EFFECTS OF THE FOOD ADDITIVE MONOSODIUM GLUTAMATE ON CISPLATIN-INDUCED GASTROINTESTINAL DYSMOTILITY AND PERIPHERAL NEUROPATHY IN THE RAT

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EFFECTS OF THE FOOD ADDITIVE MONOSODIUM GLUTAMATE ON CISPLATIN-INDUCED GASTROINTESTINAL DYSMOTILITY AND PERIPHERAL NEUROPATHY IN THE RAT

Running title: MSG neuroprotection, cisplatin & GI motility

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ABSTRACT

Background: Cisplatin is an antineoplastic drug known to produce intense vomiting, gastric dysmotility, and peripheral neuropathy. Monosodium glutamate (MSG) is a flavor enhancer with prokinetic properties potentially useful for cancer patients under chemotherapy. Our aim was to test whether MSG may improve gastrointestinal motor dysfunction and other adverse effects induced by repeated cisplatin in rats.

Methods: Male Wistar rats were exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. On the first day of weeks 1–5, rats were treated with saline or cisplatin (2 mg kg^{-1} $week^{-1}$, ip). Gastrointestinal motility was measured by radiological methods after first and fifth administrations, as well as one week after treatment finalization. One week after treatment, the threshold for mechanical somatic sensitivity was recorded. Finally, samples of stomach, terminal ileum and kidneys were evaluated in sections using conventional histology. The myenteric plexus was immunohistochemically evaluated on distal colon whole-mount preparations.

Key Results: MSG prevented the development of cisplatin-induced neuropathy and partially improved intestinal transit after the fifth cisplatin administration with little impact on gastric dysmotility. MSG did not improve the histological damage of gut wall, but prevented the changes induced by cisplatin in the colonic myenteric plexus.

Conclusion and Inferences: Our results suggest that MSG can improve some dysfunctions caused by anticancer chemotherapy in the gut and other systems, associated, at least partially, with neuroprotectant effects. The potentially useful

adjuvant role of this food additive to reduce chemotherapy-induced sequelae warrants further evaluation.

KEY WORDS

Cisplatin, monosodium glutamate, gastric emptying, intestinal transit, peripheral neuropathy, rat, myenteric.

KEY POINTS

- Antitumoral drugs such as cisplatin produce many adverse effects. In rats, repeated cisplatin induces gastrointestinal dysmotility, neuropathic signs and histological damage of gut and kidney.
- The food additive MSG included in drinking water prevented sensory neuropathy, gut dysmotility and myenteric plexus changes induced by cisplatin. Thus, neuroprotection could be involved in the preventive effects of MSG on gut motor function.
- The potential neuroprotectant effect of dietary MSG could be useful in the context of cancer chemotherapy.

1. INTRODUCTION

Chemotherapy is a non-surgical method widely used to treat cancer. The clinical use of antineoplastic drugs is associated with many side effects such as diarrhea, nausea, vomiting, anorexia, mucositis and peripheral neuropathy, both somatic and enteric[1–10]. Chemotherapy-induced anorexia may be related to the development of taste alterations[11] and loss of appetite, which may contribute to malnutrition[12].

Cisplatin is an antineoplastic drug used in the treatment of different types of cancer. It is very emetogenic and serves as reference for the preclinical development of antiemetics[13–15]. Cisplatin produces changes in gastric emptying, which appears delayed, along with distension of the stomach, which produces satiety sensation and, consequently, decreases appetite, both in humans[8] and in rats[16]. Although the current antiemetics, particularly serotonin 5-HT_3 antagonists are able to prevent/alleviate cisplatin-induced gastric dysmotility and nausea/voimiting[17–20], protection is often not complete and antiemetics may induce their own adverse effects. Other approaches might prove helpful in this context.

Monosodium glutamate (MSG) is the sodium salt of L-glutamic acid (or glutamate), an amino acid that occurs naturally, free or as part of proteins, in many foods[21,22]. MSG produces a special flavor called "umami" (tasty), which corresponds to the flavor of the foods that contain it, such as meat, mature cheeses or typical Asian dishes with soybean[21,22]. It has been used as a flavor enhancer in the food industry for decades and is currently one of the most consumed food additives, also under other names, such as glutamic acid[23] or E-621[24].

Although in laboratory animals MSG has been shown to induce obesity[25–28], from another point of view, the use of MSG incorporated into the diet, could be beneficial in the elderly and in malnourished patients, increasing the palatability of food and appetite[29,30].

Particularly, the substances with "umami" flavor could be useful as taste enhancers in cancer patients treated with chemotherapy. In these patients, of the four basic flavors (sweet, salty, bitter, acid), the ones most affected by chemotherapy are the sweet, salty and bitter flavors[31–33]. In contrast, the thresholds for detection and recognition of the "umami" taste of MSG were not modified[32,33]. Thus, MSG could be used in these patients to improve their nutritional status.

Interestingly, the presence of MSG in the gastrointestinal tract leads to the activation of vagal afferents and, consequently, of several areas of the brain related to gastric motility, which could activate gastric emptying[34]. Likewise, it facilitates duodenal motility, which also accelerates gastric emptying[35]. MSG does not only produce effects on the stomach and duodenum, but also on the colon, in which it enhances motor function, as demonstrated in preclinical studies both *in vivo*[36] and *in vitro*[37].

Thus, the aim of this work was to test, in a rat model, whether the incorporation of MSG to the diet may improve gastrointestinal motor dysfunctions induced by repeated administration of the antineoplastic drug cisplatin. The effects on cisplatin-induced mucositis and myenteric neuropathy were also evaluated.

2. MATERIALS AND METHODS

The experiments were designed and performed in accordance with the EU Directive for the Protection of Animals Used for Scientific Purpose (2010/63/EU) and Spanish regulations (Law 32/2007, RD 53/2013 and order ECC/566/2015) and were approved by the Ethical Committee at Universidad Rey Juan Carlos (URJC). All experiments were designed to minimize the number of animals used and their suffering.

2.1. Animals

Male Wistar rats (250-300 g, n = 46) were obtained from the Veterinary Unit of URJC (Madrid, Spain), and group-housed (3-4/cage) in standard transparent cages (60 cm × 40 cm × 20 cm), under environmentally controlled conditions (temperature = 20°C, humidity = 60%), with a light/dark cycle of 12 h (lights on at 8:00 am). Animals had free access to standard laboratory rat chow (Harlan Laboratories Inc.) and tap water; half of the animals were supplied with water and MSG at a dose of 4 g L^{-1}, corresponding to approximately 0.45 g kg^{-1} day^{-1} (which in turn corresponds to 5.1 g/day for a 70 kg man[36]), previously shown to prevent the development of cisplatin-induced neuropathic pain in the rat[38], without eliciting significant toxic effects[36].

2.2. Experimental protocol

Half of the rats were exposed to MSG (4 g L^{-1}) in drinking water from week 0 till one week after treatment. On the first day of weeks 1–5, the different groups of rats received one intraperitoneal (ip) injection of cisplatin (at 2 mg kg^{-1}) or saline (0.9% NaCl w/v). This dose and route of delivery are commonly used in the rat to induce a wide range of toxic effects[10,16,39–41] that are observed in humans and lie within the limits of tolerable toxicity. To reduce cisplatin-induced nephrotoxicity, 2 mL of saline

were injected subcutaneously 20 min before ip saline or cisplatin[39]. Body weight gain, food ingestion and water ingestion were measured weekly. Gastrointestinal motility was measured by radiological methods after the first and fifth cisplatin or saline administration, as well as one week after treatment finalization. One week after treatment, still during exposure to MSG, the threshold for mechanical somatic sensitivity was recorded. At the end of the study, animals were sacrificed, and different samples were obtained for further studies. The timeline of our experimental protocol is shown in Fig. 1A. The time points for evaluation of each parameter were selected based on our previous studies of cisplatin-induced side effects[10,16,17,40,42-44], to minimize the number of animals used. All evaluations were performed blindly.

2.3. Mechanical allodynia

Mechanical allodynia is a sign of peripheral neuropathy induced by cisplatin[39,40,42]. Rats were placed individually on an elevated iron mesh in a clear plastic cage and allowed to adapt to the testing environment for at least 10 min (this was also done 2-3 days before assessment in order to reduce stress). For assessment, calibrated von Frey hairs (0.9–40 g) were applied to the plantar surface of each hind paw from below the mesh floor. Each stimulus was applied for a maximum duration of approximately 2 s. This was repeated five times with 1–3 s intervals. When at least three out of five trials (60%) evoked paw-withdrawal, the force applied by that particular hair was considered as the tactile threshold. Mechanical allodynia was defined as a significant decrease in tactile threshold evoked by mechanical stimuli.

2.4. Gastric emptying and gastrointestinal transit

Gastrointestinal motor function was studied radiographically without prior fasting, as previously described[45]. On weeks 1, 5 (immediately after ip drug administration) or one week after the last ip injection, 2.5 mL of a suspension of barium sulfate (Barigraf®, 2 g mL^{-1}, t°=22°C) was intragastrically administered. Plain facial radiographs (20 ms) were obtained using a CS2100 (Carestream Dental, Madrid, Spain) digital X-ray apparatus (60 kV, 7 mA) with a focus distance manually fixed to 50±1 cm. Immobilization of the rats in prone position was achieved by placing them inside hand-made, transparent plastic tubes, which were adjusted to the size of the rat. Habituation to these restraint devices prior to commencement of the study did not significantly alter gastrointestinal motility[45]. X-rays were recorded on Carestream Dental T-MAT G/RA film (15×30 cm) housed in a hand-made cassette provided with regular intensifying screen, immediately and 1, 2, 4, 6 and 8 h (T0–T8) after contrast administration. The film cassette was located directly beneath the restraining tube. A rectangular metallic block (3 x 1 x 1 cm) was positioned aside the plastic tube in which the rat was placed so that it could serve as a reference for morphometric and densitometric analyses (see below). While taking the radiographs, the qualified investigator remained at least 2 m away from the X-ray source. Films were developed in a Kodak X-omat 2000 automatic processor. Alterations in gut motility were semiquantitatively determined from the images by assigning a compounded value to each region of the gastrointestinal tract considering the following parameters: percentage of the gastrointestinal region filled with contrast (0–4); intensity of contrast (0–4); homogeneity of contrast (0– 2); and sharpness of the gastrointestinal region profile (0–2). Each of these parameters was scored and a sum (0–12 points) was made. The X-ray images were digitized, and the size and density

of contrast were analyzed for stomach, caecum and fecal pellets, with the aid of an image analysis system (Image J 1.38 for Windows, National Institute of Health, USA, free software: http://rsb.info.nih.gov/ij/; see Figure S1, Supplementary material, for further description [46]). The number and diameter of fecal pellets within the colorectum was also determined for each rat at each time point.

2.5. Histology

Samples were obtained from the gastric body, terminal ileum and kidneys of 6-7 animals per experimental group, fixed in buffered 10% formalin and embedded in paraffin. Sections of 5 μm were stained with conventional hematoxylin-eosin (HE).

Sections were studied under a Zeiss Axioskop 2 microscope equipped with the image analysis software package AxioVision 4.6 to calculate the morphometric parameters. The analysis was made by triplicate in 5-8 random fields measured in 20-40x objective microphotographs per section and specimen.

