speed in females ($F_{(3,21)} = 6.15$, $p < 0.01$), with a dose x time interaction ($F_{(9,63)} = 3.86$, $p < 0.001$). In males there was no effect of dose ($F_{(3,21)} = 1.07$, $p = 0.383$) or the dose x time interaction ($F_{(9,63)} = 2.03$, $p = 0.059$).

In females, rotarod speed decreased at 60 min with 1.5 (26.4 ± 1.4 vs. 33.4 ± 1.6 RPM in controls, $p < 0.05$), 5 (22.9 ± 2.0 RPM, $p < 0.001$), and 15 (21.7 ± 1.7 RPM, $p < 0.001$) ng/kg. At 120 min only 15 ng/kg differed from control (24.8 ± 0.9 vs. 33.1 ± 2.6 RPM, $p < 0.05$), and at 24 h 5 ng/kg (21.6 ± 1.9 vs. 30.2 ± 2.2 RPM, $p < 0.05$) and 15 ng/kg (19.7 ± 1.1 RPM, $p < 0.01$) remained below control. In males, no dose differed from control at any time (all $p \geq 0.15$).

3.1.3. Maximal motor impairment

Figure 3 show that MMI increased with CTX-3C dose ($F_{(3,42)} = 50.30$, $p < 0.001$) and the dose response differed between sexes (sex x dose, $F_{(3,42)} = 21.24$, $p < 0.001$), without an overall sex effect ($F_{(1,42)} = 1.07$, $p = 0.306$). In females, MMI increased at 5 and 15 ($p < 0.001$) but not at 1.5 ($p = 0.97$) ng/kg. In males, MMI increased significantly at all three doses ($p < 0.001$ in all cases). Between sexes, MMI at 1.5 ng/kg was 45% lower in females than in males (Holm-adjusted $p < 0.001$) and 46% higher in females at 15 ng/kg (Holm-adjusted $p < 0.001$). No differences were found in controls or at 5 ng/kg.

3.1.4. Elevated Plus Maze

The effects of the CTX-3C on anxiety-related behavior are shown in Figure 4.

Open-arm entries (%EBA). CTX-3C decreased %EBA (dose, $F_{(3,42)} = 17.54$, $p < 0.001$) to a similar extent in both sexes (sex, $F_{(1,42)} = 0.13$, $p = 0.719$; sex x dose, $F_{(3,42)} = 0.01$, $p = 0.999$). In females, %EBA significantly decreased at 1.5 ($p < 0.01$), 5 and 15 ($p < 0.001$) ng/kg. In males, %EBA decreased at 5 ($p < 0.01$) and 15 ($p < 0.05$) ng/kg, and the reduction at 1.5 ng/kg did not reach significance.

Time in open arms (%TBA). %TBA was lower in females than in males (sex, $F_{(1,42)} = 36.28$, $p < 0.001$), with no overall effect of dose ($F_{(3,42)} = 2.36$, $p = 0.085$) or sex x dose

interaction ($F_{(3,42)}$ = 1.62, p = 0.199). In females, %TBA decreased at 5 ($p < 0.001$) and 15 ($p < 0.05$) and was unchanged at 1.5 ng/kg. In males, no dose differed from control (all $p \geq 0.72$). Females showed lower %TBA than males at 1.5 (Holm-adjusted $p < 0.05$), 5 (Holm-adjusted $p < 0.01$), and 15 (Holm-adjusted $p < 0.001$) ng/kg, but not in controls (Holm-adjusted p = 0.10).

Closed-arm entries (EBC). CTX-3C increased EBC (dose, $F_{(3,42)}$ = 38.46, $p < 0.001$), and the effect depended on sex (sex x dose, $F_{(3,42)}$ = 7.09, $p < 0.001$; sex, $F_{(1,42)}$ = 2.83, p = 0.100). In females, EBC increased at 5 and 15 ($p < 0.001$) but not at 1.5 ng/kg. In males, EBC increased at 5 ($p < 0.01$) and 15 ($p < 0.001$) but not at 1.5 ng/kg. At 15 ng/kg, EBC was 44% higher in females than in males (Holm-adjusted $p < 0.001$). The other doses did not differ between sexes after correction (Figure 4).

3.2. Effects of CTX-3C on AChE activity

The effects of the CTX-3C on AChE activity are shown in Figure 5.

In the hippocampus, AChE activity varied with dose ($F_{(3,31)}$ = 70.41, $p < 0.001$), and the dose response differed between sexes (sex x dose, $F_{(3,31)}$ = 13.10, $p < 0.001$), with no overall difference between females and males ($F_{(1,31)}$ = 1.25, p = 0.272). In females, AChE activity was lower at 1.5 (-67%, $p < 0.001$), 5 (-72%, $p < 0.001$) and 15 (-56%, $p < 0.001$) ng/kg vs. control. In males, AChE activity was lower at 1.5 (-51%, $p < 0.001$) and 5 (-42%, $p < 0.01$) ng/kg; not different at 15 ng/kg.

In the PFC, the effect of dose on AChE activity depended on sex (sex, $F_{(1,29)}$ = 2.50, p = 0.125; dose, $F_{(3,29)}$ = 22.89, $p < 0.001$; sex x dose, $F_{(3,29)}$ = 7.36, $p < 0.001$). In females, AChE activity was lower at 1.5 (-43%, $p < 0.001$) and 5 (-53%, $p < 0.001$) ng/kg; not different at 15 ng/kg vs. control. In males, AChE activity was lower at 1.5 (-30%, $p < 0.01$), 5 (-26%, $p < 0.05$) and 15 (-28%, $p < 0.05$) ng/kg.

In the striatum, AChE activity varied with dose ($F_{(3,32)}$ = 145.51, $p < 0.001$) and the dose response differed between sexes (sex x dose, $F_{(3,32)}$ = 18.73, $p < 0.001$), with no overall difference between females and males ($F_{(1,32)}$ = 1.36, $p = 0.252$). In females, AChE activity was lower at 1.5 (-62%, $p < 0.001$), 5 (-69%, $p < 0.001$) and 15 (-36%, $p < 0.001$) ng/kg vs. control. In males, AChE activity was lower at 1.5 (-61%, $p < 0.001$) and 5 (-44%, $p < 0.001$) ng/kg; higher at 15 (+34%, $p < 0.05$) ng/kg.

Control AChE activity was higher in females than in males in the hippocampus (+55%) and striatum (+33%) (Holm-adjusted $p \leq 0.002$ for both). Results for the cerebellum and brainstem are given in Supplementary Table S1.

