EREDETI KÖZLEMÉNY

ORIGINAL ARTICLE

Neurobehavioral impairments in ciprofloxacin-treated osteoarthritic adult rats

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Background and purpose – Ciprofloxacin (CIP) is a broad-spectrum antibiotic widely used in clinical practice to treat musculoskeletal infections. Fluoroquinolone-induced neurotoxic adverse events have been reported in a few case reports, all the preclinical studies on its neuropsychiatric side effects involved only healthy animals. This study firstly investigated the behavioral effects of CIP in an osteoarthritis rat model with joint destruction and pain, which can simulate inflammation-associated musculoskeletal pain. Furthermore, effects of CIP on regional brain-derived neurotrophic factor (BDNF) expression were examined given its major contributions to the neuromodulation and plasticity underlying behavior and cognition.
Methods – Fourteen days after induction of chronic osteoarthritis, animals were administered vehicle, 33 mg/kg or 100 mg/kg CIP for five days intraperitoneally. Motor activity, behavioral motivation, and psychomotor learning were examined in a reward-based behavioral test (Ambitus) on Day 4 and sensorimotor gating by the prepulse inhibition test on Day 5. Thereafter, the prolonged BDNF mRNA and protein expression levels were measured in the hippocampus and the prefrontal cortex.

Viselkedészavarok ciprofloxacinnal kezelt felnőtt osteoarthritises patkányokban

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Háttér és cél – A ciprofloxacin (CIP) egy széles spektrumú antibiotikum, amit gyakran használnak mozgásszervrendszeri fertőzések kezelésére is. Klinikai esettanulmányok beszámolnak a fluorokinolonok neurotoxikus mellékhatásairól, ugyanakkor a neuropszichiátriai mellékhatásokat vizsgáló preklinikai tanulmányok mind egészséges állatokat használnak. Ez az első olyan kísérletsorozat, amely a CIP viselkedésre gyakorolt hatásait osteoarthritises patkányokon vizsgálja, ahol az ízületi károsodás okozta fájdalom modellezheti a gyulladásos eredetű mozgásszervi fájdalmat. A kísérletsorozat vizsgálja továbbá az agyi eredetű növekedési faktor- (Brain-Derived Neurotrophic Factor, BDNF) expresszió elhúzódó, régióspecifikus változását az agyban CIP-kezelés hatására, ami neuromodulátorként hozzájárulhat a viselkedésbeli és kognitív változások kialakulásához.
Módszerek – Tizennégy nappal a krónikus osteoarthritist kiváltó monojód-acetát- (MIA) injekció után az állatok 5 napig intraperitonealisan kapták a CIP két dózisát (33 mg/kg vagy 100 mg/kg), illetve az oldószert. A kezelés negyedik napján egy jutalmazáson alapuló viselkedési tesztben (Ambitus) az állatok motoros aktivitását, motivációját és pszichomotoros tanulási képességét vizsgáltuk, az ötödik

Results – CIP dose-dependently reduced both locomotion and reward-motivated exploratory activity, accompanied with impaired learning ability. In contrast, there were no significant differences in startle reflex and sensory gating among treatment groups; however, CIP treatment reduced motor activity of the animals in this test, too. These alterations were associated with reduced BDNF mRNA and protein expression levels in the hippocampus but not the prefrontal cortex.
Conclusion – This study revealed the detrimental effects of CIP treatment on locomotor activity and motivation/learning ability during osteoarthritic condition, which might be due to, at least partially, deficient hippocampal BDNF expression and ensuing impairments in neural and synaptic plasticity.

Keywords: BDNF, ciprofloxacin, motivation, rat, psychomotor

napon pedig a szenzoros kapuzás mérésére prepulzus-gátlás- (PPI) tesztet végeztünk. Ezt követően a BDNF mRNS- és fehérje-expresszióját mértük a hippocampus és a prefrontális kéreg területén.
Eredmények – A CIP-kezelés dózisfüggően rontotta az állatok tanulási képességét, ami kapcsolatban állhat a csökkent lokomotoros és explorátoros aktivitással is. Ugyanakkor nem találtunk szignifkáns különbséget a csoportok közt a megriadási (startle) reflexben és a szenzoros kapuzásban, annak ellenére, hogy az anitbiotikummal kezelt állatok ebben a tesztben is kisebb aktivitást mutattak. A viselkedésbeli változások mellett csökkent BDNF mRNS- és fehérjeexpressziót mértünk a hippocampusban, ami viszont nem jelentkezett a prefrontális kéreg területén.
Következtetés – Az eredmények igazolták a CIP-kezelés káros hatásait a lokomotoros aktivitásra és a motivációs/tanulási képességre osteoarthritis fennállása mellett. Ez feltehetően kapcsolatban áll a hippocampus elhúzódó, csökkent BDNF-expressziójával, igazolva a neuronalis és szinaptikus plaszticitás károsodásának szerepét a neurotoxikus mellékhatások hátterében.

Kulcsszavak: BDNF, ciprofloxacin, motiváció, patkány, pszichomotoros

Ciprofloxacin (CIP) is a fluoroquinolone (FQ)-type antibiotic agent widely used in clinical practice to treat bacterial infections. However, it may also cause several adverse events, including diarrhea, arrhythmia, tendonitis, tendon rupture, pain in the extremities and neuropathy[1, 2]. In addition, neurotoxic side effects including headache, depression, seizures, sleep disorders and impaired sensory functions and acute-onset psychosis associated with CIP treatment have also been mentioned in case reports[3–6].