Histological damage of terminal ileum was evaluated in sections stained with HE using criteria adapted from Galeazzi et al[47]. A numerical score of 0–9 was assigned to each section considering general loss of mucosal architecture (graded 0–3, absent to severe), extent of inflammatory cell infiltrate (graded 0–3, absent to transmural), crypt abscess formation (0–1, absent or present), goblet cell depletion (0–1, absent or present) and muscular layer thickness (0–1, normal to reduced).

Gastric damage was evaluated by the presence of ulcers, abscesses, atrophy and dysplasia as previously described[48]. Kidneys were evaluated by the presence of tubular, glomerular and mesangial damage[49]. The analysis was independently carried out by two experienced pathologists.

2.6. Whole-mount preparations

Conventional methods for immunohistochemistry[10,50] were applied to longitudinal muscle-myenteric plexus whole-mount preparations to evaluate the effects on the myenteric plexus. Distal colon samples (2 cm long) were obtained, placed in saline and rapidly stretched and pinned on a Sylgard-coated dish (VWR, Barcelona, Spain). After conventional fixation with Zamboni's fixative and clearing with DMSO (3x10 min) and phosphate buffered saline (PBS, 3x10 min), mucosa, submucosa and circular muscle were removed. Preparations were stored at 4ºC in PBS with sodium azide (0.1%), until immunohistochemical processing.

Tissues were incubated (36 h at room temperature, RT) with a mixture of both the pan-neuronal marker HuC/D (1:500; mouse biotin-conjugated, A-21271, from Thermo Fisher Scientific, Madrid, Spain) and sheep anti-nNOS (neuronal nitric oxide synthase, 1:500; Sigma-Aldrich, AB1529, from Merck, Madrid, Spain). After washing with PBS (3x10 min), tissues were exposed for at least 3 h at RT to a mixture of streptavidin-AlexaFluor 488 (1:500, S11223, Thermo Fisher), and donkey anti-sheep-RRX (1:500; 713-295-003, from Jackson, Ely, Cambridgeshire, UK). After washing with PBS (3x10 min), preparations were dehydrated in 50%-70%-100% buffered glycerol (10 min each) and mounted on slides.

The preparations were observed under a fluorescence Zeiss Axioskop 2 microscope, equipped with the image analysis software package AxioVision 4.6. In the study, 5-7 whole-mount preparations per group, from different animals, were used. The analysis was made in 5-8 non-overlapping microphotographs per preparation, obtained with the 10x objective. Different parameters were analyzed manually or with the aid of

Image J: number of ganglia per surface unit (ganglia were considered as a set of 3 or more neurons); ganglionic size; number of HuC/D-immunoreactive (IR) cell bodies per total surface unit (neuronal density), per ganglionic surface (intraganglionic or packing neuronal density[50]) and per ganglion; and proportion of nNOS-IR relative to HuC/D-IR myenteric neurons. A semiquantitative analysis of the density of the nNOS-IR nerve fibers was also performed by assigning different values to visually different densities in the same fields evaluated previously for cell bodies and ganglia: 0 – non-existing; 1 – low; 2 – normal; 3 – high.

2.7. Compounds and drugs

MSG was obtained from Productos Químicos Manuel Riesgo SA (SS061/1000), diluted in sterile water (4 g L^{-1}) and kept at room temperature of the Veterinary Unit in transparent bottles that were changed twice a week. Barium sulfate (Barigraf® AD, Juste SAQF, Spain) was suspended in tap water and continuously hand-stirred until administration. Cisplatin was purchased from Sigma-Aldrich (Spain) and dissolved in saline (sonicated for about 15 min). Saline/cisplatin volumes were adjusted to a maximum of 2.5 mL kg^{-1}.

2.8. Statistical analysis

Data are presented as the mean values ± SEM. Normality was assessed using Kolgomorov-Smirnov test. Differences were analyzed using one- or two-way ANOVA tests (followed by *post-hoc* Tukey's multiple comparison test) and Kruskal-Wallis test (followed by *post-hoc* Dunn's test multiple comparison test) for parametric and non-parametric data, respectively. Values of $P<0.05$ were considered significantly different. Statistical analysis was performed using GraphPad Prism, v. 7.0.

3. RESULTS

3.1. Weight, intakes and somatic mechanical sensitivity

All animals had approximately the same initial weight, which progressively increased over time (Figure 1B). Cisplatin-treated animals gained the least weight throughout the experiment. MSG slightly increased body weight gain in both saline- and cisplatin-treated animals compared to the corresponding groups drinking plain water. The only statistically significant difference occurred one week after cisplatin treatment cessation between saline + water and saline + MSG groups.

Along weeks 0-5, raw average daily food and liquid intakes of the control group were around 21 g and 28.5 mL (per rat and day), respectively. Raw average liquid intake tended to increase in animals treated with MSG (34 ± 3.8 mL/rat/day), cisplatin (34.5 ± 3 mL/rat/day) or their combination (38.6 ± 1.5 mL/rat/day). However, when normalized to body weight, daily intakes were not significantly modified along the experimental weeks (Figures 1C, D).

One week after treatment cessation, mechanical sensitivity threshold was approximately 20-25 g in all animals, except cisplatin + water group. In this group, the the pressure threshold needed for paw withdrawal was significantly decreased, indicating the presence of mechanical allodynia, which was prevented by co-treatment with MSG (Figure 1E).

3.2. Gastrointestinal motor function

Semiquantitative study

In control (saline + water) rats, during the first radiographic session, gastric emptying was progressive and only a low amount of barium was still visible in the stomach 8 h

after its intragastric administration (Figure 2A). Barium content reached its maximum in the small intestine in just 1 h and this part of the gut was practically empty by 8 h (Figure 2B). Barium started to stain the caecum and the colorectum 2 and 4 h after intragastric administration, respectively. Both organs filled progressively until the end of the study (Figure 2C-D). When this experiment was performed immediately after the 5th administration (week 5) (Figure 3A-D) and 1 week after treatment finalization (week 6) (Figure 4A-D), similar curves were obtained (Figure S2, Supplementary material).

Exposure to MSG in drinking water induced minor (and probably of scarce clinical relevance) alterations in gastrointestinal motility as compared with control (saline + water) animals, irrespective of the time point evaluated (Figures 2-4).

In contrast, after its first administration, cisplatin delayed gastric emptying (Figure 2A) and filling of the small intestine (Figure 2B), without further significant modifications by MSG. Emptying of the small intestine (Figure 2B) and filling of caecum and colorectum (Figures 2C, D) were as in the control group, irrespective of treatment.

The effect of cisplatin on gastric emptying was more intense after the fifth than after the first administration (this is observed as higher values at longer time-points of the radiographic sessions in the semiquantitative study) (Figure 2A, 3A). At this time point (after fifth cisplatin administration), MSG showed a tendency to improve cisplatin-induced gastric dysmotility, although the difference did not reach statistical significance. In the small intestine, cisplatin behaved similarly to the control group after the fifth administration and MSG did not modify its effect (Figure 3B). Filling of caecum of the animals treated with cisplatin + water was significantly delayed

compared with control rats, but MSG significantly improved this parameter (Figure 3C). Filling of colorectum in cisplatin-treated animals did not suffer any statistically significant alteration after the fifth administration, irrespective of the presence of MSG (Figure 3D).

One week after treatment cessation, the motility curves were practically overlapping for all gastrointestinal regions with only two minor (and probably of scarce clinical impact) statistically significant differences compared with control animals: cisplatin slightly delayed small intestine emptying at 8 h after barium; in cisplatin + MSG group, gastric emptying was slightly delayed at 8 h after barium (Figure 4).

In summary, MSG did not significantly modify the motility curves of any gastrointestinal region at any time point when given alone. Cisplatin delayed gastric emptying after its first administration, and this effect was aggravated with treatment repetition, but practically disappeared one week after treatment cessation. Cisplatin also delayed arrival of barium to caecum (upper gastrointestinal transit) after its last administration and this was the only effect partially (but significantly) improved by cotreatment with MSG. Representative images can be seen in Figures 2E-F, 3E-F and 4E-F.

Quantitative analyses

Morphometry and densitometry curves for stomach, caecum and number of fecal pellets in the colon reflected the temporal changes found in the semiquantitative study but allowed some particular details to be studied.

Thus, maximum size of stomach (at 0 h after barium) was around 450-500 mm^2 in all groups in the three radiographic sessions, without statistically significant differences

amongst them (Figure 5A, B, C). In contrast, the maximum size of the caecum, reached at 4-8 h after barium in all sessions, was around 600-700 mm^2 in the first and last X-ray sessions for all groups (Figure 6A, B, C). However, after the fifth ip drug administration, caecum maximum size in control, MSG-only, cisplatin-only and cisplatin + MSG groups were around 670, 740, 545 and 620 mm^2, respectively. Thus, cisplatin tended to reduce this parameter, and MSG tended to increase it, whether alone or combined with cisplatin (counteracting its effect). Maximum density in either the stomach (Figure 5A', B', C') or the caecum (Figure 6A', B', C') was scarcely affected by treatments and the temporal changes paralleled those found in the semiquantitative study (Figures 2-4).

The number of fecal pellets found within the colon progressively increased from 2 to 8 h in all groups, with slight differences compared with control. Thus, after the first administration all groups showed less fecal pellets at 8 h, although the difference with control was only significant for cisplatin- and MSG-treated groups (Figure 7A). After the fifth administration, compared with control, cisplatin reduced the number of fecal pellets at 6 and 8 h, but MSG normalized this, the difference with cisplatin-only treated animals being statistically significant at 8 h (Figure 7B). One week after treatment finalization, the curves were overlapping for all treatments (Figures 7C).

After the first administration, pellets from MSG-only treated animals were slightly bigger and slightly less dense compared with the remaining groups (Figures 7A', A''). After the fifth administration, the size of fecal pellets from the animals treated with both cisplatin and MSG were slightly bigger and less dense than those from the other groups (Figure 7B', B''). One week after treatment cessation, the differences were only minor compared with control (Figure 7C', C''). Diameter of fecal pellets showed

similar tendencies as mentioned for size (Figure S3, Supplementary material). In any case, none of these changes in size, diameter or density of fecal pellets was statistically significant.

3.3. Histological analysis

The histological appearance in HE stained sections of the intestinal wall is shown in Figure 8A-D. A general damage was observed after cisplatin administration (Figure 8C), and MSG could not prevent the damage in the small intestine architecture (Figure 8D). Cisplatin-induced damage was statistically significant (Figure 8E) and affected several aspects of the intestinal wall. Thus, villi height (Figure 8F) and thickness of the submucosa were reduced, although differences were only statistically significant for the last parameter (Figure 8G). Submucosa thickness was slightly further decreased in animals treated with cisplatin + MSG (Figure 8G).

The histological appearance in HE stained sections of the gastric body and kidneys is shown in Figure S4A-H (Supplementary material). Compared with control animals and those treated only with MSG, in animals treated with cisplatin (alone or with MSG), the gastric muscle seemed to be somehow atrophic (A-D). Furthermore, tubular damage was observed in kidneys (E-H) after cisplatin administration, and MSG did not improve this effect either.

