Functional and spinal neuropeptidomic alterations in a new rat surgical model of osteoarthritic pain: A pilot study

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Background. Osteoarthritis is the leading cause of chronic joint pain, causing important productivity and economic losses. It is believed that peripheral and centralized sensitization play a role in the creation and maintenance of a chronic painful state. Different animal models have been employed for the investigation of pain mechanisms and evaluation of potential treatments, but none of them are ideal in terms of reproducibly, reliability and translational value. **Methods.** In the search for better animal model, this pilot study was performed with the goal of evaluating pain functional outcomes and spinal biomarkers between three surgical rat models of osteoarthritic pain, *i.e.* destabilization of the medial meniscus, cranial cruciate ligament transection and the combination of both, and comparing those results to the intra-articular injection of monosodium iodoacetate. Six rats were assigned to each model group and a Sham group. Static weight bearing, punctate tactile paw withdrawal threshold, and spinal neuropeptides (substance P, calcitonin gene-related peptide, bradykinin, and somatostatin) were evaluated for each group. **Results.** Both the monosodium iodoacetate and combination models induced functional alterations in static weight bearing and punctate tactile paw withdrawal threshold, the changes being more persistent in the combination group. Both also produced an increased release of pro-nociceptive and anti-nociceptive neuropeptides at different time-points. When surgical models were compared, the cranial cruciate ligament transection and destabilization of the medial meniscus models were less interesting, with temporary functional alterations, and no significant change in neuropeptides. **Discussion.** The surgical induction of osteoarthritis was accompanied by quantifiable neurophysiologic changes relating to non-physiologic pain. Comparison with the monosodium iodoacetate model showed that the interest of a surgical model, especially the combination of destabilization of the medial meniscus and cranial cruciate ligament transection, might reside in more persistent and progressive changes, a model

that may represent better the human post-traumatic osteoarthritis.

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ABSTRACT

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Discussion. The surgical induction of osteoarthritis was accompanied by quantifiable neurophysiologic changes relating to non-physiologic pain. Comparison with the monosodium iodoacetate model showed that the interest of a surgical model, especially the combination of destabilization of the medial meniscus and cranial cruciate ligament transection, might reside in more persistent and progressive changes, a model that may represent better the human post-traumatic osteoarthritis.

INTRODUCTION

Osteoarthritis (OA) is an important pathology of veterinary and human patients. It is documented as the first cause of chronic joint pain in human patients in the USA, causing tremendous decreases in productivity and economic losses (*Pomonis et al., 2005*). Currently available treatments are centered on symptom relief and, although knowledge of the disease process has significantly evolved over the past decades, the pathology and symptomatology remain only partially understood (*Fernihough et al., 2004*; *Pelletier, Martel-Pelletier & Abramson, 2001)*. It is believed that peripheral and centralized sensitization play a role in the creation and maintenance of a chronic painful state and that it is imperfectly correlated to radiographic or histologic evaluation of the affected joints *(Fernihough et al., 2004*; *Hawker, 2012*; *Im et al., 2010*; *Zhang, Ren & Dubner, 2013*).

Different animal models have been employed for the investigation of pain mechanisms and evaluation of potential treatments. The ideal animal model should be reproducible, reliable and offer the best translational value possible (*Bendele, 2001*; *Little & Smith, 2008*). Classically, the intra-articular injection of monosodium iodoacetate (MIA) in rats has been used for the evaluation of analgesic OA therapies. It relies on the disruption of chondrocyte glycolysis, causing an interruption in their metabolism and subsequent cartilage damage *(Guzman et al., 2003*; *Kobayashi et al., 2003*; *Pomonis et al., 2005*). It is believed to cause structural changes that mimic the human pathology and, although pathogenesis is different from the natural disease, weight bearing changes (*Pomonis et al. 2005*) and centralized pain were documented *(Ferland et al., 2011*; *Fernihough et al. 2004*; *Im et al., 2010*; *Zhang, Ren & Dubner, 2013*). Unfortunately, the MIA model causes temporary changes of short duration and relies on a disease mechanism different from human OA, which could limit the predictability of therapeutic effect of analgesics and disease modifying agents. Different surgical rat models have also been used with various results and outcome measures. Until now, none of the surgical model has satisfied all the desired criteria (*Barve et al., 2007*; *Bendele, 2001*; *Little & Zaki, 2012*).