Although sensory gating deficit is characteristic in neuropsychiatric disorders[7], data are not available about its FQ-associated appearance. Additionally, antimicrobial-induced cognitive side effects are often overlooked[8]. Despite the extensive use of antibiotics in musculoskeletal pain, there are a limited number of preclinical studies reporting CIP-induced neuropsychiatric adverse events (e.g., anxiogenic, depression-like behavior) in rodents[9–12]. The limitation of these studies is the investigation of healthy animals, which does not simulate a clinically relevant condition. In most cases, when CIP treatment is indicated in musculoskeletal infection, pain is a leading sign. Therefore, the first aim of the current study was to examine the influence of repeated CIP administration on different behavioral parameters (motor activity, motivation for reward, and psychomotor learning) and sensory gating in adult osteoarthritic rats, which can mimic reliably chronic pain associated with degenerative joint disease[13, 14].

ABBREVIATIONS

ANOVA: analysis of variance
BDNF: brain-derived neurotrophic factor
CIP: ciprofloxacin
CNS: central nervous system
FQ: fluoroquinolone
PFC: prefrontal cortex
PA: pulse alone
PP: prepulse–pulse pair
PPI: prepulse inhibition
SEM: standard error of the mean

The exact mechanism of FQ-mediated central nervous system (CNS) toxicity remains uncertain. Inhibition of $GABA_A$ receptors as well as activation of excitatory NMDA receptors, reduced brain serotonin and GABA levels, enhanced oxidative stress and weakened antioxidant defense system resulting in delayed mitochondrial toxicity, and elevated acetylcholinesterase, monoamine oxidase A, and monoamine oxidase B activities are postulated mechanisms[9, 15].

Brain-derived neurotrophic factor (BDNF) contributes to adult hippocampal neurogenesis and plasticity implicated in normal cognitive functions such as learning and memory[16]. A few studies have reported that antibiotic-induced dysbiosis can reduce regional BDNF mRNA expression in rodents, but there is no data available on FQ-induced BDNF alterations[17, 18]. Thus, this study was also designed to determine whether CIP treatment alters BDNF expression in the hippocampus and the prefrontal cortex (PFC), central regions involved in cognition and behavioral regulation[16].

Methods

Animals

All experiments involving animals were conducted with the approval of the Hungarian Ethics Committee for Animal Research (registration number: XIV/1248/2018), in accordance with the guidelines set by the Government of Hungary and EU Directive 2010/63EU. Male Wistar rats were group-housed (3 per cage) under a 12 h/12 h light/dark cycle and controlled temperature (22 °C ± 1 °C). Vehicle-treated control group and two groups receiving intraperitoneal injection of 33 or 100 mg/kg CIP were used (n = 12/group). The animals had free access to food and water, except the night before the Ambitus test (see below), when total food deprivation was applied to enhance motivation for obtaining food rewards. All experimental procedures were performed between 8:00 a.m. and 4:00 p.m.

Modeling of osteoarthritis and CIP administration

For induction of osteoarthritis, rats were given a single intraarticular injection of 500 µg monosodium iodoacetate (MIA, Sigma-Aldrich Kft., Budapest, Hungary) in a volume of 50 µl through the infrapatellar ligament of the right hind knee using a 27-gauge needle[14, 19]. Animals were left undisturbed for 14 days to develop mild cartilage destruction and osteoarthritis-like joint pain. Ciprofloxacin hydrochloride anhydrous (a generous gift of TEVA Gyógyszergyár Zrt., Debrecen, Hungary) was dissolved in physiological saline and injected intraperitoneally in 33 or 100 mg/kg doses (in a volume of 4 mL/kg body weight), based on earlier studies[9, 20]. Control animals received the same volume of saline. Rats were conscious and gently restrained during injections, but no signs of major distress were observed.

Experimental protocol

The pharmacological treatment was started 14 days after the induction of osteoarthritis, and lasted for five days with daily weighing. Ambitus and PPI tests were conducted on Days 4 and 5, respectively. Seven days after the cessation of antibiotic or vehicle administration, six animals in each group were randomly selected and terminated (rodent guillotine) for molecular biology studies.

Behavioral testing

Ambitus test

Locomotion, reward-motivated exploratory activity, and psychomotor learning were assessed in the Ambitus system beginning 30 min after CIP injection on Day 4. The Ambitus system is a rectangular corridor constructed of clear plexiglas on a black floor, arranged to form an 80 cm by 80 cm enclosed square (www.deakdelta.hu) allowing free movement in either direction[13]. As was revealed earlier, this is a new version of Hole-Board test, which automatically record the activity of the animals, and it could detect different behavioral impairments, including cognitive alterations (e.g. in schizophrenia animal model)[13, 21, 22]. Each of the four corridors has four side boxes, two on the internal side and two on the external side, all of equal size (5 × 5 × 5 cm) to provide food rewards (20 mg of puffed rice). Infrared beams detect the exploratory activity in side boxes and the locomotor activity in midway of each corridor with 1 ms time resolution.

Trials commenced by placing a rat at the starting point within the corridor, after which the experimenter immediately left the room. The animals were allowed to explore the corridor and collect food rewards for 5 min, and the number of food rewards eaten was recorded. The apparatus was cleaned with 70% alcohol after each animal. Animals' behavior was also recorded using an infrared video device (WCM-21VF, CNB, China) suspended above the apparatus. If an animal ate all available rewards within 5 min, the video recording was used to determine the time of task completion (last reward consumption).