3.4. Whole-mount preparations

Representative microphotographs and quantitative results for whole-mount preparations of the distal colon are shown in Figure 8H-O. Density of ganglia (Figure 8L) and density of neurons per surface unit (Figure 8M) as well as intraganglionic neuronal density (Figure S5B, Supplementary material) were slightly (but not

significantly) reduced in cisplatin-treated animals, and MSG tended to normalize these parameters. The mean number of neurons per ganglia was around 37-39 neurons in all groups except for MSG group, which had a mean value of around 30 neurons per ganglion, but the differences were not statistically significant (Figure 8N). The ganglionic size was also slightly reduced in MSG alone-treated animals compared with the other groups, but the differences did not reach statistical significance (Figure S5A). The percentage of nNOS-IR neurons was significantly increased in cisplatin-treated animals and MSG normalized these values (Figure 8O). Compared with control animals, density of nNOS-IR fibers was slightly (but not significantly) reduced in all other experimental groups (Figure S5C).

4. DISCUSSION

Here we evaluated if the food additive MSG might be useful to prevent gastrointestinal motor dysfunctions and associated mucositis and changes in myenteric innervation induced by repeated administration of the antitumoral drug cisplatin in the rat.

Cisplatin reduces body weight gain[43,45] and food intake[16,40,46,51,52]. However, anorexia was not evident, maybe because we estimated daily food intake from weekly measurements, and anorexia might be restricted to the first 24 h after each cisplatin administration, with compensatory increases afterwards[16,40]. Therefore, the mild weight reduction observed here might be better explained by other factors, such as impaired nutrient absorption due to mucositis (present results,[10,44]) and, perhaps, diarrhea, increased urination or dehydration (measurable in the rat as the dorsal fold

sign[53]), but probably not to spontaneous locomotor activity alterations, absent in previous reports[42,43].

MSG, alone or combined with cisplatin, did not significantly modify weight or food intake. Despite its sodium content, MSG alone did not produce any remarkable alterations in the kidneys or the cardiovascular system in control animals[36], and it did not exacerbate cisplatin-induced histological kidney damage either (present results). Indeed, MSG did not compromise the general health of control or cisplatin-treated animals but exerted beneficial effects *versus* some cisplatin-induced toxicities.

As previously shown by us[10,40,42,43] and others[39,54], cisplatin induced mechanical allodynia, a sign of sensory neuropathy (a relatively long-lasting chemotherapy-induced side effect with high impact on patient´s quality of life[6]), when measured 1 week after treatment finalization. This effect was sensitive to MSG administration, as in previous reports showing that MSG has significant neuroprotective effects in cisplatin-induced peripheral neuropathy[38,55]. Our previous studies with oxaliplatin demonstrate that platinum-based anti-cancer agents induce oxidative stress leading to neuronal damage and death underlying post-treatment dysfunctions[7]. MSG -induced neuroprotection may be due to improvements in the endogenous antioxidant profile, with reduction of lipid peroxidation (malondialdehyde, MDA) and in glutation (GSH) levels[56], and probably to an indirect effect on microtubules after interaction with a receptor found only on neural cells[55]. Further studies are warranted to determine the pathways, receptors and mediators involved in the neuroprotective effects of MSG, including the possibility that it might prevent platinum accumulation (another mechanism underlying long-term peripheral sensory neuropathy[57,58]).

Numerous clinical and preclinical studies[43,45,59] show that cisplatin provokes delayed gastric emptying and gastric distension, leading to satiety and, consequently, decreased appetite both in humans[8] and rats[16]. Cisplatin-induced gastric dysmotility is due to the massive release of serotonin from intestinal enterochromaffin cells. Serotonin stimulates 5-HT_3 receptors on vagal afferent fibers and enteric nerves. This relaxes the stomach, particularly the fundus[18], through the activation of the vagovagal reflex, resulting in 5-HT_3 antagonists-sensitive delayed gastric emptying and distension[17,18], as well as gastric retention of food[60,61] and probably gas[61]. As described[17,43,62], cisplatin-induced gastric dysmotility was aggravated after the fifth cisplatin injection compared with the first one but was resolved one week after treatment cessation. Indeed, serotonin-mediated cisplatin-induced gastric dysmotility is an acute event occurring within the 24 h after administration, associated with the development of acute nausea and vomiting in humans[8] (and animal species displaying the vomiting reflex, like ferrets[63]), and acute "pica" (an indirect marker of nausea in non-vomiting species[64]) in rats[16,18,40,43,45,46].

One week after treatment finalization, a tendency to muscle atrophy was detected by histological methods in the gastric body of cisplatin-treated rats, but MSG did not improve it. MSG stimulates glutamate sensors in the stomach and intestine, activating the functions of the digestive tract[22,34]. Whereas some reports showed no change in gastric emptying[65,66], others demonstrated an acceleration after oral MSG administration[22,35]. In our study, MSG tended to improve cisplatin-induced gastric dysmotility, at least after the fifth cisplatin administration. Perhaps a higher dose could be more effective. Interestingly, when MSG was included in solid food instead of water, food (but not liquid) intake improved in cisplatin-treated rats (data not

shown), highlighting that MSG may increase intake of the dietary component to which it is added (water/solid food). Whether this effect is associated with a significant improvement of cisplatin-induced gastric dysmotility is not yet known. Although mild in the present conditions, the effect of dietary MSG on gastric motility could be important in the context of chemotherapy, to somewhat mitigate its impact on patient's quality of life, by increasing appetite and reducing nausea/vomiting.

Cisplatin did not alter the motility curves of the small intestine, but significantly delayed caecum filling after the fifth administration. This could be indirectly due to the delayed gastric emptying, but also to a decrease in the motor function of the small intestine[10,44]. In animals receiving cisplatin and MSG, caecum filling was significantly improved after the fifth administration. This may be mediated by umami receptors, present along the gastrointestinal tract[37,67-69], but it can also be due to its neuroprotective effect against cisplatin, demonstrated for both somatic (sensory neuropathy[38,55]) and visceral innervation (enteric neuropathy, present results). In contrast, the colonic motility curves were practically identical for all treatment groups at all time points, and the analysis of fecal pellets revealed only minor differences after the fifth cisplatin administration. Thus, although MSG can increase colonic motility both *in vivo*[36] and *in vitro*[37], in this study it caused scarce significant effects in colon function, either alone or in animals treated with cisplatin. Other doses or routes of administration might be more efficient. Alternatively, the use of other techniques (intracolonic recording of contractions *in vivo*[36] or organ bath tests, *in vitro*[37]) might allow detecting more subtle changes in colonic motor function. This will be the aim of future studies.

Ileal samples were histologically evaluated to see if the improvements induced by MSG in small intestinal motor function were associated with improvements in the gut wall structure. As described[44], cisplatin produced a stage 4 mucositis, characterized by damage and ulceration of ileal mucosa, as well as a decrease in submucosa layer thickness (in areas without Peyer´s patches). However, mucositis was not very intense. MSG alone did not cause any effect on these parameters. Remarkably, Nakadate et al[70] found that MSG induced the formation of thinner and elongated villi, with less rough endoplasmic reticulum, which may compromise the normal function of the intestine, but they used a high dose (2 mg g^{-1}) injected subcutaneously. Similarly, Feng et al[71], described antagonistic effects of MSG (3% of food weight) in pigs, with detrimental effects on crypt growth and inflammation in the proximal intestine, but beneficial effects on the distal intestine. Importantly, these studies were focused on the relationship of obesity with diets high in MSG. In contrast, other authors have not found any deleterious effect of different MSG doses on murine intestine organoid growth patterns[72]. Noticeably, in cisplatin-treated animals, MSG did not produce any further damage in the gut wall, except for a slight but significant reduction in the submucosa thickness. Although its mechanism and functional significance needs to be more precisely established, this might simply reflect the general deterioration that could not be overcome by MSG (as shown for the kidneys).

Thus, considering the anti-neuropathic effect of MSG (present results and those previously reported[38,55]) and that repeated cisplatin induces an enteric neuropathy affecting the myenteric plexus[10], we hypothesized that the improvement of intestinal motor function induced by MSG in cisplatin-treated animals could be more related to neuroprotection of gut innervation. Therefore, we analyzed the myenteric plexus of

distal colon whole-mount preparations from the different experimental groups (due to technical issues, ileal tissue could not be used in this study). At the dose used, cisplatin significantly increased the proportion of nNOS-IR neurons, mainly involved in the inhibitory motor pathways of intestinal motility. This is the most consistent feature of the enteric neuropathy induced in the myenteric plexus by repeated cisplatin (previously demonstrated in rat colon and ileum by immunohistochemistry and molecular expression studies[10,44], respectively) and oxaliplatin[73,74] (demonstrated by immunohistochemistry in mouse colon), and underlies the intestinal motility unbalance toward inhibition/relaxation produced by both antineoplastic drugs[10,44,73,74]. Importantly, co-treatment with MSG led to normal proportions of nNOS-IR neurons and tended to normalize the other parameters (i.e., ganglionic and neuronal densities) also slightly affected by the relatively low dose of cisplatin used here. Although more research is needed to determine how other myenteric neuronal populations may be affected by cisplatin, MSG or their combination, as well as the mechanisms involved, our data suggest that the neuroprotective effect of MSG is not restricted to somatic peripheral innervation but may also preserve gut innervation (at least intrinsic colonic motor innervation) and explain, at least partially, gastrointestinal motor dysfunction improvement.

4.1. Concluding remarks

In a rat model, gut dysmotility and neuropathic signs induced by repeated administration of the antitumoral drug cisplatin, were improved by incorporation of the food additive MSG in drinking water, at a dose of 4 g L^{-1}. The beneficial effects of MSG on small intestinal transit did not involve mucositis improvement, suggesting that motor components could be more directly involved. Indeed, the proportion of

nNOS myenteric neurons, altered by cisplatin, was normalized by cotreatment with MSG.

Our results suggest that MSG could be considered as a useful adjuvant to improve some dysfunctions caused by anticancer chemotherapy, particularly those involving neurotoxicity.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

RA designed the study and provided financial support. YLT, GV, LLG, RG and JAU performed the experiments and analyzed the data. YLT, RA, and JAU wrote the manuscript. KN provided essential intellectual input. All authors reviewed and approved the final version of the manuscript.

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FIGURE LEGENDS

Figure 1. Experimental protocol and effect of cisplatin and monosodium glutamate (MSG) on general health parameters in the rat. As shown in A (experimental protocol), body weight gain (B), solid food intake (C), liquid intake (D) and threshold for mechanical sensitivity to von Frey hairs (E) were measured in rats intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment (week 6). Experimental groups were: saline + water (dotted line or striped bar, n=12), saline + MSG (blue line/bar, n=11), cisplatin + water (red line/bar, n=8) or cisplatin + MSG (black line/bar, n=7). The threshold for mechanical sensitivity was recorded one week after treatment (n=12-14/group). Data represent the mean ± SEM. * $p<0.05$, **** $p<0.0001$ vs saline + water; #### $p<0.0001$ vs cisplatin + water (two-way ANOVA followed by Tukey´s *post-hoc* test in B-D; one-way ANOVA followed by Tukey´s post hoc test in E).

Figure 2. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat after first cisplatin administration. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered immediately after the first intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11),

cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. ***p<0.001 vs saline + water; ^^ p<0.01, ^^^ p<0.001 vs saline + MSG (two-way ANOVA followed by Tukey´s *post-hoc* test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.