In the search for better animal models, this pilot study was performed with the goal of evaluating pain functional outcomes and spinal biomarkers between three surgical rat models of OA pain, *i.e.* destabilization of the medial meniscus (DMM), cranial cruciate ligament transection (CCLT)

and the combination of both (Combo), and comparing those results to the MIA model. The use of DMM was previously studied in mice for structural and biomarker assessment (*Das et al., 2010*; *Inglis et al., 2008)*. To our knowledge, it is the first application of this surgical model in rats. It was selected because of the ease of induction and standardization compared to the meniscectomy. Consequently, the Combo model appears as a new surgical OA model in rats. The research hypothesis was that surgical OA induction would be accompanied by quantifiable neurophysiological modifications compatible with the presence of chronic non-physiologic pain.

MATERIAL AND METHODS

Animals

The study protocol was approved by the Université de Montréal Animal Care and Use Committee (No. rech-1766), in accordance with the recommendations of the Canadian Council on Animal Care.

Female Sprague-Dawley rats (n = 30) were obtained from Charles River Canada (St.-Constant, Québec, Canada). Mean body weight was 400 g and ages ranged from four to eight months old (skeletal maturity).

The study was conducted at ArthroLab Inc. (Saint-Basile-le-Grand, Québec, Canada) in a standardized environment and with routine maintenance according to ArthroLab Inc. SOP AC7011-3.

Group description

Rats (n = 6 per group) were randomly assigned to one of the five treatment groups that included three different surgical OA models, one Sham surgical model and one MIA model. Groups were as follows: (1) Sham; (2) DMM; (3) CCLT; (4) Combo for the combination of DMM and CCLT rats; (5) MIA.

Induction of OA

Anesthesia and analgesia. For the four surgical groups, on day (D) 0, 0.02 mg/kg of buprenorphine (Buprenex®, Reckitt Benckiser, Richmond, VA, USA) was administered

intramuscularly as premedication, anesthesia was induced with isoflurane (IsoFlo®, Abbott Animal Health, Montreal, Québec, Canada) in O_2 in an induction box and maintained with 2% isoflurane in O_2 mixture with a face mask. At the end of the surgical procedures, a periarticular block of bupivacaine 0.25% (Marcaine®, McKesson Canada, St.-Laurent, Québec, Canada) at a dose of 0.05-0.1 mL per stifle (< 1 mg/kg) was performed. For the MIA group, similar procedure was conducted, with the exception of the periarticular bupivacaine block.

Intra-articular MIA injection. In the subjects of the MIA group, an intra-articular injection of 2 mg of MIA (Sigma-Aldrich, St.-Louis, MO, USA) dissolved in 50 µL of 0.9% sterile saline was performed through the right infrapatellar ligament using a previously described technique (*Fernihough et al., 2004*; *Guingamp et al., 1997*; *Otis et al., 2016*; *Vermeirsch et al., 2007*).

Surgical procedures. All procedures were performed on the right stifle following preparation for aseptic surgical technique. For the surgical groups, a medial skin incision followed by a medial parapatellar arthrotomy was used. The patella was luxated laterally, the pertinent articular structures were identified and the designated procedure was performed. Then, the patella was anatomically reduced and the surgical site closure was performed in successive planes using 5-0 polyglactin 910 (Vicryl®, Ethicon, Somerville, NJ, USA). In the subjects of the Sham group, all intra-articular structures were left intact after the arthrotomy. In the animals of the DMM group, the medial cranial meniscotibial ligament was identified and transected using a #15 blade as previously described in mice (*Glasson, Blanchet & Morris, 2007*). Spontaneous caudomedial retraction of the medial meniscus was observed, proof of complete transection of the ligament. In the rats of the CCLT group, the cranial cruciate ligament was transected with a #11 blade as previously described (*Williams et al., 1982*) and the complete transection was confirmed by cranial drawer motion. In the subjects of the Combo group, first the DMM was performed followed by the CCLT.

Functional evaluations

Rats were acclimatized to the evaluation environments at D-14, D-7, D-5 and D-3, spending five to ten minutes in each of the two apparatus used for functional pain assessment, according to a recent validation in rats (*Otis et al., 2016*). Assessment time-points differed between the surgical groups (D-1 = baseline, D14, D28 and D42) and the MIA group (D-1 = baseline, D3, D7, D14

and D21). Selected functional pain assessment methods have been recently determined as reliable (reproducible, repeatable) and sensitive to pain OA detection in rats using the MIA model (*Otis et al. 2016*). Functional evaluation observers were completely blinded to OA induction, and experimental design.