3.3. Effects of CTX-3C on total antioxidant capacity (TAC)

The effects of the CTX-3C on TAC are shown in Figure 6.

In the hippocampus, the effect of dose on TAC depended on sex (sex, $F_{(1,28)}$ = 11.70, $p < 0.01$; dose, $F_{(3,28)}$ = 15.33, $p < 0.001$; sex x dose, $F_{(3,28)}$ = 3.61, $p < 0.05$). In females, TAC was higher at 15 (+45%, $p = 0.001$) ng/kg and not different at 1.5 and 5 ng/kg vs. control. In males, TAC was higher at 1.5 (+24%, $p < 0.001$), 5 (+19%, $p < 0.001$), and 15 (+24%, $p < 0.001$) ng/kg.

In the PFC, the effect of dose on TAC depended on sex (sex, $F_{(1,29)}$ = 21.66, $p < 0.001$; dose, $F_{(3,29)}$ = 12.87, $p < 0.001$; sex x dose, $F_{(3,29)}$ = 9.00, $p < 0.001$). In females, TAC was higher at 15 (+30%, $p < 0.001$) ng/kg and not different at 1.5 and 5 ng/kg vs. control. In males, TAC was higher at 1.5 (+31%, $p < 0.001$), 5 (+18%, $p < 0.05$), and 15 (+20%, $p < 0.01$) ng/kg.

In the striatum, TAC varied with dose, without a sex x dose interaction (sex, $F_{(1,27)}$ = 0.47, $p = 0.500$; dose, $F_{(3,27)}$ = 32.92, $p < 0.001$; sex x dose, $F_{(3,27)}$ = 1.99, $p = 0.139$). In females, TAC was higher at 1.5 (+22%, $p < 0.05$), 5 (+27%, $p < 0.01$), and 15 (+54%, p

< 0.001) ng/kg vs. control. In males, TAC was higher at 1.5 (+49%, $p < 0.001$), 5 (+43%, $p < 0.001$), and 15 (+55%, $p < 0.001$) ng/kg.

Control TAC did not differ between sexes in any region (Holm-adjusted $p > 0.15$). Females had lower TAC than males in the PFC at 1.5 ng/kg (−25%, Holm-adjusted $p < 0.001$) and 5 ng/kg (−20%, Holm-adjusted $p < 0.001$) and in the hippocampus at 1.5 ng/kg (−21%, Holm-adjusted $p < 0.01$) (female vs. male). At 15 ng/kg the values were similar in both sexes. Results for the cerebellum and brainstem are given in Supplementary Table S2.

3.4. Effects of C-CTX-1 on AChE and TAC

3.4.1. AChE activity

In the hippocampus, AChE activity varied with dose ($F_{(3,18)} = 19.91$, $p < 0.001$). It was lower at 0.15 (-44%, $p < 0.001$), 0.7 (-56%, $p < 0.001$), and 1.5 (-60%, $p < 0.001$) ng/kg vs. control. In the PFC, AChE activity varied with dose ($F_{(3,17)} = 57.49$, $p < 0.001$). It was significantly lower at 0.15 (-23%, $p < 0.05$) and 1.5 (-72%, $p < 0.001$) ng/kg and not different at 0.7 ng/kg vs. control. In the striatum, AChE activity varied with dose ($F_{(3,17)} = 18.89$, $p < 0.001$), being lower at 0.15 (-54%, $p < 0.001$) ng/kg and not different at 0.7 and 1.5 ng/kg vs. control (Figure 7A, C, and E). Cerebellum and brainstem are given in Supplementary Table S1.

3.4.2. TAC

In the hippocampus, TAC varied with dose ($F_{(3,16)} = 14.77$, $p < 0.001$), being significantly lower at 0.15 (-28%, $p < 0.01$), 0.7 (-42%, $p < 0.001$), and 1.5 (-34%, $p < 0.001$) ng/kg vs. control. In the PFC, TAC varied with dose ($F_{(3,16)} = 13.86$, $p < 0.001$); it was lower at 0.15 (-37%, $p < 0.001$) and 0.7 (-23%, $p < 0.01$) ng/kg, not different at 1.5

ng/kg vs. control. In the striatum, TAC varied with dose ($F_{(3,16)} = 15.00$, $p < 0.001$), being significantly lower at 0.15 (-44%, $p < 0.001$) and 0.7 (-29%, $p < 0.01$) ng/kg and not different at 1.5 ng/kg vs. control (Figure 7B, D, and F). Cerebellum and brainstem are given in Supplementary Table S2.

4. Discussion

A single intraperitoneal dose of CTX-3C in the low ng/kg range, far below the reported mouse LD_{50}, altered motor and anxiety-related behavior and reduced AChE activity in several brain regions of rats, and the effects depended on sex and dose. C-CTX-1 reduced AChE activity and TAC in female rats at doses ten times lower than those of CTX-3C. To our knowledge, these are the first data on these endpoints with CTX-3C and C-CTX-1 after systemic administration in rats. They indicate that doses at about 1% of the mouse LD_{50} or lower can produce measurable neurobehavioral and biochemical changes.

Motor effects differed between the two measures and between sexes. In females, the MMI increased at 5 and 15 ng/kg but not at 1.5 ng/kg, which suggests a threshold between these doses. Females also fell from the rotarod at a lower speed 60 min after injection at all doses, at 120 min with 15 ng/kg and at 24 h with 5 and 15 ng/kg, so the impairment persisted at least one day. Control females improved relative to their baseline, whereas those given 5 and 15 ng/kg fell below it. In males, the speed at which they fell did not change, yet the MMI was already increased at 1.5 ng/kg and did not rise further with dose, which indicates a plateau.

The MMI, defined as the decline in performance from the baseline value to the lowest score, appears to capture a loss of coordination that average performance does not reflect. Locomotor hyperactivity can also modify rotarod performance, so these motor

outcomes should be read together with the open-field data below. Motor deficits on the rotarod have been described in male mice given P-CTX-1 at 0.26 ng/g intraperitoneally, together with trembling during the first week (Au et al. 2016) while recurrent impairments in motor coordination and grip strength were observed during a 4-month follow-up period (Asthana et al. 2018) , and the toxin reduced the spontaneous firing of motor cortex neurons and the EEG activity (Kumar et al. 2017). Our doses of CTX-3C were at least 17 times lower than 0.26 ng/g, so impairment in females 24 h after 5 ng/kg is consistent in direction with these reports but occurred at a much lower dose. Additionally, the studies cited used P-CTX-1, only males, and did not compare doses below 0.26 ng/g.