Figure 3. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat after fifth cisplatin administration. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered immediately after the fifth intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * p<0.05, ** p<0.01, ***p<0.001 vs saline + water; ^^ p<0.01, ^^^ p<0.001 vs saline + MSG; # p<0.05, ## p<0.01 vs cisplatin + water (two-way ANOVA followed by Tukey´s *post-hoc* test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.

Figure 4. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat one week after cisplatin treatment finalization. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered one week after cisplatin treatment finalization and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * $p<0.05$, ** $p<0.01$ vs saline + water (two-way ANOVA followed by Tukey´s *post-hoc* test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.

Figure 5. Morphometric and densitometric analysis of the effect of cisplatin and monosodium glutamate (MSG) on the rat stomach. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (A, A') or the fifth (B, B') administration or one week after treatment (C, C'). Gastric size (A, B, C) and gastric density (A', B', C') were analyzed with an image processor (Image J). Experimental groups were: saline + water (dotted line, n=12),

saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * p<0.05, ** p<0.01, ***p<0.001 vs saline + water; ^ p<0.05, ^^ p<0.01, ^^^ p<0.001 vs saline + MSG (two-way ANOVA followed by Tukey´s *post-hoc* test).

Figure 6. Morphometric and densitometric analysis of the effect of cisplatin and monosodium glutamate (MSG) on the rat caecum. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (A, A') or the fifth (B, B') administration, or one week after treatment (C, C'). Caecum size (A, B, C) and caecum density (A', B', C') were analyzed with an image processor (Image J). Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * p<0.05, *** p<0.001 vs saline + water; # p<0.05, ### p<0.001 vs cisplatin + water (two-way ANOVA followed by Tukey´s *post-hoc* test).

Figure 7. Quantitative, morphometric and densitometric analysis of the effect of cisplatin and monosodium glutamate (MSG) on the rat fecal pellets. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (A, A', A'') or the fifth (B, B', B'') administration, or

one week after treatment (C, C', C''). Fecal pellets were counted (A, B, C) and their size (A', B', C') and density (A'', B'', C'') were analyzed with an image processor (Image J). Experimental groups were: saline + water (dotted line or striped bar, n=12), saline + MSG (blue line/bar, n=11), cisplatin + water (red line/bar, n=12) or cisplatin + MSG (black line/bar, n=11). Data represent the mean ± SEM. * p<0.05, ** p<0.01 vs saline + water; ### p<0.001 vs cisplatin + water (two-way ANOVA followed by Tukey´s *post-hoc* test for Number; one-way ANOVA followed by Tukey´s post hoc test for Morphometry and Densitometry).

Figure 8. Effect of cisplatin and monosodium glutamate (MSG) on the structure of the rat small intestinal wall and distal colon myenteric plexus. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment (week 6). Samples were obtained on week 6. Experimental groups were: saline + water (A, H, H', striped bar, n=5-7), saline + MSG (B, I, I', blue bar, n=7), cisplatin + water (C, J, J', red bar, n=5-7) or cisplatin + MSG (D, K, K', black bar, n=7). Histological samples of the small intestine were embedded in paraffin and sections stained with HE (representative images in A-D). Histological damage (E), villi size (F) and submucosa thickness (G) were evaluated. Whole mount distal colon longitudinal muscle-myenteric plexus preparations were processed to detect the neurons expressing HuC/D (pan-neuronal marker) and neuronal nitric oxide synthase (nNOS, mainly present in myenteric neurons involved in inhibitory motor circuits). With the aid of Image J, different parameters were evaluated: density of ganglia (L) and HuC/D-IR neurons (M) vs serosal surface unit, number of neurons/ganglion (N) and proportion (%) of nNOS-IR vs HuC/D-IR

neurons (O). Data represent the mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001 vs saline + water; # p<0.05, ### p<0.001 vs cisplatin + water (Kruskal-Wallis followed by Dunn's *post-hoc* test, E-G; one-way ANOVA followed by Tukey´s *post-hoc* test, L-O). Bar 100 µm.

SUPPLEMENTARY MATERIAL – FIGURES LEGENDS

Figure S1. Procedure to measure organ size with Image J. Before taking any X-ray, a 30 mm-long metallic block is placed by the animal so that it can be used as a scale bar for morphometric and densitometric analyses. With Image J (Image J 1.38 for Windows, National Institute of Health, USA, free software: http://rsb.info.nih.gov/ij/) we set its length as our scale and then select the region of interest from which the area can be automatically obtained. In the example, the size of this particular stomach is 512.094 mm^2. This bar also serves as a reference for densitometry (100% for its color intensity).

Figure S2. X-Ray analysis of GI motility in control rats along time. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) for 5 consecutive weeks (weeks 1-5). Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (continuous black line) or the fifth administration (dotted black line), or one week after treatment finalization (dotted purple line). Stomach size (E), stomach density (F), caecum size (G) and caecum density (H) were analyzed with an image processor (Image J). Data

represent the mean ± SEM (n=12). * p<0.05, ** p<0.01 vs first administration; ^^^ p<0.001 vs fifth administration (two-way ANOVA followed by Tukey´s *post-hoc* test).

Figure S3. Effect of cisplatin and monosodium glutamate (MSG) on the diameter of the rat fecal pellets within the colon (as seen on the X-rays). The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast. Fecal pellets diameter found within the colon was analyzed with an image processor (ImageJ), immediately after the first (A) or the fifth (B) administration, or one week after treatment cessation (C). Experimental groups were: saline + water (striped bar, n=12), saline + MSG (blue bar, n=11), cisplatin + water (red bar, n=12) or cisplatin + MSG (black bar, n=11). Data represent the mean ± SEM (two-way ANOVA followed by Tukey´s *post-hoc* test).

Figure S4: Effect of cisplatin and monosodium glutamate (MSG) on the general histological structure of the rat gastric body and kidney. Representative figures in HE of the gastric body (A-D) and kidney (E-H) of rats intraperitoneally administered with saline (2.5 mL kg^{-1}) (A, B, E, F) or cisplatin (2 mg kg^{-1}) (C, D, G, H) for 5 consecutive weeks (weeks 1-5) and exposed (B, D, F, H) or not (A, C, E, G) to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Histological samples embedded in paraffin sections were obtained one week after treatment. Bar: 100 µm. Experimental groups were: saline + water (A, E), saline + MSG (B, F), cisplatin + water (C, G) or cisplatin + MSG (D, H).

Figure S5: Effect of cisplatin and monosodium glutamate (MSG) on the structure of the rat distal colon myenteric plexus. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or cisplatin (2 mg kg^{-1}) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to one week after treatment. Whole mount distal colon longitudinal muscle-myenteric plexus preparations were processed to detect the neurons expressing HuC/D (pan-neuronal marker) and neuronal nitric oxide synthase (nNOS, mainly present in myenteric neurons involved in inhibitory motor circuits). With the aid of Image J, different parameters were evaluated (see methods for further details): ganglion size (A), intraganglionic neuronal density (B), and density of nNOS-IR nerve fibers (C). Experimental groups were: saline + water (A, striped bar, n=5), saline + MSG (B, blue bar, n=7), cisplatin + water (C, red bar, n=5) or cisplatin + MSG (D, black bar, n=7). Bars show mean values ± SEM (one-way ANOVA followed by Tukey´s *post-hoc* test).

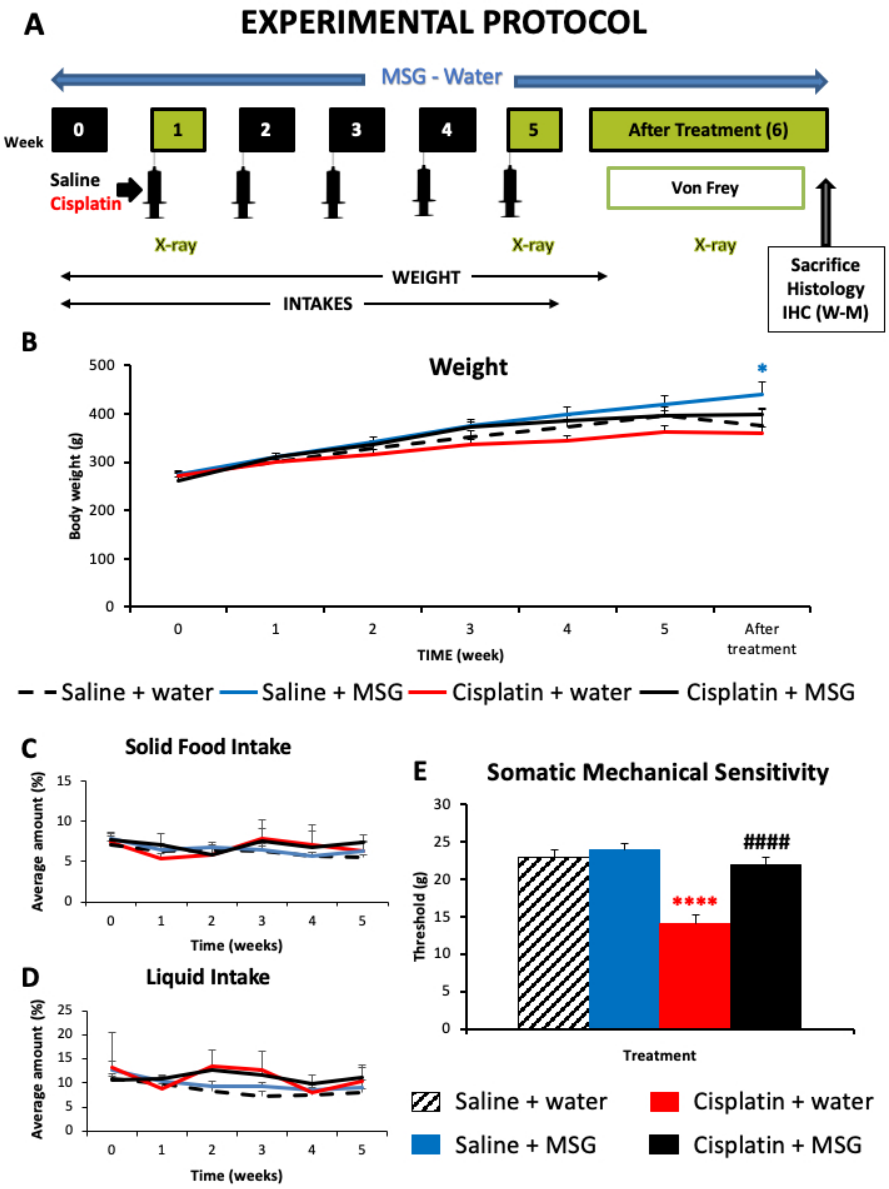

Figure 1. Experimental protocol and effect of cisplatin and monosodium glutamate (MSG) on general health parameters in the rat. As shown in A (experimental protocol), body weight gain (B), solid food intake (C), liquid intake (D) and threshold for mechanical sensitivity to von Frey hairs (E) were measured in rats intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment (week 6). Experimental groups were: saline + water (dotted line or striped bar, n=12), saline + MSG (blue line/bar, n=11), cisplatin + water (red line/bar, n=8) or cisplatin + MSG (black line/bar, n=7). The threshold for mechanical sensitivity was recorded one week after treatment (n=12-14/group). Data represent the mean ± SEM. * $p<0.05$, **** $p<0.0001$ vs saline + water; #### $p<0.0001$ vs cisplatin + water (two-way ANOVA followed by Tukey´s post-hoc test in B-D; one-way ANOVA followed by Tukey´s post hoc test in E).