The weight distribution through the right and left stifle was assessed using an Incapacitance Meter® (IITC Life Science Inc., Woodland Hills, CA, USA) to measure static weight bearing (SWB) distribution in the two hind limbs as previously published (*Otis et al., 2016*). The force exerted by each hind limb was measured and analyzed in grams, but reported in percentage of total body weight (%BW) to normalize the data. Rats were allowed to acclimate to the testing apparatus and when stationary, readings were taken over a 3-s period. Triplicates were taken simultaneously for each limb at each time point.

Then, tactile sensitivity was assessed using the Electronic von Frey anesthesiometer® (IITC Life Science Inc., Woodland Hills, CA, USA) with a standardized filament (0.7mm^2 polypropylene Supertip) to obtain punctate tactile paw withdrawal threshold (PWT). Rats were placed in a grillage-bottom cage on an elevated stand and allowed to acclimatize for one minute. The operator then applied the filament with continuous progressive pressure to the center of the plantar surface of the paw until the animal lifted the paw. Both hind paws were tested three times, in a randomized order, and with a refractory period of one minute between each trial (*Otis et al,. 2016)*.

Euthanasia and spinal cord collection

Euthanasia was performed by decapitation following isoflurane overdose (after the last functional evaluation day, D21 for the MIA and D42 for the surgical groups) after which collection of the spinal cord was achieved by a saline flush technique (*Otis et al., 2016*). Samples were snap frozen in cold hexane, stored individually and kept at -80°C pending neuropeptidomic analysis.

Neuropeptidomics

Central sensitization mechanisms include various biochemical processes such as increased spinal release of neurotransmitters and neuromodulators, as well as an increased excitability of

postsynaptic neurons. Recently, spinal release of substance P (SP) and calcitonin gene-related peptide (CGRP) was detected in the MIA-induced OA pain model in rats (*Kobayashi et al., 2003*; *Otis et al., 2016*). In the present study, SP, CGRP, bradykinin (BK) and somatostatin (SST) were analyzed by high performance liquid chromatography-mass spectrometry and expressed in fmol/mg of spinal cord homogenates (1:5 w/v in 0.25% TFA solution) according to a previously described technique (*Otis et al., 2016*).

Statistical analysis

The %BW and PWT data were expressed as the average of the three trials of each paw. The symmetry index was used only to statistically confirm the impressions given by the graphs, when necessary.

The normality of the data (Shapiro-Wilk test) and the homogeneity of variance were confirmed using the absolute values of the residuals of the mixed model, when appropriate. Unless indicated otherwise, hypothesis were two-sided and alpha-value was set at 0.05.

For each model, the first tested hypothesis was that there was at least one evaluation day when the outcome was different from the baseline. A linear mixed model for repeated measures was used. Multiple comparisons were performed using the Dunnett procedure. Then, the surgical models that presented a significant change over time were compared. The second hypothesis was that at least one model differed. The alpha-value was set at 0.1 at that time to maximize the chances of significant results in a comparative pilot study setting. It is acceptable to set a higher alpha value, when the goal of the study is to find an effect that could lead to a promising scientific discovery. This allows to increase the power and consequently decrease the risk of Type II error, but it also increases the chances of making a Type I error (*i.e.,* saying there is a difference when there is not) (*Curran-Everett & Benos, 2004*). Data were processed using a linear mixed model for repeated measures, except for the neuropeptides data, which were analyzed with the unpaired exact Wilcoxon test following a non-parametric Kruskal-Wallis one-way analysis of variance. Tukey adjustment was used to obtain adjusted (adj)-*P*-values for multiple comparisons.

194 RESULTS

195 All animals lived until the day of euthanasia and there were no significant complication
196 following the surgical procedures or intra-articular injections. The %BW and PWT data of one
197 rat of the CCLT group were excluded from statistical analyses due to non-relevant baseline
198 values. Collection of the spinal cord was unsuccessful in one rat of the MIA group.
199