In the open field, crossings increased with dose in both sexes, from 15 ng/kg in females and from 5 ng/kg in males, but the sex x dose interaction was not significant, so the difference in threshold between sexes is not supported statistically. In the EPM, the %EBA decreased in both sexes and the %TBA decreased only in females (Figure 4). This pattern resembles the anxiety-like behavior that Wang et al. (2017) described in rats given repeated low doses of P-CTX-1. However, CAE increased with the dose in both sexes, in parallel with open-field crossings. Because %EBA is a ratio, it falls when CAE rises even if open-arm entries do not change, so part of the pattern may reflect hyperlocomotion rather than a change in anxiety. The reduction in %TBA, a measure that is less dependent on the number of entries, was seen only in females, and these results should be described as altered exploratory and anxiety-related behavior rather than as anxiety.

AChE activity decreased at 1.5 and 5 ng/kg in the hippocampus, striatum and PFC of both sexes (to 28-58% of control in hippocampus and striatum). These regions were chosen *a priori* for their relation to the behaviors assessed, and reduced AChE activity would prolong the synaptic action of acetylcholine, thereby enhancing cholinergic

signaling; this may contribute to alterations in motor control, exploration and anxiety-like behavior (Colovic et al. 2013; Petrov et al. 2018; Sienkiewicz-Jarosz et al. 2000). CTXs were first thought to be anticholinesterases, but later work showed that their main action is the activation of VGSCs, not the inhibition of the enzyme (L'Herondelle et al. 2020), so direct action on AChE is unlikely to explain our results. The reduction could instead be a downstream consequence of that primary action. Activation of VGSCs depolarizes neurons and increases acetylcholine release, as shown in other models. C-CTX-1 increased spontaneous and evoked quantal acetylcholine release at the frog neuromuscular junction (Mattei et al. 2010) , and P-CTX-1 triggered acetylcholine release from *Torpedo* cholinergic synaptosomes (L'Herondelle et al. 2020; Vale et al. 2015). A cholinergic component is also supported by the relief of the gastrointestinal and cardiovascular signs of CFP with atropine (L'Herondelle et al. 2020) and by the reduction of choline and the imbalance between excitatory and inhibitory neurotransmitters found in the mouse motor cortex after P-CTX-1 (Kumar et al. 2017). A sustained increase in cholinergic activity could lead to lower AChE activity, but we did not test this, and it remains a hypothesis.

Our findings also show that the AChE response was not linear. At 15 ng/kg activity returned to control values in the female PFC and the male hippocampus and increased significantly in the male striatum (+34%), whereas in the female hippocampus (-56%) and striatum (-36%) it remained low (Figure 5). Homeostatic adaptation to the sustained sodium-channel activation caused by CTX is a plausible explanation for this loss of effect at the highest dose. In cultured cortical neurons, CTX-3C depolarized the membrane, decreased firing and shifted synaptic transmission toward inhibition, and after 24 h it induced synaptic scaling with downregulation of glutamate receptors (Martín, Vale, Hirama et al. 2015; Martín, Vale, Rubiolo et al. 2015). The effects of CTX-3C on gene

expression also changed with the duration of exposure (Rubiolo et al. 2018). These findings come from *in vitro* systems and cannot be applied directly to our data, but they show that neurons respond to CTXs with compensatory mechanisms that may produce non-linear dose and time relationships.

The doses at which AChE inhibition was observed coincided with those that altered behavior, although the correspondence was not complete. In females, 1.5 ng/kg significantly reduced AChE activity in the hippocampus and the striatum, without altering the MMI, the number of crossings, or ECA. In males, a dose of 15 ng/kg restored AChE levels in the hippocampus and increased levels in the striatum. However, this same dose caused the greatest increase in the number of crossings. Therefore, the hyperlocomotion observed at the highest dose does not appear to depend on AChE inhibition in the regions analyzed. The proposed adaptation for AChE would not explain the behavioral threshold observed in females or the plateau observed in males, phenomena that we describe, but for which we have no explanation. CTX act on VGSCs, increasing neuronal excitability and the release of neurotransmitters (Nicholson & Lewis 2006). So, an effect, direct or indirect, on cholinergic neurons could be a possible mechanism, but we did not evaluate this. AChE inhibition is a hypothesis to explain the observed changes, not an established cause.

TAC increased following the administration of CTX-3C in both sexes but with a different dose dependence. In males, an increase was observed starting at a dose of 1.5 ng/kg in the PFC, hippocampus, and striatum. In females, by contrast, the increase was only observed at 15 ng/kg in the PFC and the hippocampus, whereas in the striatum, it occurred starting at 1.5 ng/kg (Figure 6). At the 15 ng/kg dose, the values were similar in both sexes. Given that there were no differences in the control values, the disparity between the sexes lies in their sensitivity to the dose rather than in the maximum response.

An increase in TAC is more consistent with a compensatory response than with a protective mechanism. It could reflect an upregulation of antioxidant systems or a modification in non-enzymatic antioxidants in response to the metabolic load resulting from sustained neuronal excitability. We did not measure reactive oxygen species (ROS), oxidative damage, or individual antioxidants, so this remains a hypothesis. Furthermore, TAC, on its own, is not an indicator of oxidative stress. Evidence regarding the redox effects of CTXs is limited and inconsistent. It has been shown that, in human astrocytes, CTX-1B increased LDH release but did not significantly increase the generation of ROS (D'Mello et al. 2017).

Sex modified the response for most of the variables studied. The sex x dose interaction was significant for MMI, performance on the rotarod, ECA, AChE activity in the hippocampus, PFC, and striatum, as well as for TAC in the PFC and hippocampus. However, it was not significant for open-field activity, %EBA, %TBA, or TAC in the striatum. Furthermore, females in the control group exhibited higher AChE activity than males in the control group in both the hippocampus (+55%) and the striatum (+33%), whereas TAC in the control groups showed no sex-based differences. The observed sex differences could be related to toxicokinetics, hormonal influences on the cholinergic system, or sex-dependent expression of cholinergic components. Estradiol modulates AChE activity in the rat brain, with effects varying by brain region and age (Martins et al. 2012), and alters central cholinergic function in female rats (Lapchak et al. 1990). Additionally, cerebral oxidative metabolism varies according to sex and the phase of the estrous cycle (Arias et al. 2010). Consequently, estrogens could therefore have contributed to the observed differences, particularly regarding AChE. However, we did not measure hormone levels or the phase of the estrous cycle, so this remains a hypothesis that our design cannot test and that we cannot separate from the other explanations. To

our knowledge, sex-based differences in the effects of CTXs have not been compared previously. The *in vivo* studies we reviewed used only males (e.g., (Asthana et al. 2018; Au et al. 2016; Kumar et al. 2017; Patel et al. 2015; Soto de Jesus et al. 2026; Wang et al. 2017; Zhang et al. 2013), and the one study that included mice of both sexes did not report a comparison between them (Raposo-García et al. 2022).