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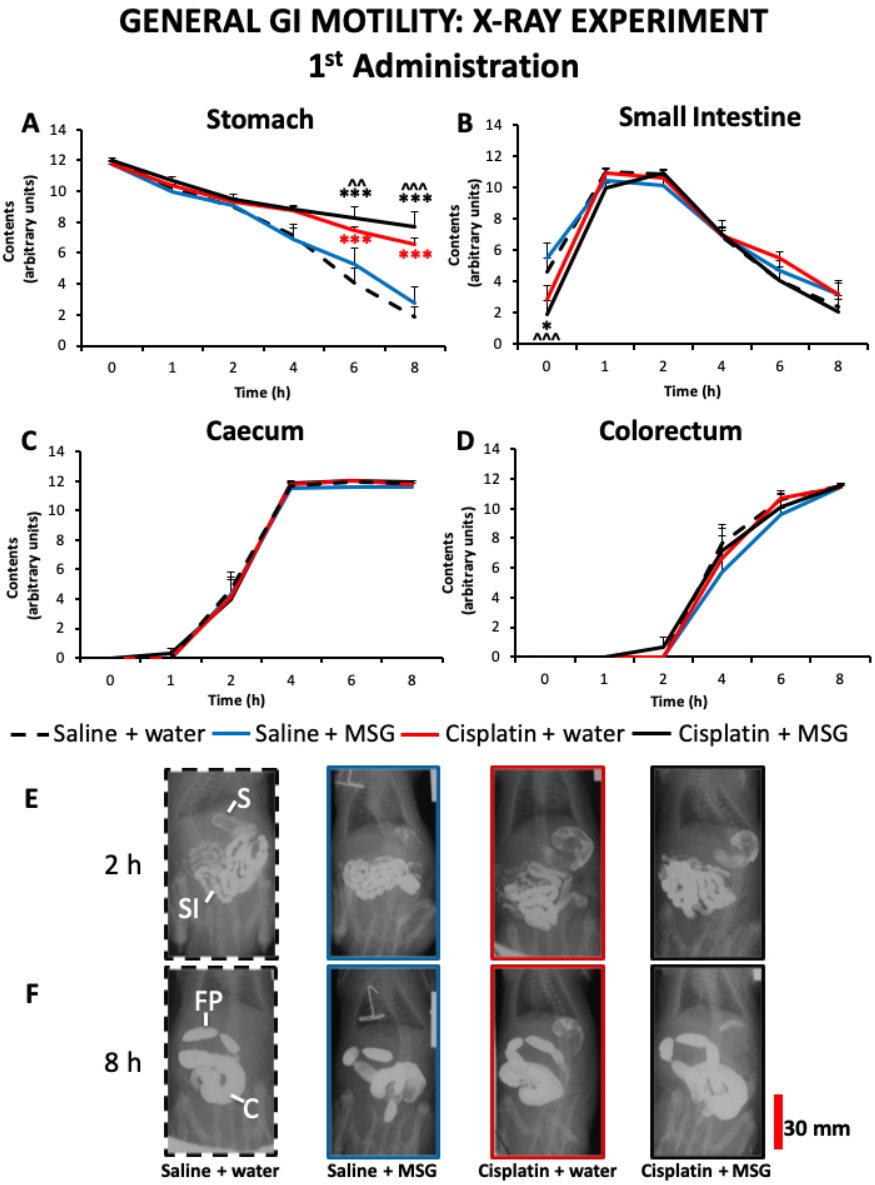

Figure 2. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat after first cisplatin administration. The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered immediately after the first intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. ***p<0.001 vs saline + water; ^^ p<0.01, ^^^ p<0.001 vs saline + MSG (two-way ANOVA followed by Tukey´s post-hoc test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.

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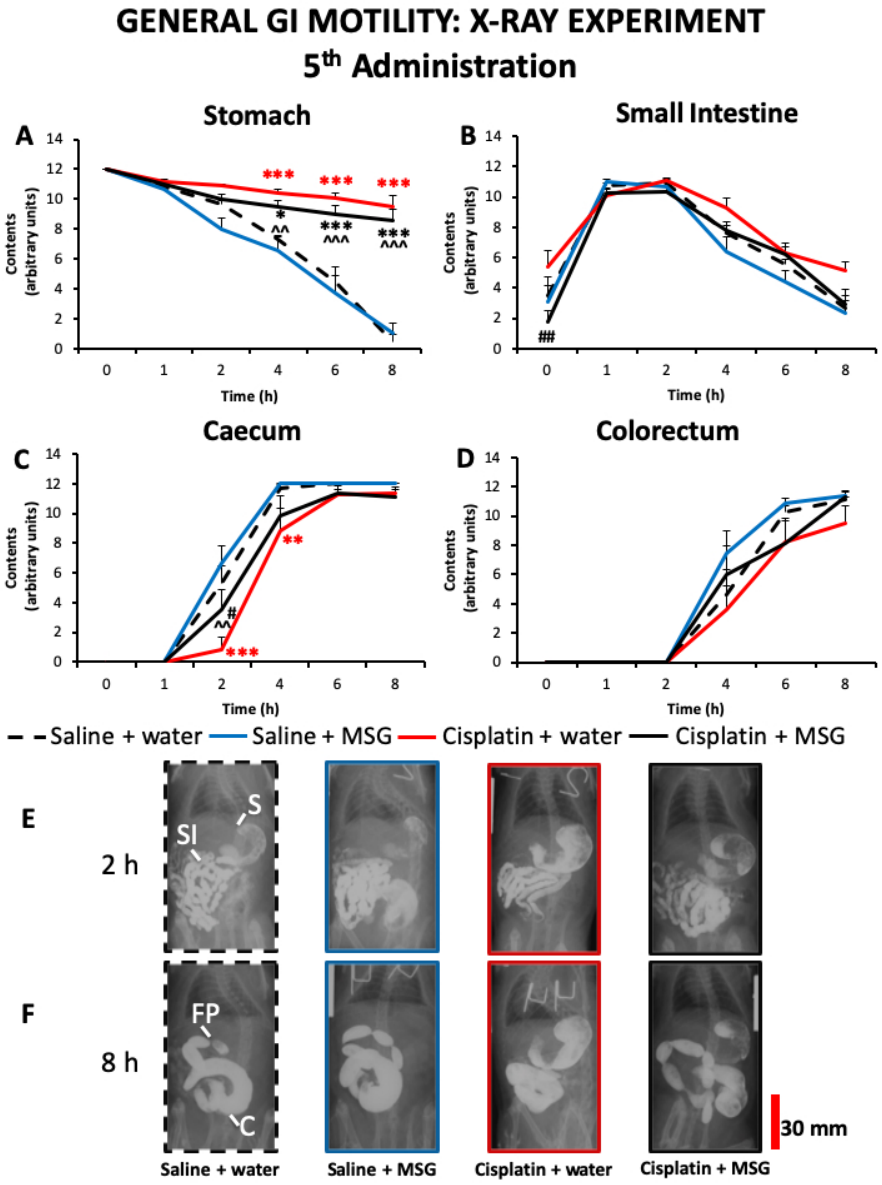
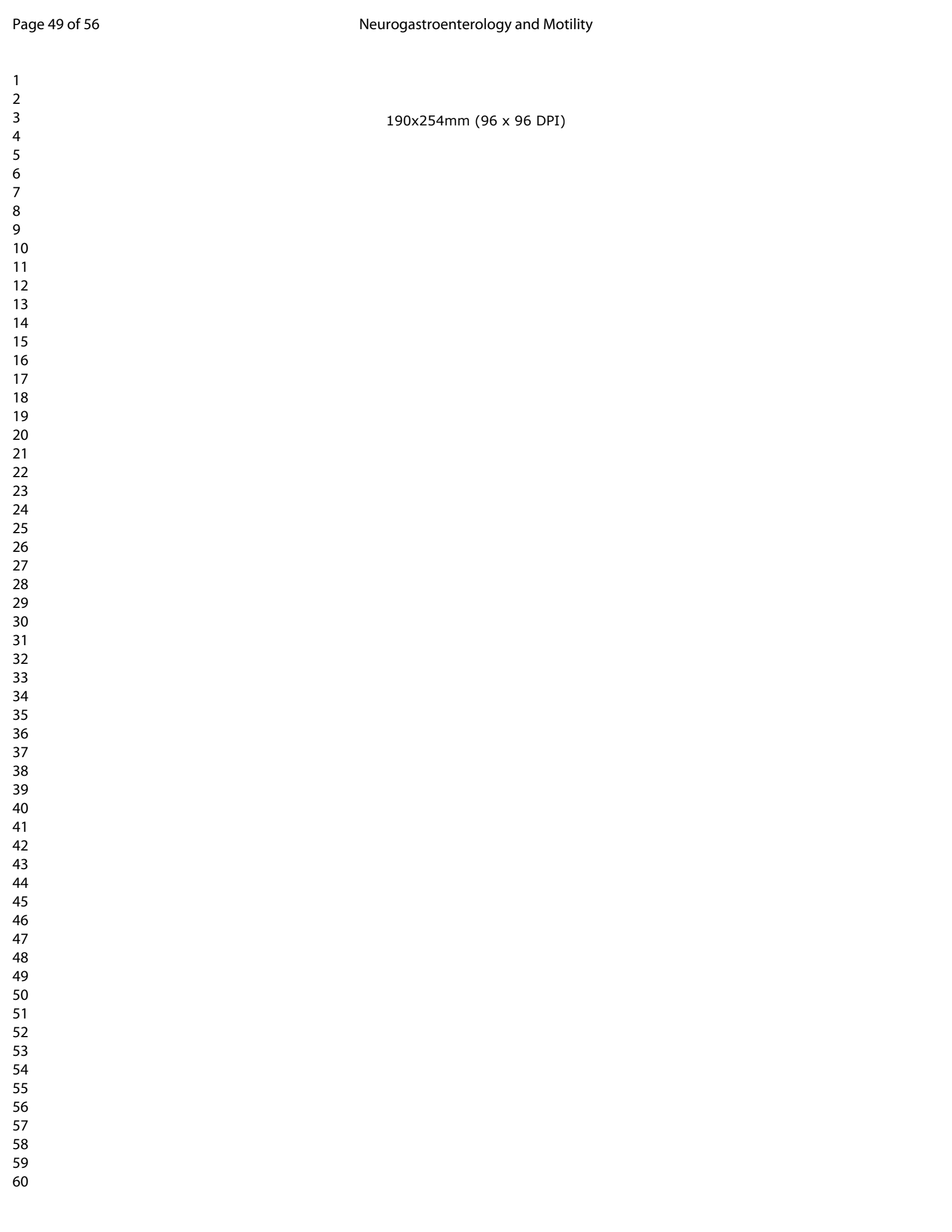