200 **Functional evaluations**
201 The values from all groups except for DMM rats, presented a significant change over time for
202 the right hind limb (RHL) %BW (Table 1). Values decreased for all groups at the second
203 evaluation time-point and tended to increase afterwards (Fig.1). Within the surgical models, only
204 the Combo model data were still significantly lower than the baseline values at D28 (Table 1). A
205 Type III day effect was noted (P=0.004) for the surgical models, no group effect, and this
206 indicated that globally, an alteration in the %BW of the RHL was detected over time but the
207 analysis was not sensitive enough to detect the apparently more severe change in the Combo
208 group (Fig.1). Interestingly, there was an increase in the %BW of the left (non-affected) hind
209 limb in the MIA group at D3 and D7 and the asymmetry SWB distribution confirmed a
210 significant weight shift to the left side for these time-points (P<0.001). In the surgical groups,
211 this phenomenon was not observed and the SWB distribution was not significantly different from
212 baseline.
213 For the right hind paw (RHP) PWT, changes in time were significant for all groups except the
214 Sham group (Table 2). Values decreased for all groups at the second evaluation time-point and
215 tended to increase afterwards (Fig.2). The Combo group continued to show a significantly
216 persistent decrease in RHP PWT values until the last evaluation day (Table 2). A Type III effect
217 of the day (P=0.014) and group (P=0.064) was present when surgical models were compared
218 using normal distribution compound symmetry with heterogeneous day covariance structure
219 mixed model. This indicated that globally, an alteration in PWT was detected over time and the
220 statistical analysis was sensitive enough to detect a larger alteration in the Combo group than in
221 the DMM group (P=0.053). Interestingly, the difference between groups was not significant for
222 the Combo when compared to the CCLT and Sham groups (Fig.2). There was a simultaneous
223 increase in the PWT of the left hind paw and decrease of PWT of the RHP on D14 for the DMM

group and on D3 and D7 for the MIA group. The asymmetry distribution of the PWT showed a significant weight shift to the left side at D3 and D7 (P<0.001) for the MIA group only. In the surgical groups, this was not observed and the PWT distribution was not significantly different from baseline.

Biomarkers

Compared to the Combo group, all other surgical groups presented significantly lower values for CGRP (Sham adj-P=0.002; CCLT adj-P=0.007; DMM adj-P<0.001) (Table 3). The concentration of SST in the Combo group was significantly higher compared to Sham and CCLT groups (adj-P=0.088 and 0.017, respectively). The spinal concentrations of SP and BK presented a Type-III significant group effect (P=0.095, and 0.028, respectively), but the analysis was not sensitive enough to detect the difference between surgical groups. Values of all neuropeptides, except SP (P=0.476), were significantly higher in the MIA model compared to Combo group (adj-P<0.02).

DISCUSSION

In the search for an animal model of osteoarthritic pain that would allow the best therapeutic evaluation and translation to the human specie, this study allowed some interesting comparisons between the MIA model and different surgical models, particularly the Combo model. Because the duration of evaluation of the chemical (MIA) and surgical models was different (up to D21 for MIA; up to D42 for surgical models), as well as the time-points distribution, the comparison between the MIA and Combo models calls for prudence. Nevertheless, the main results are that:

1. Both MIA and Combo models induced functional alterations in %BW and PWT, these changes lasting for a longer period of time in the Combo group.
2. Both MIA and Combo models induced an increased release of pro-nociceptive (CGRP) and anti-nociceptive (SST) neuropeptides.

Functional evaluations

This pilot study highlighted a limited interest in the CCLT and DMM models, as their functional alterations were of short duration, and the change in neuropeptides non significant, compared to Sham. Interestingly, the functional changes induced by the CCLT and DMM models, were not so different from the Sham group. This suggests that their functional alterations were most likely the result of joint inflammation associated with the arthrotomy, and not the consequence of significant biomechanical instability. It could be argued that the CCLT and DMM models could have shown alterations resulting from biomechanical instability if the rats had been more mobile and active, which was not part of the current study design (*Appleton et al., 2007*). Hence, they could remain interesting models in specific study settings. However, the changes induced by both the MIA intra-articular injection and Combo surgery led to biomechanical (SWB), sensory (PTW) and nociceptive neuropeptides changes in the same research context.