C-CTX-1 inhibited AChE and lowered TAC in female rats at 0.15 to 1.5 ng/kg, ten times below the doses of CTX-3C. This is notable because its mouse LD_{50} (3.6 μg/kg) is higher than that of CTX-3C (1.3-2 μg/kg) and its toxicity equivalency factor is lower (0.1 versus 0.2) (EFSA CONTAM Panel 2010), so a lower potency would have been expected. Relative potency depends on the preparation and endpoint. In cerebellar neurons CTX-3C was the most potent toxin at blocking peak sodium currents, whereas P-CTX-1B was more effective at blocking potassium currents (Pérez et al. 2011), and C-CTX-1 acts at low nanomolar concentrations on both sodium and potassium channels at the neuromuscular junction (Mattei et al. 2010). The LD_{50} and the equivalency factors summarize acute lethality in mice, and they need not predict potency on neural endpoints after systemic exposure in rats. *In vitro* data point in the same direction. C-CTX-1 and CTX-3C inhibited human Nav1.6 sodium currents and reduced Neuro-2a viability with similar potency (relative potency about 0.7 versus CTX-1B), in contrast with the equivalency factors derived from mouse lethality (Raposo-García et al. 2023). The lower lethality of C-CTX-1 therefore need not reflect lower activity at the molecular target, which is compatible with finding effects at low doses.

C-CTX-1 reduced AChE activity and TAC in the three regions analyzed, with large and consistent effects (Figure 7). However, the profile was not monotonic: in striatal AChE and in PFC and striatal TAC the effect was greatest at the lowest dose, whereas PFC AChE showed its largest decrease at the highest dose. The lack of a significant effect

at the higher doses should not be read as absence of effect. Each dose group had only four to five animals, so the tests had limited power to detect moderate changes, and in the three regions analyzed every non-significant value was a decrease, in the same direction as the significant ones. For example, striatal AChE at the two higher doses was reduced by about 20%, a change similar in size to the smallest decrease that reached significance in the PFC (Figure 7). These values suggest a smaller or more variable effect, not a null one. A smaller effect at higher doses is also compatible with the non-linear response described above for CTX-3C, although our design cannot distinguish a compensatory adaptation from variability.

On the other hand, a direct comparison with CTX-3C is limited to 1.5 ng/kg, the only shared dose, and we did not make one, so the difference in effective doses should not be taken as evidence that C-CTX-1 is more toxic. The finding is relevant because C-CTX-1 is the analogue mainly responsible for the autochthonous ciguatera outbreaks detected in the European Union, such as those in the Canary Islands and Madeira (Canals et al. 2021), so a toxin of lower estimated potency may still affect the nervous system at doses far below those suggested by its LD_{50} (the highest dose we used was about 0.04% of its mouse LD_{50}). A single intraperitoneal dose in rats cannot be extrapolated to dietary exposure in humans, but these results support the inclusion of C-CTX-1 in neurotoxicological studies of ciguatera.

5. Limitations of the study

This study has some limitations. Group sizes were small (6-7 animals per group in behavior and 4-7 in biochemistry), so results close to $p = 0.05$ are fragile, and we did not correct for multiple comparisons across the three behavioral tests or the brain regions. Toxins were given by a single intraperitoneal injection, and biochemical variables were

measured at one time point, so we cannot describe their time course or reversibility. The intraperitoneal route differs from oral exposure through contaminated fish, and the dose comparison relies on mouse LD_{50} values because none has been reported in rats. The estrous cycle was not monitored, so the phase of each female at the time of treatment is unknown.

6. Conclusions

The administration of single systemic doses of CTX-3C (1.5, 5, and 15 ng/kg) altered motor behavior, anxiety levels, and brain AChE activity in rats. These effects were sex-dependent and did not show a linear relationship with the dose. Meanwhile, C-CTX-1 (0.15, 0.7, and 1.5 ng/kg, reduced AChE levels and TAC in females. These results support the inclusion of both sexes in toxicological studies on CTXs and indicate that the effects of low systemic doses of these toxins on the nervous system warrant more comprehensive evaluation. Future research should include the correlation of biochemical and behavioral data within the same animals, studies on the temporal progression of effects and oral exposure, behavioral assessment of C-CTX-1 in both sexes, and the examination of markers for oxidative damage and cholinergic function beyond just AChE activity.

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Authorship

MB-C: design of the study, acquisition, analysis and interpretation of data. Drafting the article and revising it critically for important intellectual content. Final approval of the version to be submitted. CC-F: acquisition of data, analysis and interpretation of data. LB: acquisition of data, analysis and interpretation of data. RD: The conception and design of the study, analysis and interpretation of data. Drafting the article and revising it critically for important intellectual content. Final approval of the version to be submitted. LRFF: The conception and design of the study, analysis and interpretation of data. Drafting the article and revising it critically for important intellectual content. Final approval of the version to be submitted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used Claude (Anthropic) to assist with the statistical analysis of the dataset, the generation of figures, and language editing and translation of parts of the text. The study design, the choice of endpoints and the interpretation of the findings were determined by the authors. The statistical approach was specified by the authors. All figures were generated directly from these data. The authors reviewed and verified all content, including the statistical output and every cited reference, and take full responsibility for the accuracy and integrity of the published article.

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Figure legends

Figure 1. Effect of CTX-3C on locomotor activity in the open field. Number of line crossings in female (A) and male (B) rats after intraperitoneal administration of vehicle (control) or CTX-3C (1.5, 5 or 15 ng/kg). Bars show mean ± SEM and circles individual animals (n = 7 for controls, n = 6 for each CTX-3C dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control of the same sex (Dunnett's test after two-way ANOVA). The sex × dose interaction p value is shown in panel B.

Figure 2. Effect of CTX-3C on rotarod performance in female (A) and male (B) rats. Rotarod speed (RPM) at baseline and 60 min, 120 min, and 24 h after administration of vehicle (control) or CTX-3C (1.5, 5 or 15 ng/kg). Symbols show mean ± SEM (n = 7 for controls, n = 6 for each CTX-3C dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control of the same sex at the same time (Dunnett's test); asterisks are colored according to dose (light, 1.5 ng/kg, medium, 5 ng/kg, dark, 15 ng/kg).