Two types of tasks were applied during the study. In Task 1 (Trial 1 and 2), all inside and outside boxes were baited (16 rewards), while in Task 2 (Trial 3 and 4), only the inside boxes were baited (8 rewards). In this way, the motor requirements were similar but the reward contingency was altered. All the rats performed two sessions of each task 1 min apart and tasks were separated by 3 hours. Multiple behavioral parameters related to locomo-

Table 1. *Behavioral parameters analyzed in the Ambitus test*

	Parameter	Task	Calculation (by definition)
Locomotor activity	Overall locomotion (N°)	Task 1 & 2	Total number of corridor visits up to 300 s
	Discovered corridors (N°)	Task 1 & 2	Number of different corridors (max. 4) visited
Exploration	Overall external exploration (N°)	Task 1 & 2	Total number of external box visits up to 300 s
	Overall internal exploration (N°)	Task 1 & 2	Total number of internal box visits up to 300 s
Cognitive abilities	Effective exploration (%)	Task 1	(number of rewards eaten) × (100)/(discovered internal and external boxes (max. 16)
		Task 2	(number of rewards eaten) × (100)/(discovered internal boxes (max. 8)
	Learning capacity (%)	Task 1	(number of rewards eaten) × (cut-off time (300)) × 100/(number of rewards (16)) × (time till the consumption of the last reward)
		Task 2	(number of rewards eaten) × (cut-off time (300)) × 100/(number of rewards (8)) × (time till the consumption of the last reward)

tor and exploratory activities and cognitive ability were measured **(Table 1)**.

PPI test

The PPI of the acoustic startle response was measured 30 min after CIP or vehicle injection on Day 5. After a 7.5 min habituation to the 60 dB background white noise in a startle chamber (Startle and Fear combined system, Panlab, S.L./Harvard Apparatus, Barcelona, Spain), rats were exposed to two different trial types: pulse alone (PA) trials of 40 ms 115 dB pulses, and prepulse–pulse pair (PP) trials in which a 20 ms 85 dB prepulse was followed by a 40 ms 115 dB startle pulse with a latency of 150 ms. Both trial types were applied 20 times in a random order. The interstimulus intervals ranged from 7 to 13 s. The degree of startle reactions to PA and PP, and the motor activity of the animals during the habituation phase (7.5 min), and during the acoustic stimulation phase skipping the acoustic startle response (7.5 min) were also assessed. Since body weight can confound the amplitude of the electrical signal related to motion and startle reactions, these parameters were normalized to body weight (yielding relative startle reflex and relative activity), and the PPI was calculated using the following equation: PPI (%) = 1 − (*startle response for PP*)/(*startle response for PA*) × 100.

Real-time quantitative reverse-transcriptase PCR (RT-PCR) studies

Tissue isolation

The brain was rapidly removed; the PFC and the hippocampus were isolated immediately on dry ice, placed into RNAlater solution (Sigma-Aldrich, Hungary), flash-frozen in liquid nitrogen, and stored at −70°C until total RNA extraction.

Total RNA preparation

Total cellular RNA was isolated by extraction with guanidinium thiocyanate-acid-phenol-chloroform according to the procedure of *Chomczynski* and *Sacchi*[23]. After precipitation with isopropanol, RNA was washed with 75% ethanol and resuspended in diethyl pyrocarbonate-treated water. RNA purity was controlled at an optical density of 260/280 nm with BioSpec Nano (Shimadzu, Japan); all samples exhibited an absorbance ratio in the range of 1.6–2.0. RNA quality and integrity were assessed by agarose gel electrophoresis.

RT-PCR

Reverse transcription and amplification of the PCR products were performed by using the TaqMan RNA-to-C_T-Step One Kit (Thermo Fisher Scientific, Hungary) and an ABI StepOne Real-Time cycler. Reverse-transcriptase PCR amplifications were performed as follows: at 48°C for 15 min and at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. The generation of specific PCR products was confirmed by melting curve analysis. The following primers were used: assay ID Rn02531967_s1 for *BDNF* and Rn00667869_m1 for *β-actin* (Thermo Fisher Scientific, Hungary) as the endogenous control. All samples were run in triplicate. The fluorescence intensities of the probes were plotted against PCR cycle number. The amplification cycle displaying the first significant increase

in fluorescence signal was defined as the threshold cycle (C_T).

Western blot analysis

25 µg of protein per well was subjected to electrophoresis on 4-12% NuPAGE Bis-Tris Gel in XCell SureLock Mini-Cell Units (Thermo Fisher Scientific, Hungary). Proteins were transferred from gels to nitrocellulose membranes, using the iBlot Gel Transfer System (Thermo Fisher Scientific, Hungary). The antibody binding was detected with the WesternBreeze Chromogenic Western blot immunodetection kit (Thermo Fisher Scientific, Hungary). The blots were incubated on a shaker with BDNF polyclonal antibody (cat. no sc-546), β-actin (cat. no sc-8432) monoclonal antibody (both Santa Cruz Biotechnology, California, 1:200) in the blocking buffer. Images were captured using the EDAS290 imaging system (Csertex Ltd., Hungary), and the optical density of each immunoreactive band was determined using Kodak 1D Images analysis software. Optical densities were calculated in arbitrary units after local area background subtraction.

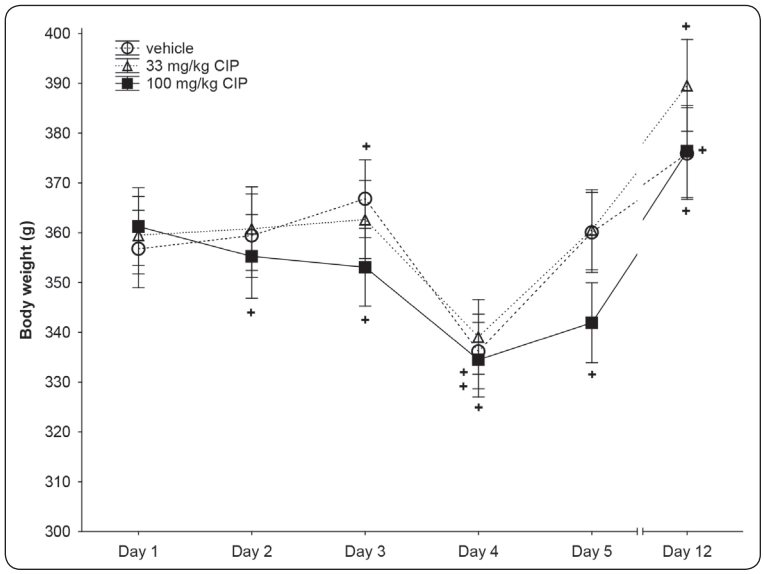

Figure 1. *Time-course of the body weight changes in saline, 33 mg/kg and 100 mg/kg dose CIP-treatment groups. Data are presented as mean ± SEM (n=12 rats/group). The symbol "+" indicates a significant difference compared to Day 1 by ANOVA with post hoc LSD test*