The significant weight shift to the left hind paw on SWB in the MIA group could be interpreted as an early occurring but non-persistent biomechanical change since the %BW values for the MIA group were not different from baseline after D7. This phenomenon was not observed in the surgical groups and could constitute a major difference between the MIA and surgical models. This contralateral weight shift could be indicative of major discomfort in the (affected) right hind limb with the rat seeking to relieve itself from this acute insult, whereas the more progressive

damage in the Combo model does not produce such intense and early pain. The increase in the left hind PWT contemporary to the decrease of the RHP values could be explained by two hypotheses. First, the significant weight shift to the left hind paw at D3 and D7 in MIA could be responsible for the elevation of the PWT of the left paw of an animal being ‘’less responsive’’: the animal being very reluctant to bear weight on the painful limb during the inflammatory phase of the MIA model (*Fernihough et al., 2004*; *Guingamp et al., 1997*) artificially increases the PWT on the contralateral limb. With regards to the early occurrence of such SWB and PWT shift in the MIA model, it could also reflect early peripheral sensitization, leading subsequently or concomitantly to central sensitization. Second, diffuse descending pain inhibition mechanisms (*Beaulieu, 2005*; *Felson, 2005*; *Le Bars, Dickenson & Besson, 1979*) could be activated very efficiently by the initial strong inflammation present in the MIA-treated stifle and be less intense as time passes and inflammation subsides. It is uncertain at this point if the biomechanical, neurological and/or inflammatory component are responsible for those results. Nonetheless, it constitutes a significant difference between the MIA and the surgical models.

The changes persisted until the last RHP PWT evaluation time-point in the Combo group and only until D14 for the MIA group. This could be an indication for the capacity of the Combo model to induce a more persistent tactile allodynia compared to the other models in this study. The group effect in the RHP PWT showed that the Combo model sensitization was more severe than in the DMM model, because it induced more tactile allodynia. It would be expected that the Combo model would also be more severe than the CCLT model although the difference was not statistically significant in this study, likely because of a low statistical power (Type-II statistical error). The same explanation applies for the absence of statistical difference between surgical groups for RHL SWB.

Biomarkers

As neuromodulators, SP and CGRP are important players in peripheral and centralized sensitization in inflammatory arthritis and OA (*Otis et al,. 2016*; *Schaible et al., 2009*). Both SP and CGRP were higher in the Combo model than in the other surgical models, but only CGRP reached statistical significance. CGRP is accepted as an important mediator in subchondral (*Aso et al., 2016)* and central (*Otis et al., 2016*) OA pain signalling using the MIA rat model. This

suggests that there was induction of neuronal plasticity at the central level for the Combo model too.

The significantly lower SST in the Sham and CCLT groups compared to the Combo group is interesting as it could indicate a greater potential of the Combo model to induce allodynia. SST has not been evaluated specifically in osteoarthritic conditions. It was mostly studied for inflammatory conditions like rheumatoid arthritis and asthma *(Pintér, Helyes & Szolcsányi, 2006*). With the hypothesis that the inflammatory component of the disease is likely to be a major contributor to the pathological pain, it would be expected that if a model causes more inflammation, it could induce more allodynia. Additionally, such SST spinal release could be associated with an increased descending nociceptive inhibition (*Bär et al., 2004*; *Pintér, Helyes & Szolcsányi, 2006)*. This phenomenon of increased inflammation and concomitant inhibitory pain modulation could be monitored by the quantification of SST in a research setting. Finally, BK has been studied in multiple species and reported to be involved in OA pain (*Meini & Maggi, 2008)*. However it was not possible to detect significant change in the current study.

The significantly higher values of CGRP, SST and BK in the MIA model could indicate that it causes more pain and has a greater potential for allodynia induction. But, the comparison with the Combo group is limited since the time frame for both groups was different as was the time of spinal cord collection. Both the MIA and the surgical models are expected to require time to develop significant articular lesions and neuronal plasticity (*Orita et al., 2011*). Previous studies in surgical models showed that at least six weeks might be required (*Hayami et al., 2006*). The maximal potential for pathological pain induction of the Combo model might not have been reached at D42 (*Ferland et al., 2011*).

Following this pilot study, it was calculated that, to reach a statistical power of 80% with an alpha-value of 0.05, 12 rats per group would be required to document an 8% difference in the SWB and 10 g in the PWT. Those numbers reflect the difference documented between the Sham and Combo group.

CONCLUSION

In conclusion, the surgical induction of OA was accompanied by quantifiable neurophysiological changes associated with pain, as shown by functional analysis, spinal neuropeptides and

comparison with the current gold standard of OA pain in rats, the MIA model. The research hypothesis was confirmed and objectives reached. The Combo model can induce changes compatible with chronic pain and comparison with the MIA model indicates more persistent changes potentially useful for the evaluation of therapeutic modalities. Indeed, the limited (in time) alterations in the 2 mg MIA model reduce drastically the accessible window for assessing any therapeutic efficacy. Moreover, the changes observed in the Combo surgical model seem more progressive and consequently present higher degree of face validity with natural post-traumatic OA. Prospective studies with a larger number of animals, a longer duration, multiple time points evaluation of the histologic, functional, epigenomic and neuroproteomic changes would help to obtain a better characterization of the Combo model. Validation with therapeutic intervention should also be performed.