Figure 3. Effect of CTX-3C on maximum motor incoordination (MMI) in the rotarod test in female (A) and male (B) rats. MMI = (baseline score − lowest score) × 100 / baseline score. Bars show mean ± SEM and circles individual animals (n = 7 for controls, n = 6 for each CTX-3C dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control of the same sex (Dunnett's test after two-way ANOVA). The sex x dose interaction p value is shown in panel B.

Figure 4. Effect of CTX-3C on behavior in the elevated plus maze. Open-arm entries (%EBA; A, B), time in open arms (%TBA; C, D) and closed-arm entries (EBC; E, F) in female (A, C, E) and male (B, D, F) rats. %EBA = EBA/(EBA + EBC) × 100; %TBA =

TBA/(TBA + TBC) × 100, where EBA/EBC are entries and TBA/TBC time in open/closed arms. Bars show mean ± SEM and circles individual animals (n = 7 for controls, n = 6 for each CTX-3C dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control of the same sex (Dunnett's test after two-way ANOVA). The sex x dose interaction p value is shown in the right-hand panels.

Figure 5. Acetylcholinesterase (AChE) activity in the hippocampus (A, B), PFC (C, D) and striatum (E, F) of female (A, C, E) and male (B, D, F) rats treated with vehicle (control) or CTX-3C (1.5, 5 or 15 ng/kg). Bars show mean ± SEM and circles individual animals (control, n = 6-7; CTX-3C, n = 4-5 per dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. the control group of the same sex (Dunnett's test after two-way ANOVA on log-transformed data). The sex x dose interaction p value is shown in the male panels.

Figure 6. Total antioxidant capacity (TAC) in the hippocampus (A, B), PFC (C, D) and striatum (E, F) of female (A, C, E) and male (B, D, F) rats treated with vehicle (control) or CTX-3C (1.5, 5 or 15 ng/kg). Bars show mean ± SEM and circles individual animals (control, n = 6-7; CTX-3C, n = 4-5 per dose). *p < 0.05, **p < 0.01, ***p < 0.001 vs. the control group of the same sex (Dunnett's test after two-way ANOVA on log-transformed data). The sexx× dose interaction p value is shown in the male panels.

Figure 7. Acetylcholinesterase (AChE) activity (A, C, E) and total antioxidant capacity (TAC; B, D, F) in the hippocampus (A, B), PFC (C, D) and striatum (E, F) of female rats treated with vehicle (control) or C-CTX-1 (0.15, 0.7 or 1.5 ng/kg). Bars show mean ± SEM and circles individual animals (control, n = 6-7; C-CTX-1, n = 4-5 per dose). Values are absolute values (TAC was measured with a different kit and its own control group, so

TAC values are not comparable with those of Figure 6). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control (Dunnett's test after one-way ANOVA on log-transformed data).