Figure 3. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat after fifth cisplatin administration. The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered immediately after the fifth intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * p<0.05, ** p<0.01, ***p<0.001 vs saline + water; ^^ p<0.01, ^^^ p<0.001 vs saline + MSG; # p<0.05, ## p<0.01 vs cisplatin + water (two-way ANOVA followed by Tukey´s post-hoc test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.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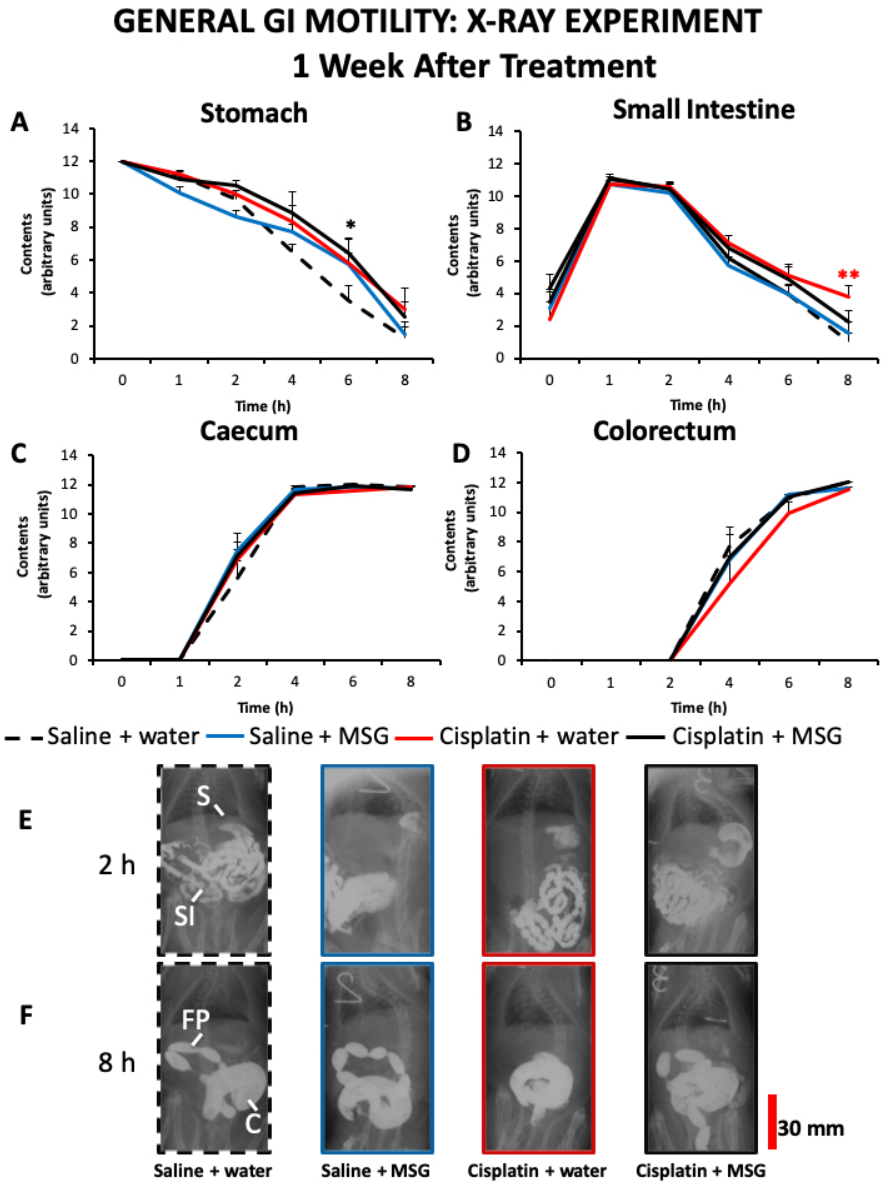

Figure 4. Effect of cisplatin and monosodium glutamate (MSG) on gastrointestinal motility in the rat one week after cisplatin treatment finalization. The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered one week after cisplatin treatment finalization and X-rays were taken 0, 1, 2, 4, 6 and 8 h after barium administration. Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * $p<0.05$, ** $p<0.01$ vs saline + water (two-way ANOVA followed by Tukey´s post-hoc test). E-F: Representative radiographic images obtained for the different treatment groups at 2 and 8 h after contrast administration. S, stomach; SI, small intestine; C, caecum; FP, fecal pellets (in colorectum). Scale bar: 30 mm.

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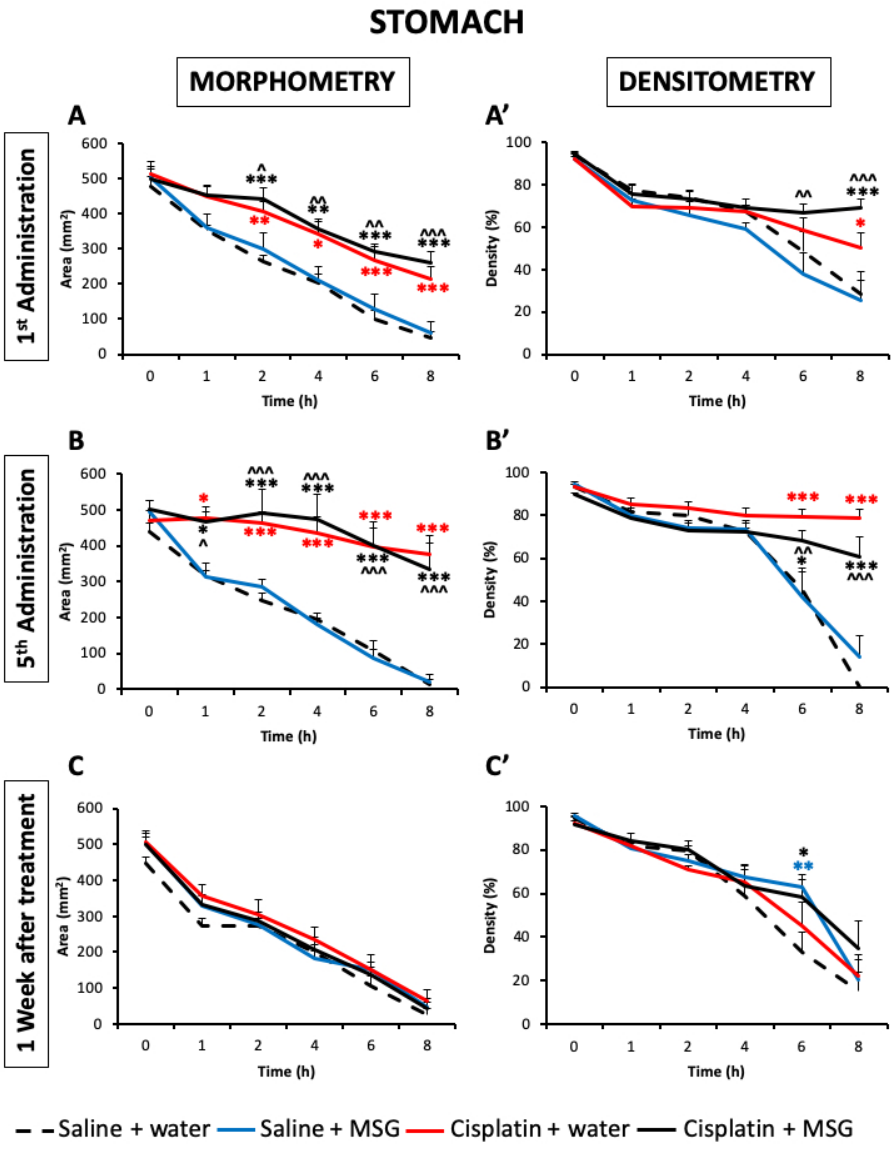

Figure 5. Morphometric and densitometric analysis of the effect of cisplatin and monosodium glutamate (MSG) on the rat stomach. The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (A, A') or the fifth (B, B') administration or one week after treatment (C, C'). Gastric size (A, B, C) and gastric density (A', B', C') were analyzed with an image processor (Image J). Experimental groups were: saline + water (dotted line, n=12), saline + MSG (blue line, n=11), cisplatin + water (red line, n=12) or cisplatin + MSG (black line, n=11). Data represent the mean ± SEM. * p<0.05, ** p<0.01, ***p<0.001 vs saline + water; ^ p<0.05, ^^ p<0.01, ^^^ p<0.001 vs saline + MSG (two-way ANOVA followed by Tukey´s post-hoc test).

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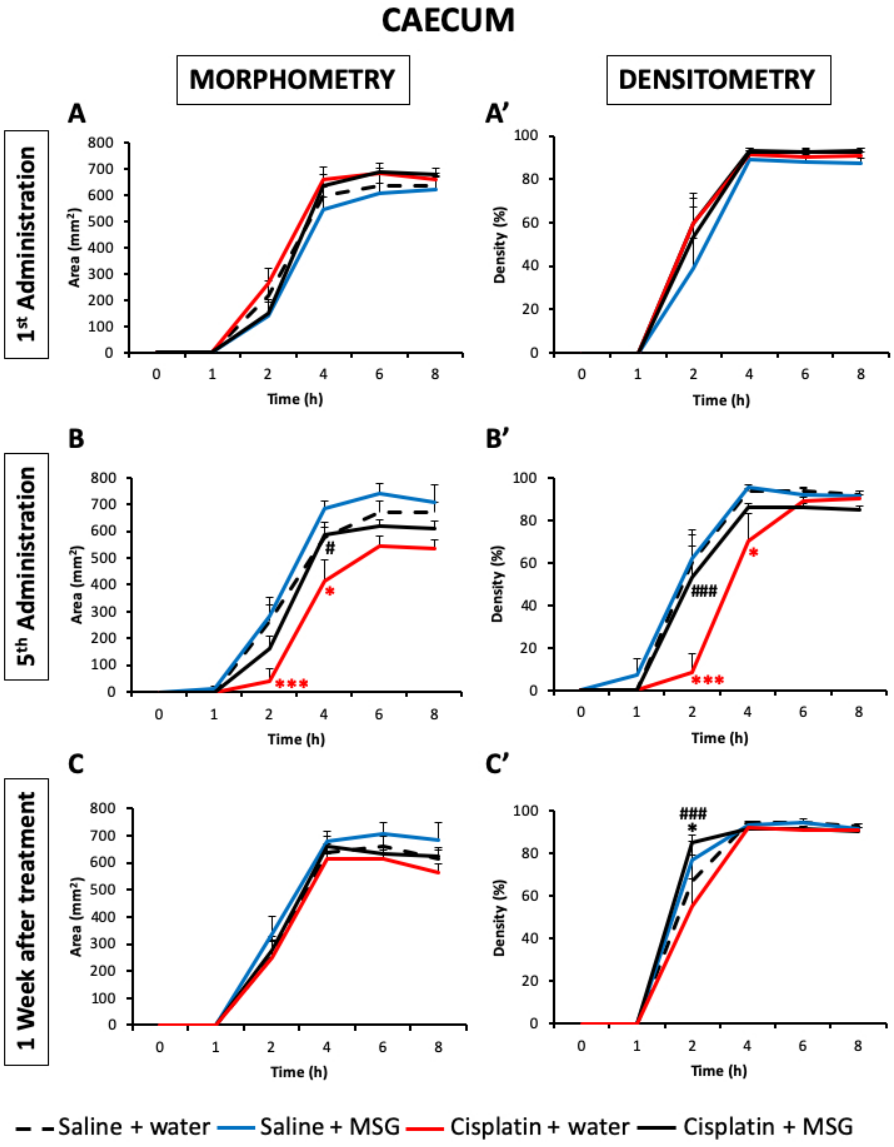