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FIGURE LEGENDS

Figure 1. Percentage body weight (%BW) (mean ± standard deviation)) of the right hind limb for the static weight bearing by day (D).

Time is distributed differently for the surgical (D-1, D14, D28 and D42) and the MIA (D-1, D3, D7, D14, D21) groups. A star indicates a day when there is a statistically significant decreased value compared to its baseline (see Table 1 for details).

Figure 2. Paw withdrawal threshold (PWT) (mean ± standard deviation)) of the right hind paw by day (D).

Time is distributed differently for the surgical (D-1, D14, D28 and D42) and the MIA (D-1, D3, D7, D14, D21) groups. A star indicates a day when there is a statistically significant decreased value compared to its baseline (see Table 2 for details).

Table 1(on next page)

Table 1 - Static weight bearing for the right hind limb.

Testing time effect and specific comparison *vs*. baseline of the static weight bearing for the right hind limb.

Testing time effect and specific comparison *vs*. baseline of the static weight bearing for the right hind limb.

Experimental groups	Type III test of fixed effects ProbF	Day	Adjusted *P*-value (diffences of least squares means, standard error)
Sham	**0.041**	14	**0.022** (-13.93, 4.61)
CCLT	**0.028**	14	**0.006** (-14.49, 2,98)
DMM	0.599		
Combo	**<0.001**	14	**<0.001** (-18.33, 2.83)
		28	**0.003** (-14.01, 3.60)
MIA	**<0.001**	3	**<0.001** (-19.74, 4.11)
		7	**0.006** (-15.17, 4.19)

Notes: For each group, the best structure of the covariance model was assessed using a graphical method (plots of covariance *vs.* lag in time between pairs of observation compared to different covariance model), and using information criteria that measure the relative fit of competing covariance model: normal distribution, compound symmetry covariance structure (Sham, DMM and MIA groups); heterogeneous compound symmetry covariance structure (CCLT group), and type-1 auto regressive covariance structure (Combo group). For the baseline to specific day comparison, adjusted *P*-value for multiple comparisons was obtained using the Dunnett procedure. A bold font highlights a significant difference.

Table 2 (on next page)

Table 2 - Paw withdrawal threshold for the right hind paw.

Testing time effect and specific comparison *vs*. baseline of the paw withdrawal threshold for the right hind paw.

Testing time effect and specific comparison *vs*. baseline of the paw withdrawal threshold for the right hind paw.

Experimental groups	Type III test of fixed effects ProbF	Day	Adjusted *P*-value (diffences of least squares means, standard error)
Sham	0.061		
CCLT	**0.036**	14	**0.014** (-23.47, 6.90)
DMM	**0.049**	14	**0.043** (-14.81, 5.52)
Combo	**0.009**	14	**0.005** (-31.64, 8.32)
		28	**0.047** (-21.97, 7.96)
		42	**0.017** (-26.26, 8.14)
MIA	**<0.001**	3	**<0.001** (-34.34, 5.68)
		7	**0.004** (-25.20, 6.83)
		14	**0.049** (-19.07, 7.29)

Notes: For each group, the best structure of the covariance model was assessed using a graphical method (plots of covariance *vs.* lag in time between pairs of observation compared to different covariance model), and using information criteria that measure the relative fit of competing covariance model: normal distribution, compound symmetry covariance structure (Sham, CCLT, DMM, Combo and MIA groups). For the baseline to specific day comparison, adjusted *P*-value for multiple comparisons was obtained using the Dunnett procedure. A bold font highlights a significant difference.

Table 3(on next page)

Table 3 - Neuropeptide spinal concentrations.

Between-groups comparison of neuropeptide spinal concentrations (mean ± standard deviation) in surgical and chemical models of osteoarthritis pain in rats.

Between-groups comparison of neuropeptide spinal concentrations (mean ± standard deviation) in surgical and chemical models of osteoarthritis pain in rats.