Figures

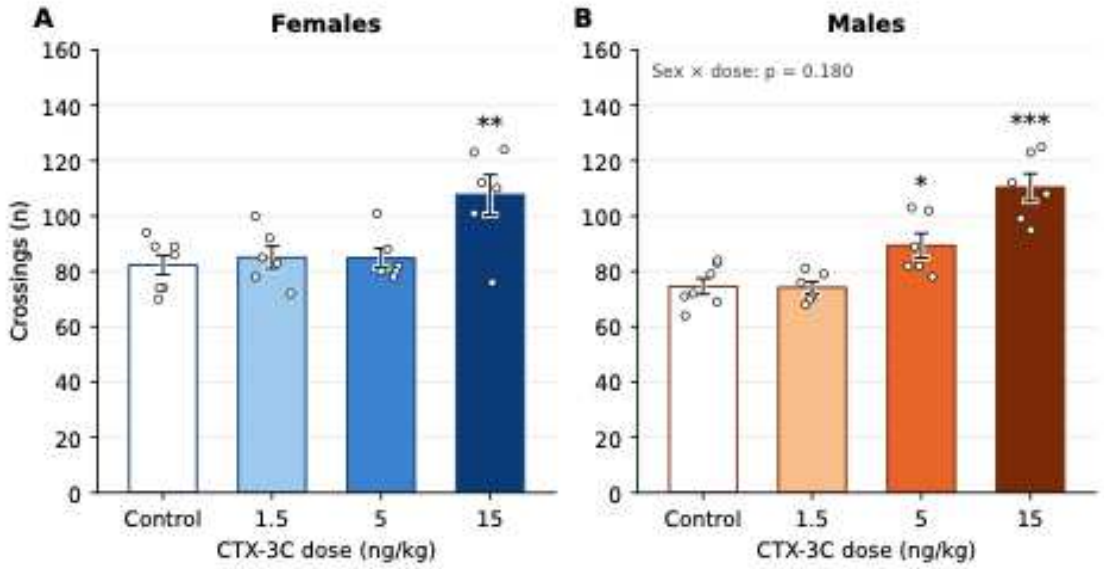


Figure 1

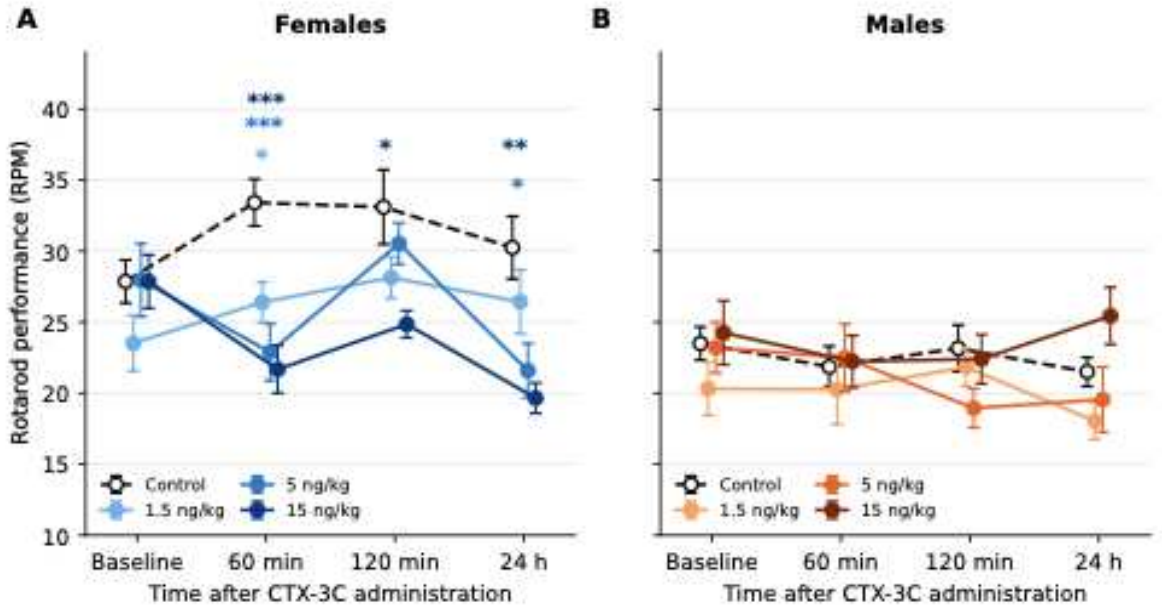


Figure 2

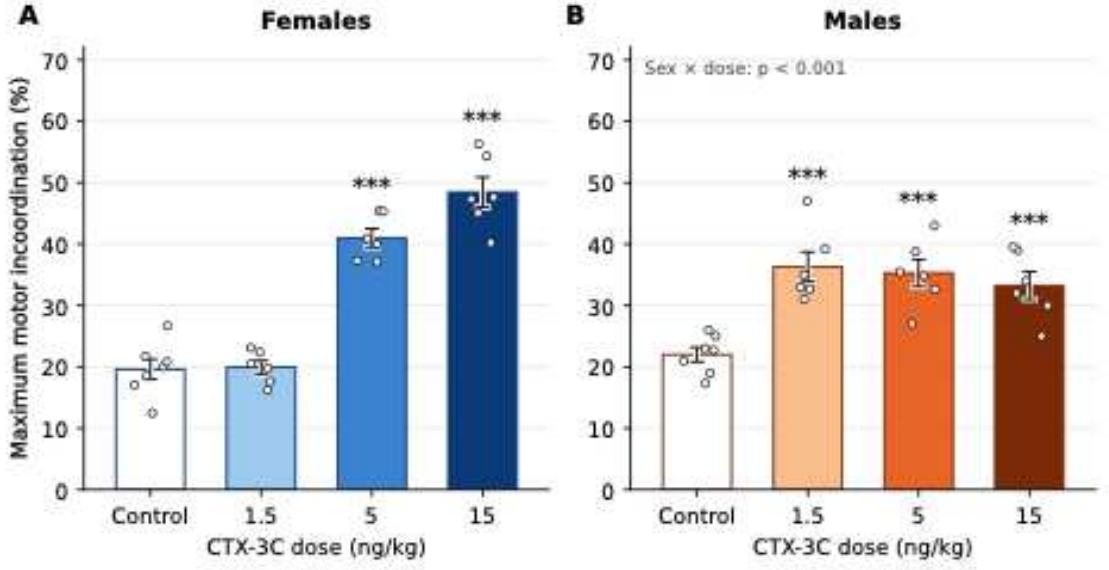


Figure 3

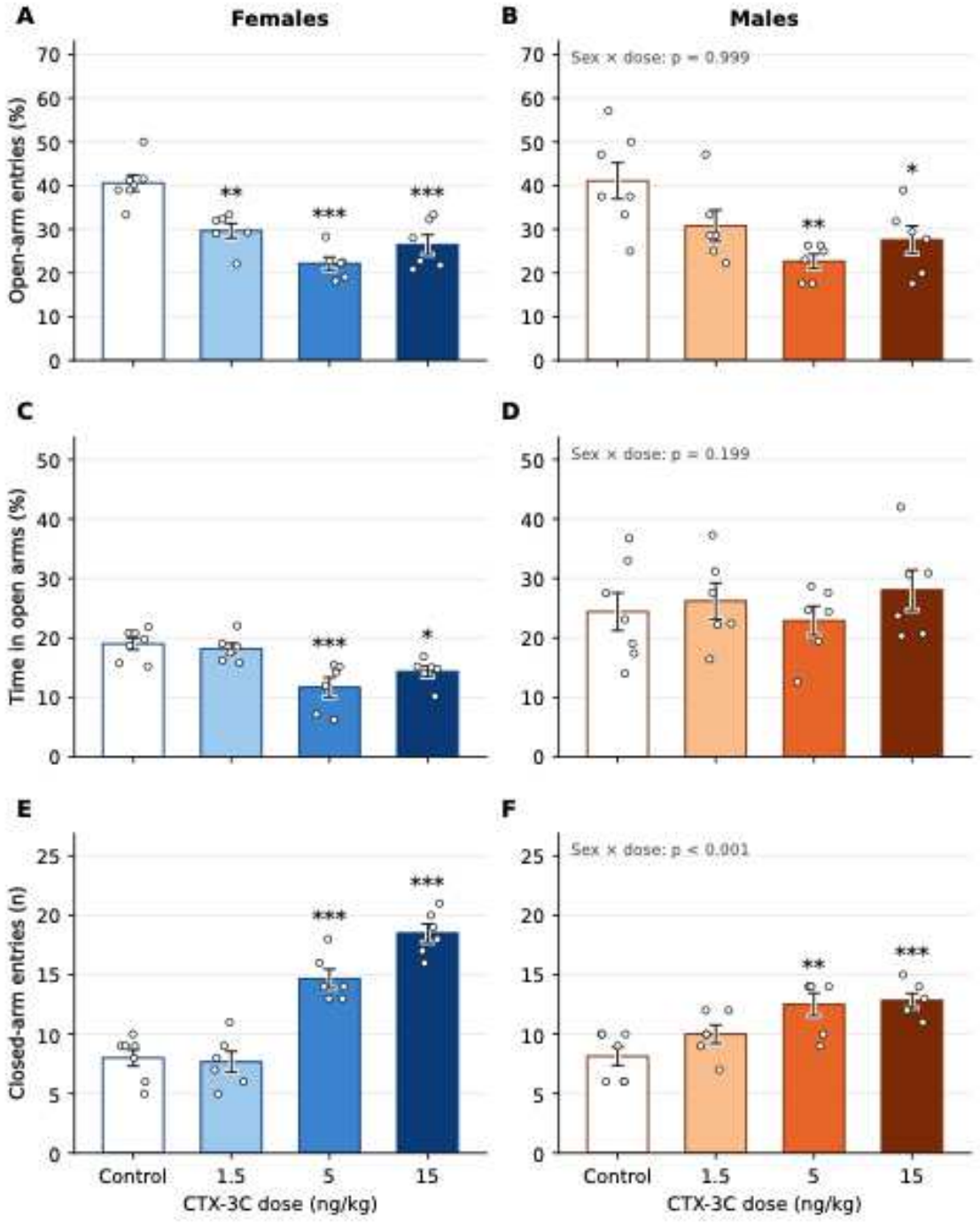

A
Females
B
Males
Open-arm entries (%)
Sex × dose: p = 0.999
C
D
Time in open arms (%)
Sex × dose: p = 0.199
E
F
Closed-arm entries (n)
Sex × dose: p < 0.001
Control
1.5
5
15
CTX-3C dose (ng/kg)