Statistical analysis

Data are expressed as mean ± SEM. Repeated measures ANOVA was used to evaluate changes in body weight and the time-dependent effects of trial and treatment as factors during the Ambitus test. One-way ANOVA was used to evaluate PPI test results with treatment group as the main factor. Unpaired t-tests were used to compare BDNF expression. All post hoc comparisons were performed using the Fisher LSD test. A $p < 0.05$ was considered significant for all tests. All analyses were performed using Statistica 13.4.0.14 (TIBCO Software Inc., USA).

Results

Body weight

Repeated measures of ANOVA showed significant effects of time ($F_{(5,165)} = 42.02$, $p < 0.0001$) and time × treatment interaction ($F_{(10,165)} = 1.90$, $p < 0.05$; **Figure 1**). The post hoc comparison revealed that while the body weight of the control animals increased significantly on Day 3 compared to baseline, a significant weight loss was detected in the 100 mg/kg CIP-treated group on Days 2 and 3 compared to Day 1. A one-night food deprivation period before the Ambitus test on Day 4 significantly reduced body weight in both groups. Body weight of the 100 mg/kg CIP group returned to normal (equivalent to the saline group) 7 days after cessation of treatment.

Ambitus test

Locomotion-related parameters

Regarding the overall locomotor activity of the animals in the Ambitus test **(Figure 2A)**, ANOVA revealed significant effect of trial ($F_{(3,132)} = 20.70$, $p < 0.0001$) and treatment ($F_{(2,132)} = 11.84$, $p < 0.0001$), i.e. it significantly decreased by repetition in all groups compared to Trial 1, and also dose-dependently in CIP-treated groups **(Figure 2B)**. Thus, the 100 mg/kg CIP-treated animals showed the lowest locomotor activity, that was significantly lower compared to the control group (except during Trial 3).

Regarding the number of corridors entered during Ambitus trials, repeated measures ANOVA revealed significant effect of trial ($F_{(3,132)} = 7.74$, $p < 0.0001$) and treatment ($F_{(2,132)} = 4.57$, $p < 0.05$). All animals entered each of the 4 corridors during Trial 1 **(Figure 2C)**, but the number of explored corridors decreased in CIP-treated groups when the task was repeated after a one-minute break (Trial 2), indicating that motivation for discovery was reduced by CIP after this short delay. The activity of 100 mg/kg CIP-treated animals recovered during the introduction of Task 2 (Trial 3) conducted after a 3-h delay, but it reduced again by repetition (Trial 4) as confirmed by post hoc analysis. In contrast, the control animals did not show this zig-zag pattern in this type of motor activity.