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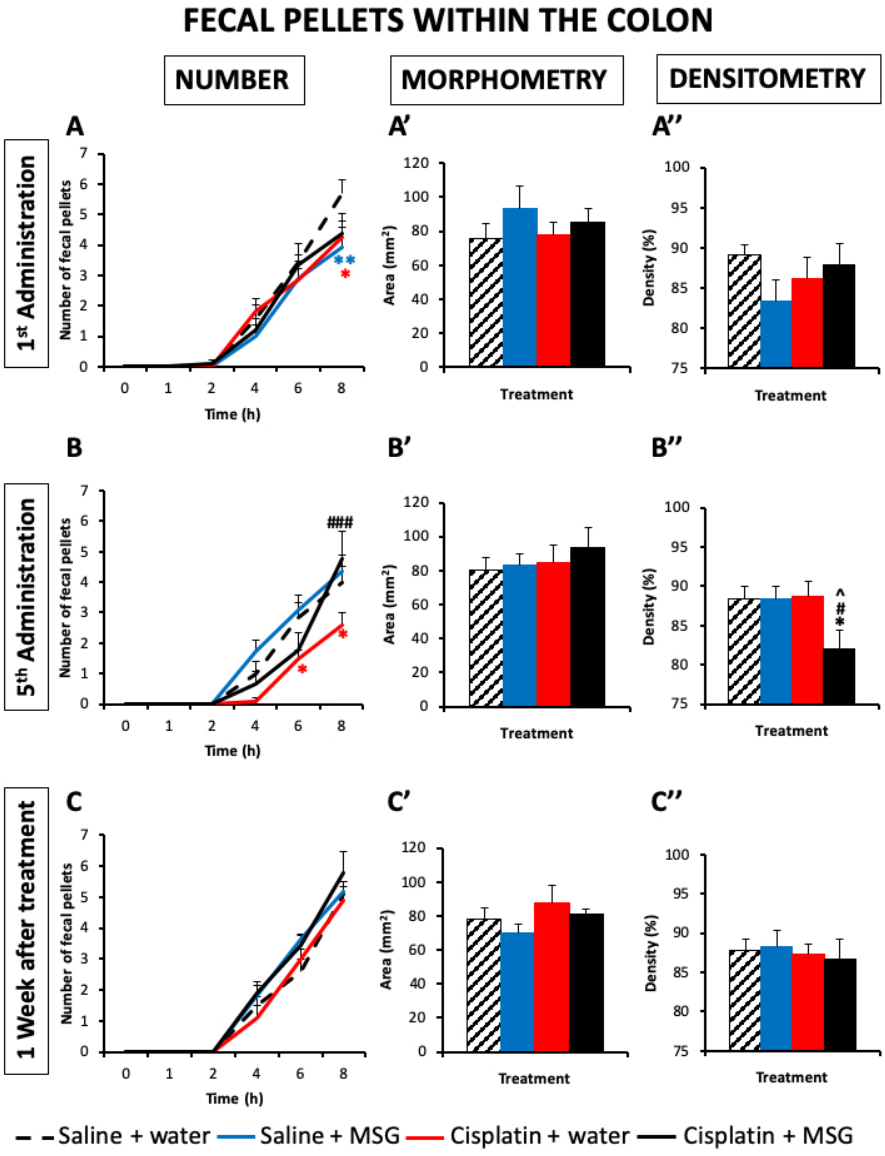

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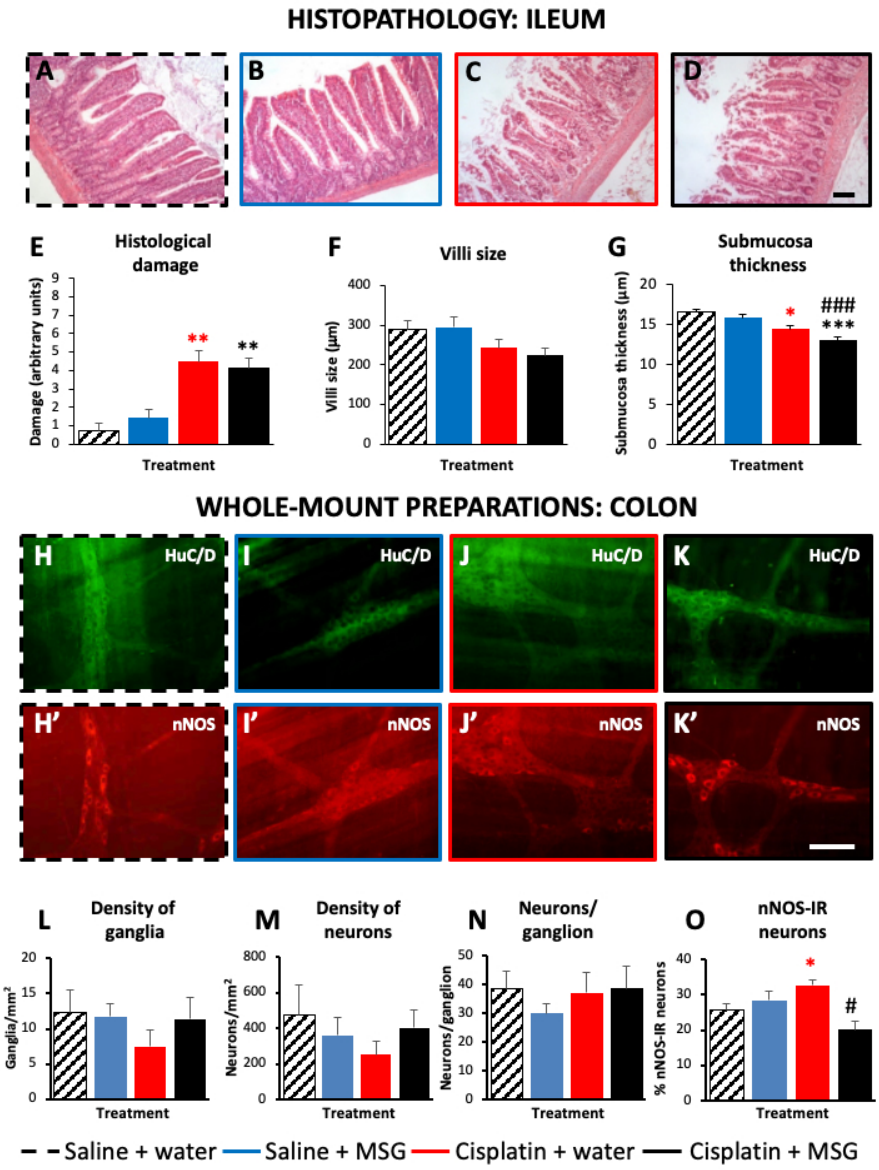

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(Kruskal-Wallis followed by Dunn’s post-hoc test, E-G; one-way ANOVA followed by Tukey´s post-hoc test, L-O). Bar 100 µm.

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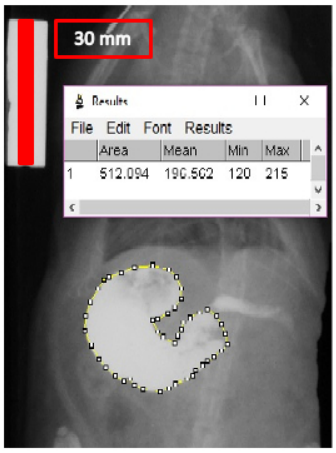
PROCEDURE TO MEASURE ORGAN SIZE WITH IMAGE J



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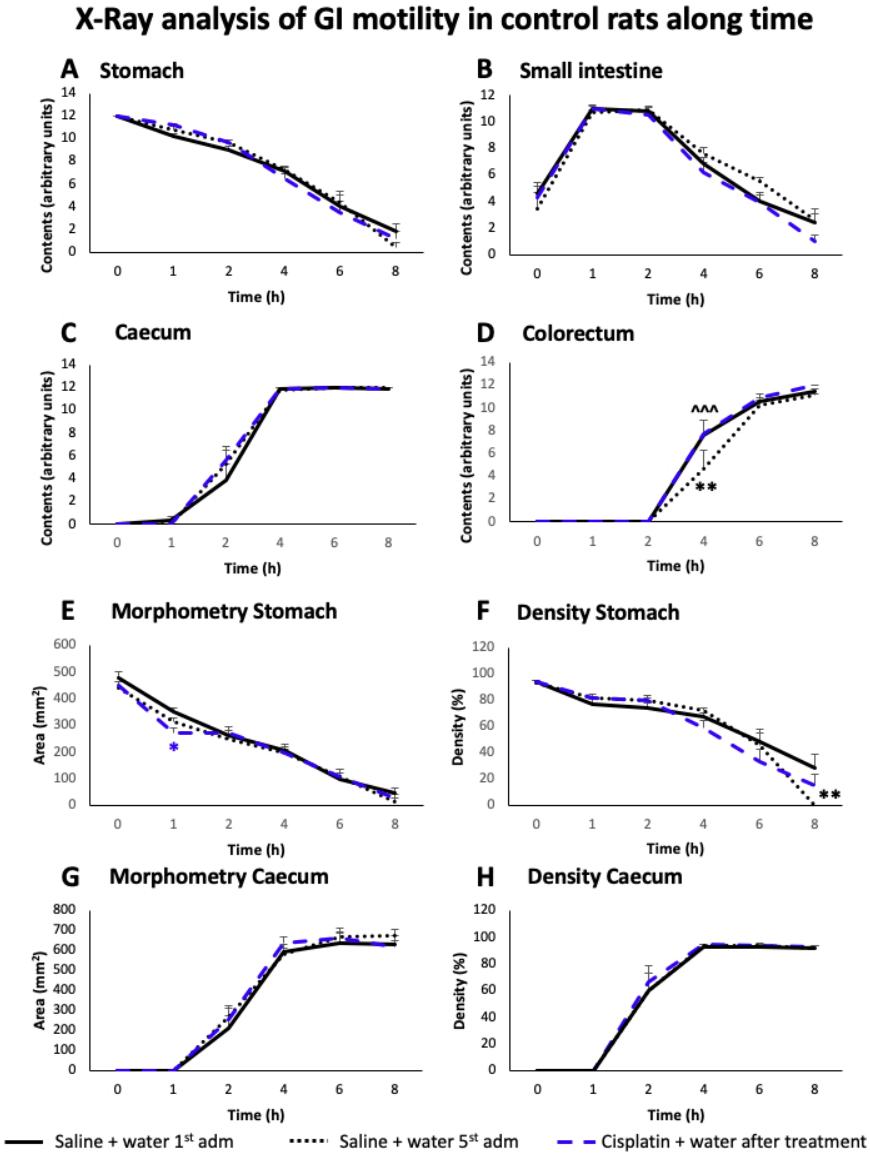

Figure S2. X-Ray analysis of GI motility in control rats along time. The rats were intraperitoneally administered with saline (2.5 mL kg−1) for 5 consecutive weeks (weeks 1-5). Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast, immediately after the first (continuous black line) or the fifth administration (dotted black line), or one week after treatment finalization (dotted purple line). Stomach size (E), stomach density (F), caecum size (G) and caecum density (H) were analyzed with an image processor (Image J). Data represent the mean ± SEM (n=12). * p<0.05, ** p<0.01 vs first administration; ^^^ p<0.001 vs fifth administration (two-way ANOVA followed by Tukey´s post-hoc test).