		Neuropeptides (fmol/mg)			
Experimental groups	*n*	SP	CGRP	BK	SST
Sham	6	112 ± 12 [a]	569 ± 42 [a]	213 ± 15 [a]	339 ± 23 [a]
CCLT	6	118 ± 18 [a]	593 ± 58 [a]	183 ± 15 [a]	325 ± 28 [a]
DMM	6	104 ± 16 [a]	546 ± 42 [a]	191 ± 14 [a]	351 ± 23 [a,b]
Combo	6	135 ± 31 [a]	**725 ± 105** [b]	195 ± 20 [a]	**379 ± 45** [b]
MIA	5	147 ± 11 [a]	**1065 ± 153** [c]	**354 ± 12** [b]	**722 ± 44** [c]

Notes: Between-group comparison was conducted using a non-parametric Kruskal-Wallis one-way analysis of variance with post-hoc analysis, when required, using the unpaired exact Wilcoxon test following. Tukey adjustment was used to obtain adjusted *P*-values for multiple comparisons. Different letters indicate statistically significant difference highlighted by bold font.

Figure 1(on next page)

Fig 1 - Percentage body weight (%BW) (mean ± standard deviation)) of the right hind limb for the static weight bearing by day (D).

Time is distributed differently for the surgical (D-1, D14, D28 and D42) and the MIA (D-1, D3, D7, D14, D21) groups. A star indicates a day when there is a statistically significant decreased value compared to its baseline (see Table 1 for details).

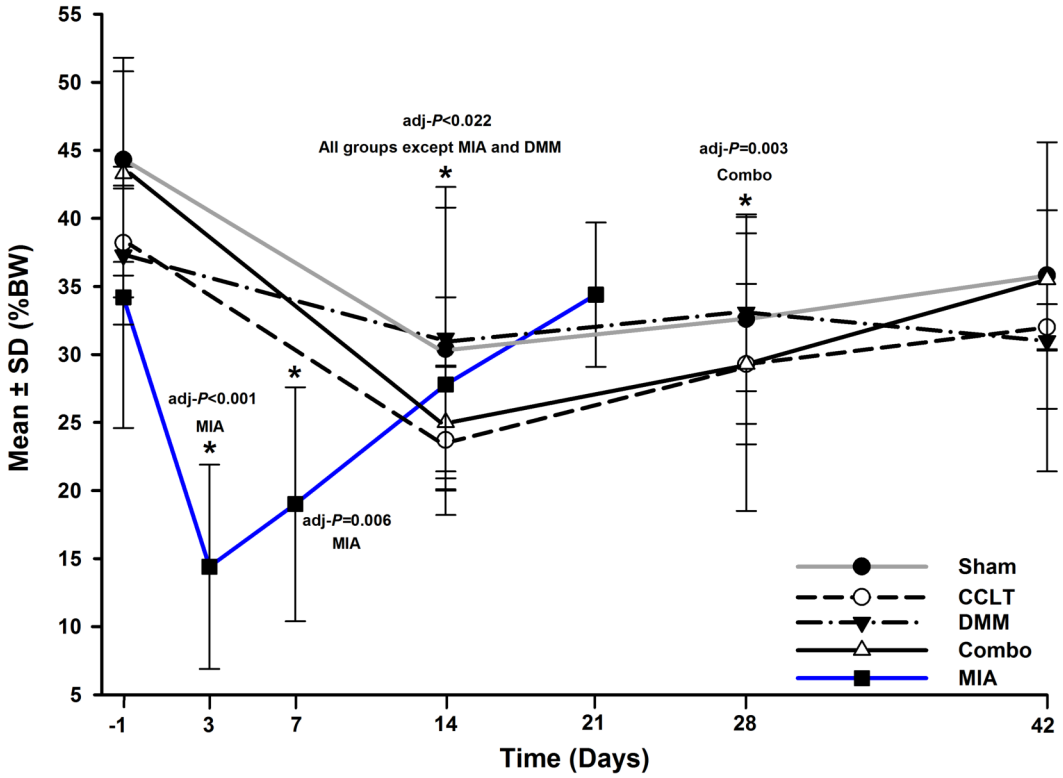

Figure 2(on next page)

Fig 2 - Paw withdrawal threshold (PWT) (mean ± standard deviation)) of the right hind paw by day (D).

Time is distributed differently for the surgical (D-1, D14, D28 and D42) and the MIA (D-1, D3, D7, D14, D21) groups. A star indicates a day when there is a statistically significant decreased value compared to its baseline (see Table 2 for details).