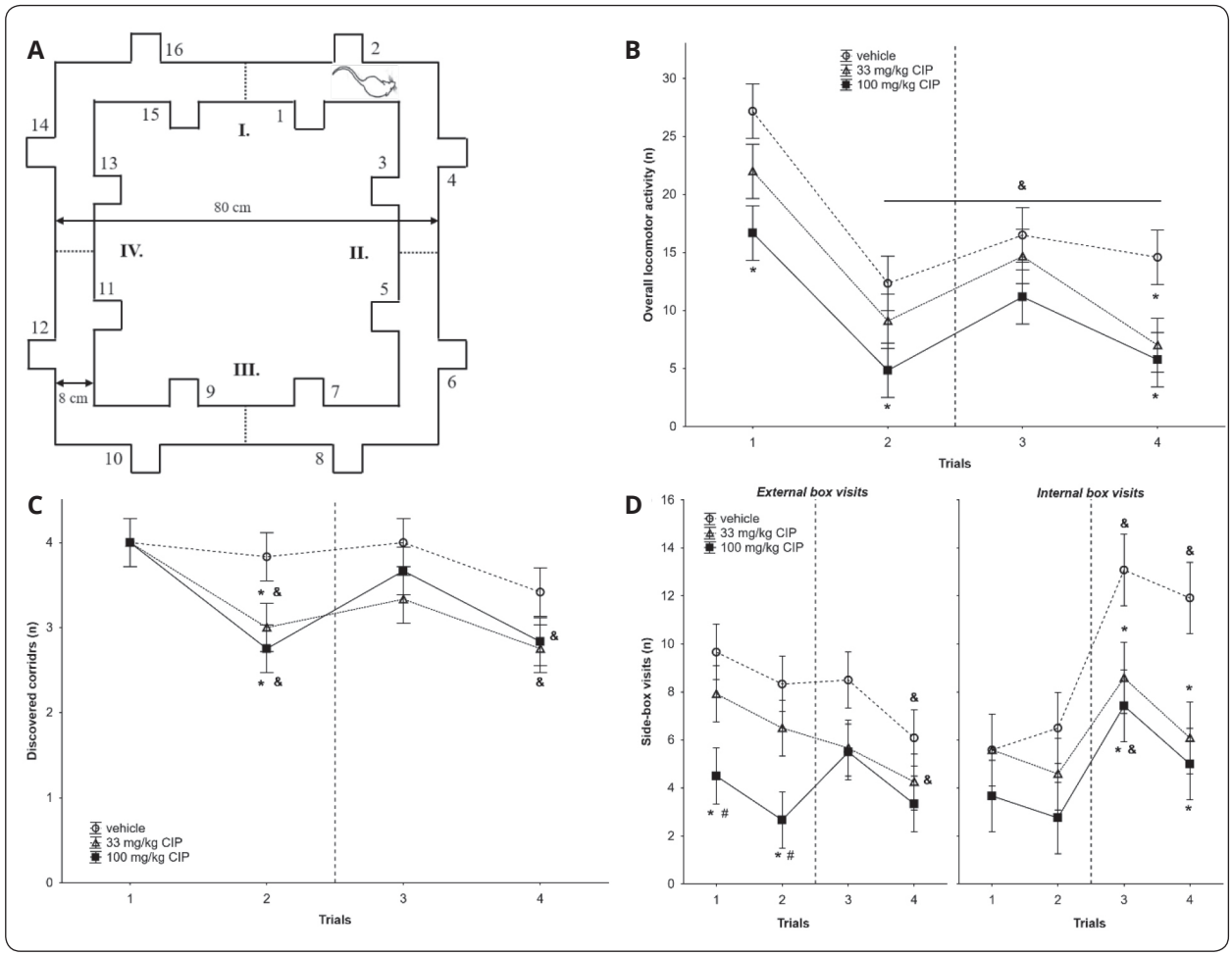

Figure 2. *The Ambitus apparatus* **(A)***. Ground plan of the corridor with side-boxes (1-16). In the middle of the 4 corridors (I-IV) dashed lines indicate photo beams. The rat shows the starting point of the animals in each trial. The height of the apparatus is 50 cm. Overall locomotor activity* **(B)** *and number of discovered corridors* **(C)** *by trial in the different groups. Changes in the exploratory activity* **(D)** *toward the external and internal side boxes by trial. Data are presented as mean ± SEM (n=12 rats/group). The symbols indicate significant differences by LSD post hoc test compared to the vehicle-treated control group (*), compared to the first trial (&), and between the CIP groups (#). The reference line indicates the introduction of Task 2*

Exploration-related parameters

Administration of CIP also markedly altered the number of rewarded external side-box visits during Ambitus trials, suggesting reduced reward-dependent motivation for exploration, as evidenced by significant main effects of trial ($F_{(3,132)} = 3.14$, $p < 0.05$) and treatment ($F_{(2,132)} = 12.70$, $p < 0.0001$) **(Figure 2D)**. Thus, when both the external and internal boxes were rewarded (Task 1, Trial 1 and 2), animals treated with 100 mg/kg CIP showed significantly less interest in the external boxes. This difference was not observed during Task 2, as both the vehicle and low-dose CIP groups also showed significantly reduced exploration of the external (non-rewarded) boxes.

Internal box visits also differed significantly among groups, with significant main effects of trial ($F_{(3,132)} = 7.77$, $p < 0.0001$) and treatment ($F_{(2,132)} = 9.68$, $p < 0.001$) **(Figure 2D)**. During Task 1, there were no significant differences among the groups, but during Trials 3 and 4, CIP treated groups demonstrated significantly lower exploratory activity of the rewarded internal boxes compared to controls.

Parameters related to cognitive abilities

The effective exploration ratio differed significantly among groups, with main effects of both treatment ($F_{(2,119)} = 9.46$, $p < 0.001$) and trial ($F_{(3,119)} = 7.96$, $p < 0.0001$) **(Figure 3A)**. The post hoc comparison showed that the

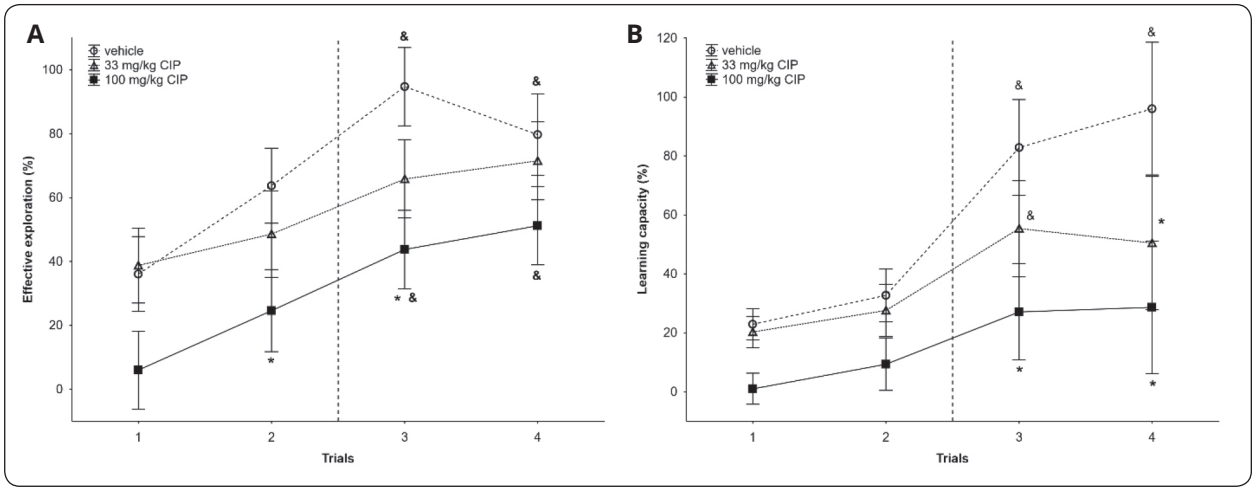

Figure 3. *The effective exploration* **(A)** *and motivation index* **(B)** *by trial in the different groups. Data are presented as mean ± SEM (n=12 rats/group). The symbols indicate significant differences by LSD post hoc test compared to the vehicle-treated control group (*) and compared to the first trial (&). The reference line indicates the introduction of Task 2*

higher CIP dose caused significantly lower values in most trials, despite the moderate improvements by repetition.