Figure 4

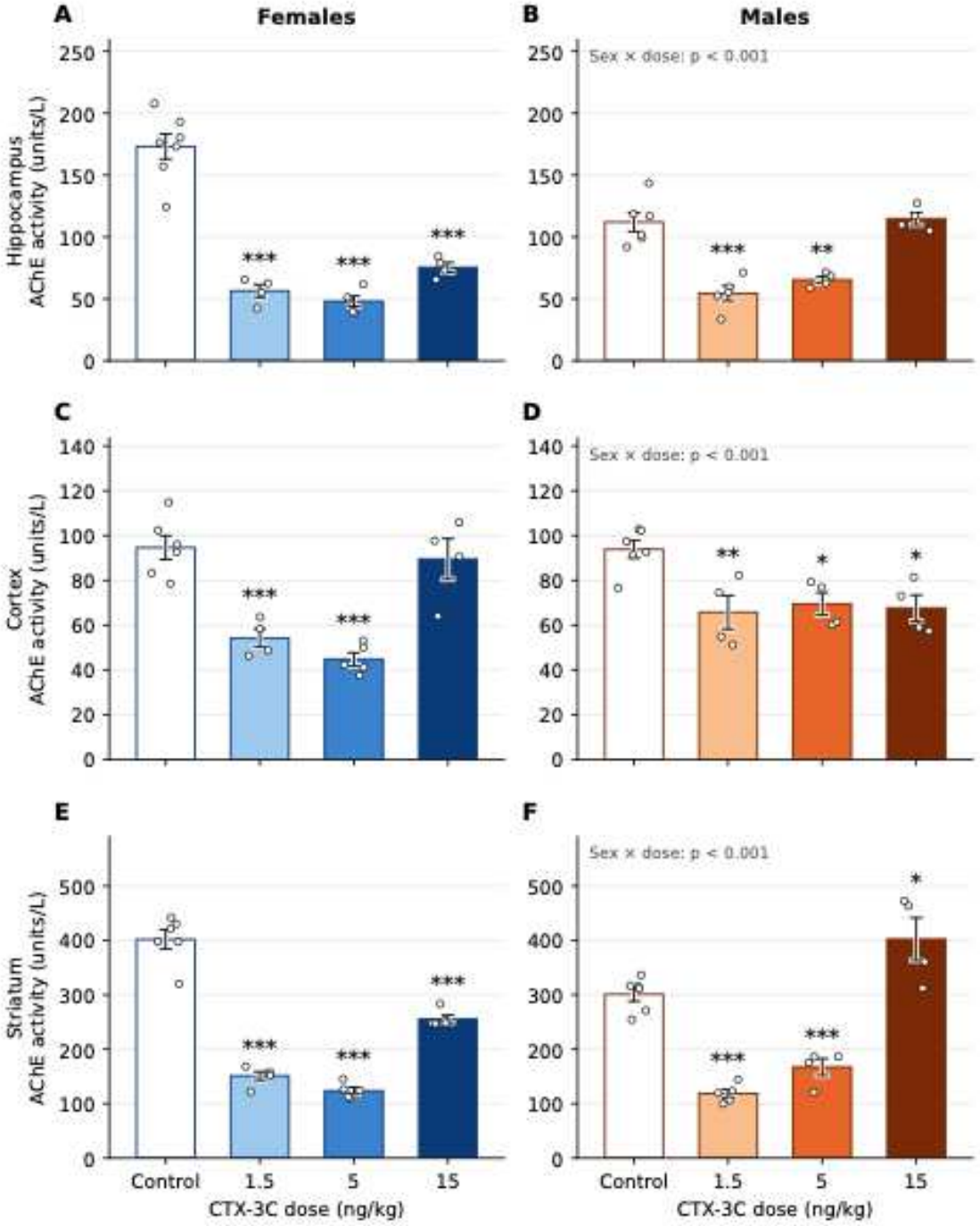

A
Females
B
Males
Sex × dose: p < 0.001
Hippocampus
AChE activity (units/L)
Cortex
AChE activity (units/L)
Striatum
AChE activity (units/L)
C
D
E
F
Control
1.5
5
15
CTX-3C dose (ng/kg)