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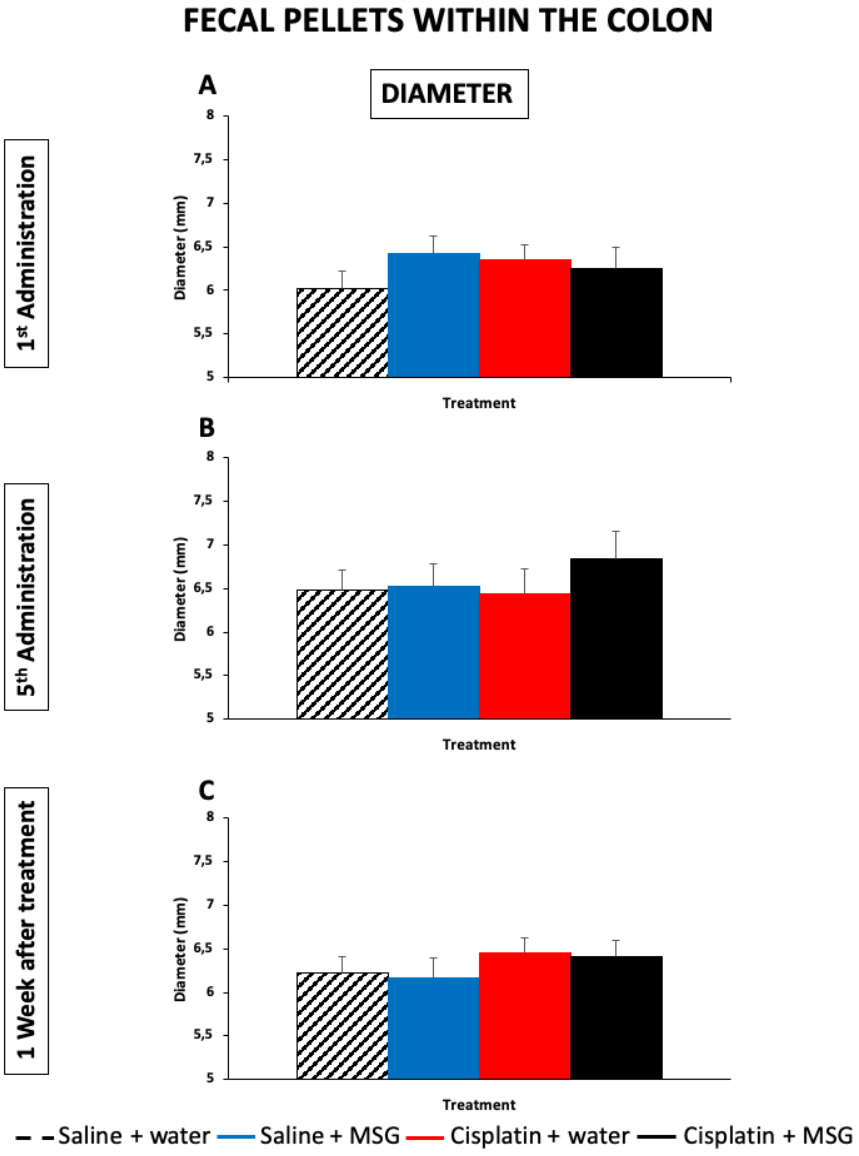

Figure S3. Effect of cisplatin and monosodium glutamate (MSG) on the diameter of the rat fecal pellets within the colon (as seen on the X-rays). The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Barium sulfate (2.5 mL, 2 g mL−1) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6 and 8 h after contrast. Fecal pellets diameter found within the colon was analyzed with an image processor (ImageJ), immediately after the first (A) or the fifth (B) administration, or one week after treatment cessation (C). Experimental groups were: saline + water (striped bar, n=12), saline + MSG (blue bar, n=11), cisplatin + water (red bar, n=12) or cisplatin + MSG (black bar, n=11). Data represent the mean ± SEM (two-way ANOVA followed by Tukey´s post-hoc test).

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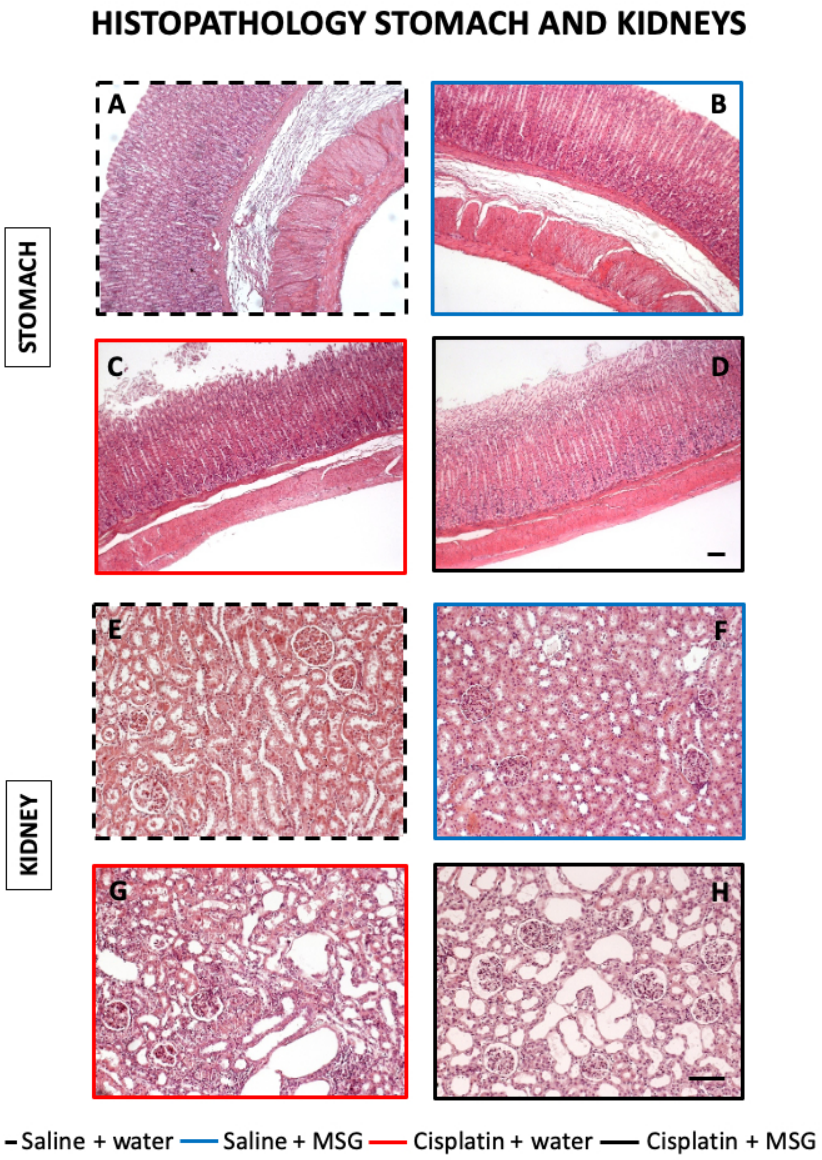

Figure S4: Effect of cisplatin and monosodium glutamate (MSG) on the general histological structure of the rat gastric body and kidney. Representative figures in HE of the gastric body (A-D) and kidney (E-H) of rats intraperitoneally administered with saline (2.5 mL kg−1) (A, B, E, F) or cisplatin (2 mg kg-1) (C, D, G, H) for 5 consecutive weeks (weeks 1-5) and exposed (B, D, F, H) or not (A, C, E, G) to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Histological samples embedded in paraffin sections were obtained one week after treatment. Bar: 100 µm. Experimental groups were: saline + water (A, E), saline + MSG (B, F), cisplatin + water (C, G) or cisplatin + MSG (D, H).

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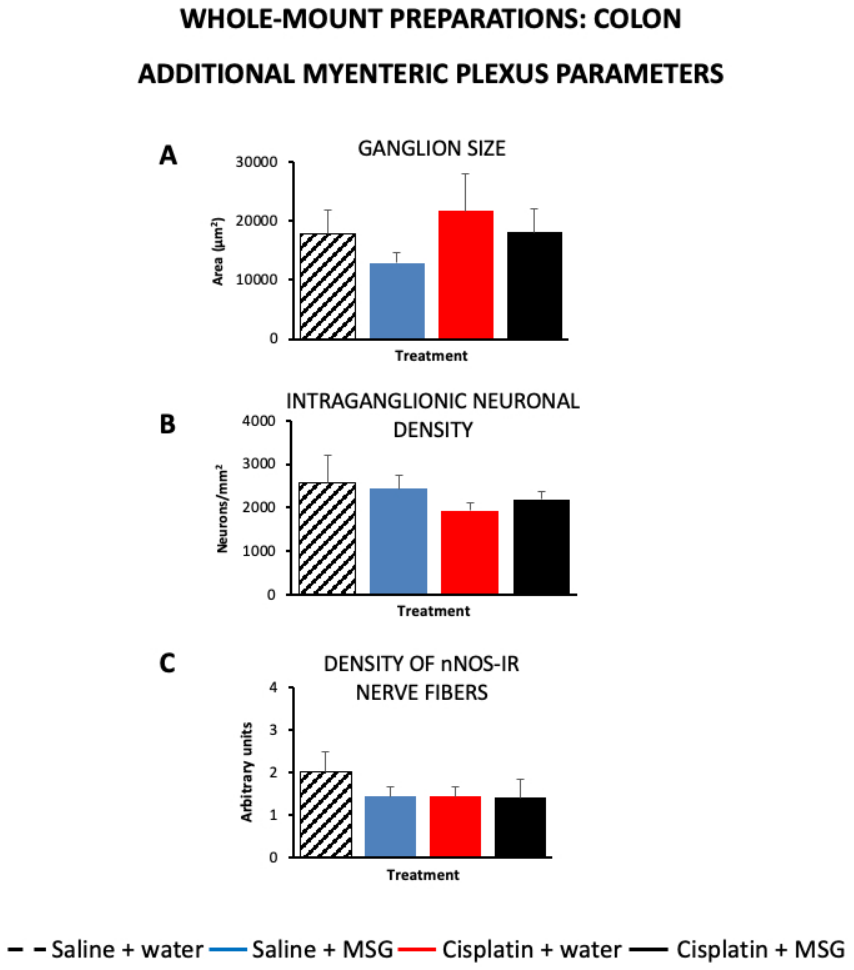

Figure S5: Effect of cisplatin and monosodium glutamate (MSG) on the structure of the rat distal colon myenteric plexus. The rats were intraperitoneally administered with saline (2.5 mL kg−1) or cisplatin (2 mg kg-1) for 5 consecutive weeks (weeks 1-5) and exposed or not to MSG (4 g L-1) in drinking water from week 0 to one week after treatment. Whole mount distal colon longitudinal muscle-myenteric plexus preparations were processed to detect the neurons expressing HuC/D (pan-neuronal marker) and neuronal nitric oxide synthase (nNOS, mainly present in myenteric neurons involved in inhibitory motor circuits). With the aid of Image J, different parameters were evaluated (see methods for further details): ganglion size (A), intraganglionic neuronal density (B), and density of nNOS-IR nerve fibers (C). Experimental groups were: saline + water (A, striped bar, n=5), saline + MSG (B, blue bar, n=7), cisplatin + water (C, red bar, n=5) or cisplatin + MSG (D, black bar, n=7). Bars show mean values ± SEM (one-way ANOVA followed by Tukey´s post-hoc test).

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