Regarding the learning capacity, it also differed significantly among groups, with significant main effects of treatment ($F_{(2,33)} = 3.58$, $p < 0.05$) and trial ($F_{(3,99)} = 11.49$, $p < 0.0001$) **(Figure 3B)**. The post hoc analysis revealed significantly lower values for the CIP-treatment groups, especially upon introduction of Task 2, compared to control animals. Since no animals ate all rewards from the explored boxes during the Trial 1 and 2, a very low learning capacity was detected in all groups. The control animals showed significant improvement in this parameter during the following trials, while the high dose of CIP perfectly inhibited the beneficial effects of repetition.

PPI test

In all groups, the acoustic startle response was suppressed by the prepulse ($F_{(1,33)} = 169.6$, $p < 0.0001$ by ANOVA) **(Figure 4A)** and there were no group differences in the magnitude of this reduction (%PPI) as confirmed by ANOVA **(Figure 4B)**.

Regarding the relative motor activity, ANOVA revealed significant effects of treatment ($F_{(2,33)} = 7.99$, $p < 0.005$), phase ($F_{(1,33)} = 76.01$, $p < 0.001$) and treatment × phase interaction ($F_{(2,33)} = 4.73$, $p < 0.05$) **(Figure 4C)**, thus it was reduced in all groups over the test period compared to the habituation phase. The post hoc comparison revealed that the 100 mg/kg CIP-treatment group demonstrated significantly reduced motor activity during the habituation phase compared to vehicle and 33 mg/kg CIP treatments and lower motor activity could be observed during the test phase, too.

RT-PCR and Western blot studies

BDNF mRNA and protein expression were determined seven days after the cessation of pharmacological treatment. CIP treatment did not modify significantly the BDNF mRNA and protein expression in the PFC in the applied doses compared to the vehicle treated group **(Figure 5A)**. However, a dose-dependent decrease in mRNA expression was observed in the hippocampus. The 100 mg/kg CIP treatment significantly reduced hippocampal protein expression, too, compared to both the vehicle and the low-dose CIP group. Thus the changes in mRNA and protein level show correlation by CIP dose. These results refer to a response selective for the hippocampus and also indicate a delayed long-lasting effect of a short antibiotic treatment **(Figure 5B)**.

Discussion

This study demonstrates that repeated CIP treatment reduced dose-dependently locomotion, reward-based exploratory activity, and psychomotor learning in a clinically relevant model of osteoarthritis, responses that may be analogous to some of the neurobehavioral and neuropsychiatric effects of CIP in patients[8, 24]. The differences compared to the control animals enhanced by repetition of the cognitive test, suggesting that single, short-lasting investigation of the animals can not reveal the detrimental effects of CIP. In contrast, we found no effects on

sensory gating, suggesting that this model does not replicate the reported psychogenic effects of CIP treatment[4–6]. We also found reduced expression of BDNF mRNA and protein in the hippocampus, which may underlie the observed behavioral effects by impairing neurogenesis, neuronal processing and synaptic plasticity[16]. It should also be mentioned that the high dose CIP significantly reduced body weight in the osteoarthritic animals, which might also suggest enhanced impairments in their well-being compared to vehicle treated animals.

A number of acute-onset CNS adverse effects such as psychosis and anxiety have been reported during FQ treatment, primarily during CIP treatment[4–6, 24], as it is the most frequently prescribed member of this drug class. There are only a limited number of preclinical studies on CIP-induced central adverse effects investigating only healthy animals[9, 10, 12, 25]. Significant changes in locomotor activity have been reported in quinolone-treated rats compared to control animals, but the nature of the changes has varied markedly across studies, including increased spontaneous locomotor activity in rats[9], increased ethanol-induced hypermotility in mice[25]. In contrast, significantly depressed locomotor activity and signs of anxiety and fear was observed in rats after CIP treatment assessed by different tests[11, 12]. Performance on psychomotor learning tasks is affected by multiple factors, such as mood (e.g. anxiety, fear), task complexity, motivation, and environmental familiarity or perceived safety[26]. Only a few data are available on the effects of FQs on cognitive function, and unfortunately the results originated from healthy subjects or animals obtained by simple and/or single learning tests[27–29]. The impaired task performance detected in CIP-treated osteoarthritic rats could be explained by a motivational deficit for exploratory activity as evidenced by the reduced number of side-box entries and reward (food) retrievals in the Ambitus test. However, the decreased effective exploration ratio suggests attentional deficit, too, which also might impair cognitive function. Moreover, control animals demonstrated improvement by repetition, while high-dose CIP-treated animals did not, suggesting learning deficit.

Despite the extensive use of antimicrobials in clinical practice, few preclinical studies have examined the mechanisms underlying the observed psychogenic effects. PPI test is used to assess psychotic symptoms either in patients and preclinical practice. Some antibiotics can disrupt sensory gating[30–33]. For instance, either periconceptional exposure of Wistar rats to a non-absorbable antibiotic (succinylsulfatiazole) resulted in a 1.5- to 2-fold decrease in startle inhibition[30], or cefepime, a fourth-generation cephalosporin, induced a PPI deficit via antagonism of $GABA_A$ receptors[31]. Conversely, the second generation tetracycline analog minocycline rescued PPI impairments in rodent models of schizophrenia[32, 33]. FQs are the most commonly associated antibiotic class with