Figure 5

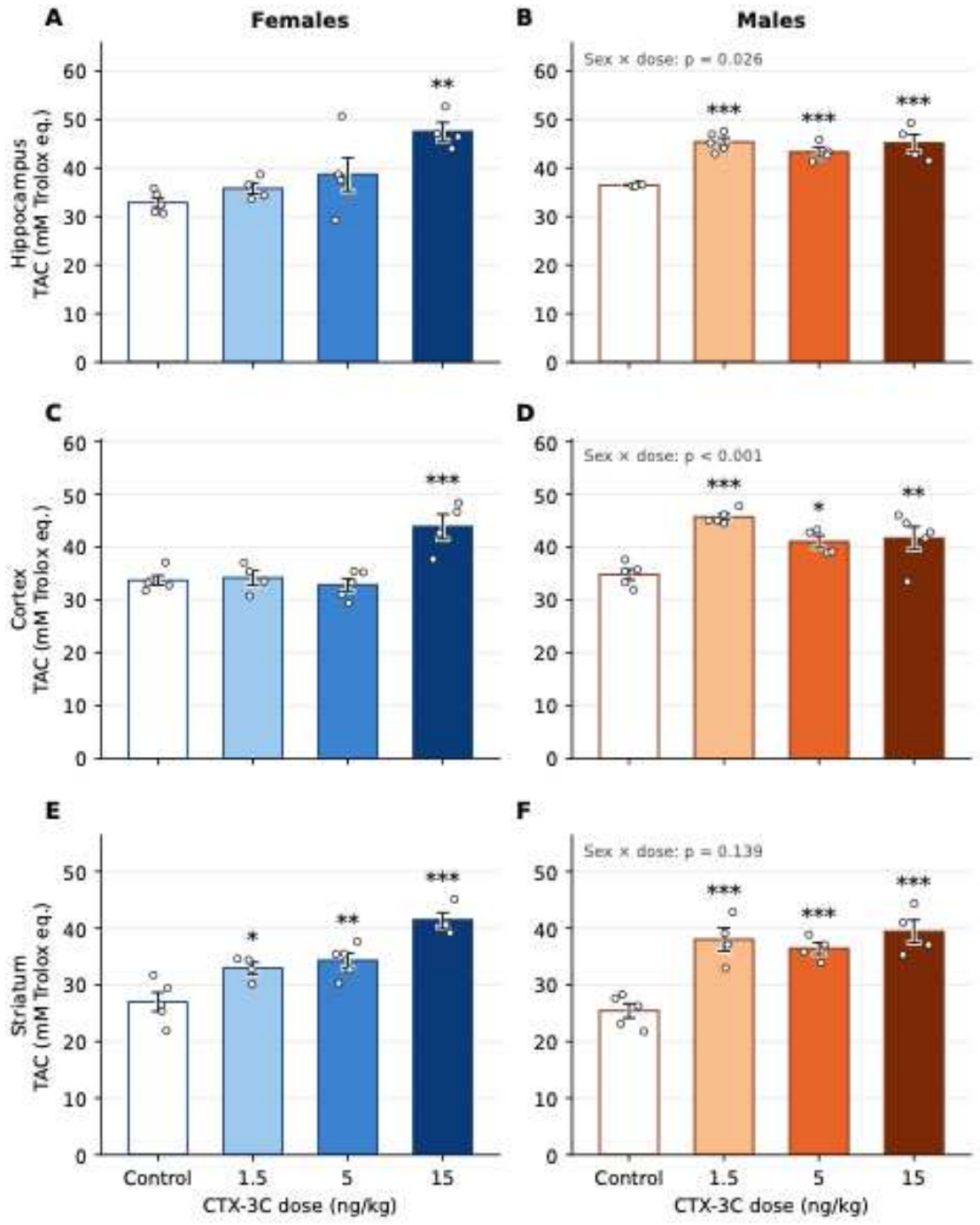

A
Females
B
Males
Sex × dose: p = 0.026
C
D
Sex × dose: p < 0.001
E
F
Sex × dose: p = 0.139
Hippocampus TAC (mM Trolox eq.)
Cortex TAC (mM Trolox eq.)
Striatum TAC (mM Trolox eq.)
Control
1.5
5
15
CTX-3C dose (ng/kg)

Figure 6

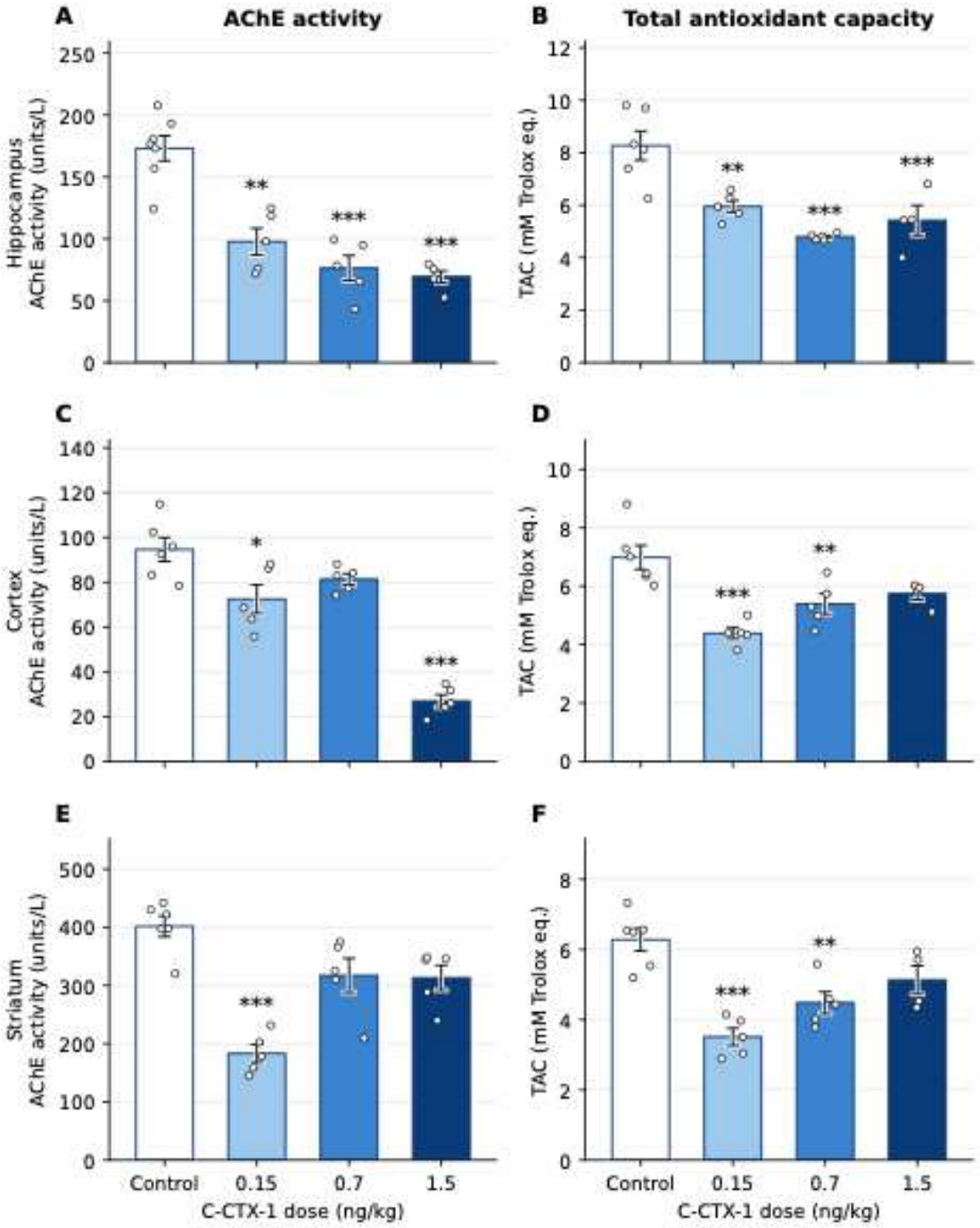

A
AChE activity
B
Total antioxidant capacity
Hippocampus
AChE activity (units/L)
TAC (mM Trolox eq.)
C
D
Cortex
AChE activity (units/L)
TAC (mM Trolox eq.)
E
F
Striatum
AChE activity (units/L)
TAC (mM Trolox eq.)
Control
0.15
0.7
1.5
C-CTX-1 dose (ng/kg)

Figure 7

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuplementarytablesAChETAC.docx