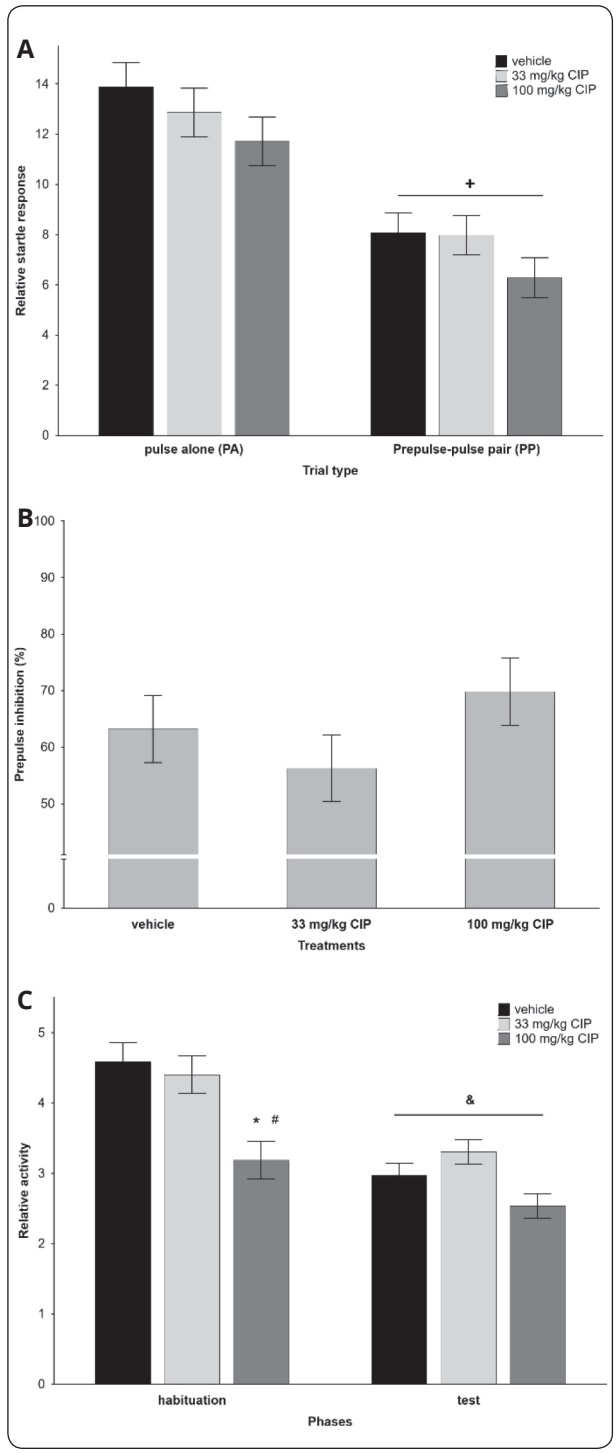

Figure 4. *The relative acoustic startle response by trial type* **(A)***, sensory gating process indicated by % PPI by treatment group* **(B)** *and the relative activity during the PPI test by phase (C; habituation vs. test). Data are presented as mean ± SEM (n=12 rats/group). The symbols indicate significant differences by LSD post hoc test between trial types (PA vs. PP, +) compared to the vehicle-treated control group (*), between CIP-treated groups (#), and between phases (&)*

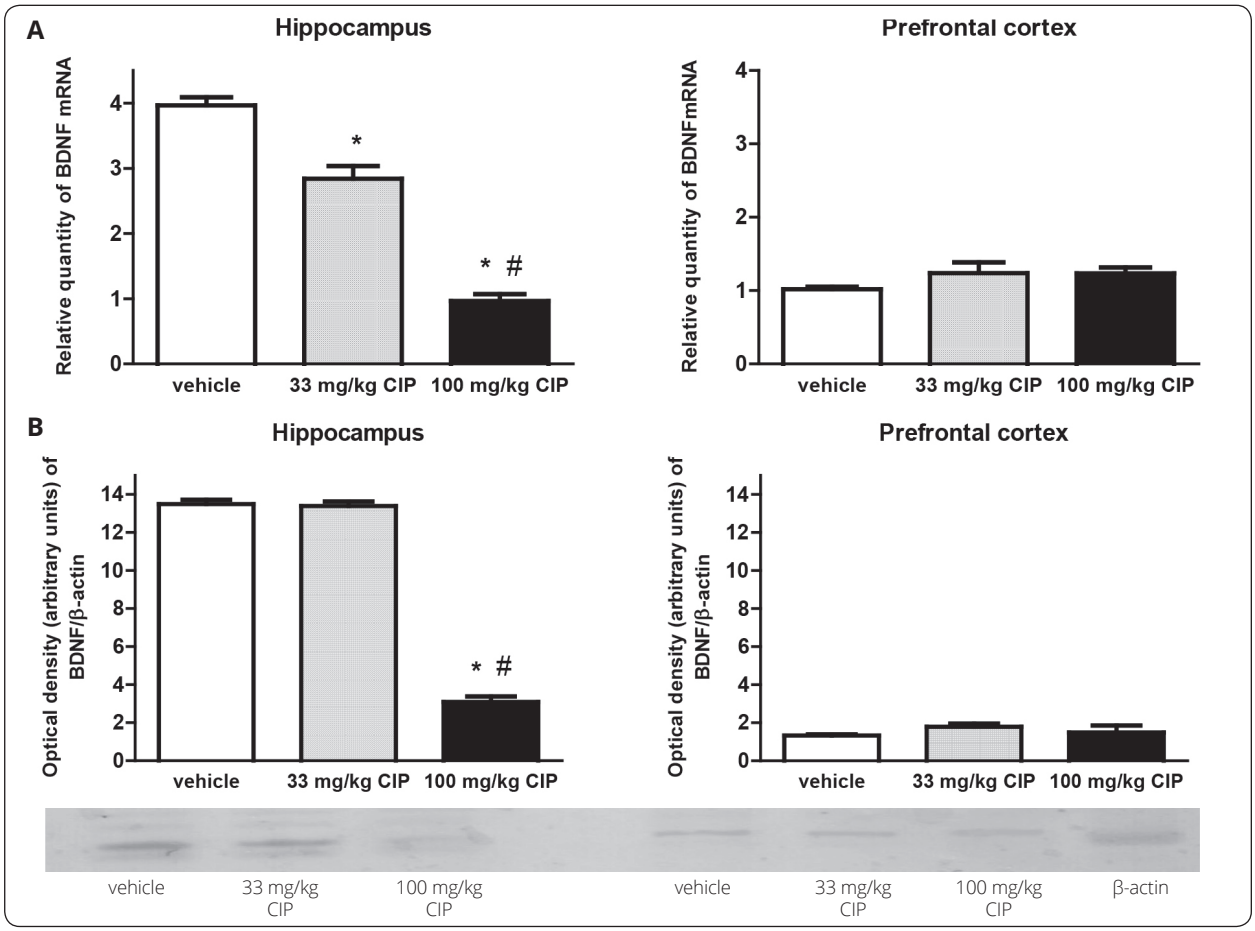

Figure 5. *Results of RT-PCR and Western immunoblotting experiments. The changes of mRNA (***A***, top panels, n=6/group) and protein expressions (***B***, lower panels, n=4/group) of BDNF in the hippocampal region and PFC samples of vehicle and CIP-treated animals. Data are presented as mean ± SEM. The symbols indicate significant differences (p < 0.001) by LSD post hoc test compared to vehicle-treated control (*) and between the CIP-treated rat brain samples (#)*

psychosis in humans[34], but only one preclinical study has examined similar responses in rodents[35]. This study investigated the perinatal and postnatal developmental toxicity of a FQ (DW-116), and it found no influence on the acoustic startle response, in agreement with our results[35]. However, the relative acoustic startle response was moderately reduced by high-dose CIP.

Multiple pathogenesis of quinolone-induced direct neuronal adverse effects have been proposed, most of them with pharmacodynamic mechanism[8]. Acute psychotic reactions and grand mal convulsions were reported following topical CIP application, too, suggesting direct neuronal effects[36, 37]. By displacing GABA from its receptors, decreased GABAergic inhibition may occur to shift the excitatory–inhibitory balance toward hyperexcitation, excitotoxicity, and lead to altered CNS functions. Decreases in brain serotonin and GABA levels have also been observed in CIP-treated rats[9]. The structural similarities of FQs to kynurenic acid, an endogenous glutamate receptor ligand, also suggest direct enhancement of neuronal excitability[38]. In addition, it has been proposed that FQs can chelate extracellular magnesium, thereby removing its channel blocking effect on NMDA receptors and driving neuronal hyperexcitability[39]. Enhanced oxidative stress and weakened antioxidant defense system may also contribute to these signs, as CIP treatment increased the accumulation of the lipid peroxidation product malondialdehyde, reduced the level of the endogenous antioxidant glutathione, and suppressed catalase activity in rat brain[9].

In most reported clinical cases, it is not clear if the observed antibiotic-related neural impairment is due to direct neuroactive properties of the drug or to alternations in endogenous microbiota (dysbiosis)[34]. Although, dysbiosis-related altered gut-brain communication might also be involved in systemic adverse effects. There are a number of potential pathways involved in the crosstalk between gut microbiota and brain[40], such as modulation of the immune system, HPA axis, and tryptophan metabolism, and production of short-chain fatty acids with

neuroactive properties. An association between gut dysbiosis and impaired learning and memory in rodents is well established; however, unlike our present study, these investigations administered antibiotic cocktails by oral gavage to animals without any condition to indicate this treatment[17, 41, 42]. Further, only two study included CIP in the antibiotic cocktail[42, 43]. Both maternal and adult treatment of mice led to persistent impairment in novel object recognition, and this effect was partially reversed by probiotic administration, strongly supporting a contribution of antibiotic-induced gut dysbiosis to these neurological, cognitive, and behavioral effects.

Evidence from rodent models suggests that microbiota depletion can alter neuronal BDNF gene expression, particularly in the hippocampus[34]. Several studies have found reduced hippocampal BDNF expression correlated with impaired cognition[17, 18, 44], although others have found no change[45] or even a modest increase[46]. Alterations in hippocampal BDNF gene products have also been reported in germ-free animals[47], further suggesting the direct neurotoxic effects of antibiotics by disrupting normal hippocampal BDNF signaling. In the present study, CIP was administered via intraperitoneal injection rather than orally, bypassing the gut. Therefore, our results are more consistent with direct effects on neural function, however further molecular-biological studies are required to reveal the precise role of BDNF alterations after CIP treatment in the neuropsychiatric funtions.

This study investigated the effects of CIP only in osteoarthritic animals without healthy control group, which might be considered as a limitation. Although, the deteriorations observed in CIP treated animals compared to the saline treated group suggests that the paradigm is appropriate to consider the consequence of an antibiotic treatment with appropriate indication. While, it can not be excluded that the lower performance in the Ambitus test really reflects the cognitive impairment or a motor deficit due to increased pain in CIP-treated animals, the moderate improvement of the locomotor and exploratory activities by the introduction of a new task in these animals could at least partially go against enhanced pain sensation. Furthermore, the decrease in exploration by repetition using the same task also signs the motivational deficit of the CIP-treated animals. In conclusion, this study firstly reported the detrimental effects of CIP treatment on neurobehavioral parameters and BDNF transcripts in a clinically relevant condition for the indication of antibiotic treatment. However, further experimental and clinical studies are required to ascertain the exact mechanisms of its memory impairing potential.

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