Received: 4 September 2020 | Revised: 1 January 2021 | Accepted: 1 February 2021

DOI: 10.1096/fj.202002009R

RESEARCH ARTICLE

THE FASEB JOURNAL

Systematic analysis reveals that colony housing aligns gait profiles and strengthens link between histological and micro-CT bone markers in rat models of osteoarthritis

Yu-Mei Chang[1] | **Stephanie Menges**[2] | **Andreas Westhof**[2] | **Kerstin Kleinschmidt-Doerr**[2] | **Christian Brenneis**[2] | **Andrew A. Pitsillides**[1]

[1]Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK

[2]Merck Healthcare KGaA, Darmstadt, Germany

Correspondence
Yu-Mei Chang, Royal Veterinary College, 4 Royal College Street, London NW1 0TU, UK.
Email: ychang@rvc.ac.uk

Abstract

Osteoarthritis (OA) etiopathogenesis is complex with strong environmental/lifestyle determinants that, in laboratory animals, extend to social context and stress levels. This study seeks to identify whether colony housing of rats exerts a social impact on locomotion behaviors to influence alignment between symptomatic (gait) and structural (bone micro-CT measures, cartilage morphometry, and histology) OA outcome measures. Rats were randomly allocated to conventional (type IV; n = 48) or rat colony cage (RCC; n = 30) housing, further randomized to OA surgical models (ACLT + tMx, MMT or DMM) or no surgery (control), and maintained for 19 weeks during which multiple gait recordings were made. Standard histological grading and bone micro-CT data were collected at necropsy. Principal component analysis was used to summarize the variation in gait, micro-CT or histology. Linear mixed effects model or two-way ANOVA was employed to evaluate the impact of the housing system, surgery and time on gait, or micro-CT and histology components Analyses reveal that RCC exaggerates trends in gait change via a combined effect of the housing system and surgery. Intriguingly, RCC-housed nonoperated control rats showed similar gait changes to rats subjected to surgery; the latter exhibited significant structural joint changes in both systems. Stronger correlation between histological and micro-CT bone changes were found in medial and lateral tibia joint compartments of rats housed in RCC system. This study has established that rat social housing exaggerates outcomes in traditional histological measures of OA, generates stronger links between histology and micro-CT bone changes and removes gait differences as a variable in their etiology.

KEYWORDS

colony housing, gait, histology, micro-CT, osteoarthritis

Abbreviations: ACLT + tMx, anterior cruciate ligament transection with resection of the medial meniscus; DMM, destabilization of the medial meniscus; MIP, maximum intensity projection; MMT, medial meniscal transection; OA, osteoarthritis; PCA, principal component analysis; PCs, principal components; RCC, rat colony cage.

1 | INTRODUCTION

Osteoarthritis (OA) is a very common contributor to years lived with disability and the life-time risk of symptomatic knee OA is >40%.[1] OA is now recognized (by the FDA) as a "serious" worldwide disease, with an unmet medical need for therapies that modify the underlying pathophysiological changes across diverse joint tissues.[2] Etiology is clearly complex, with risk factors for OA development including age, obesity, repetitive physical activities, and joint injury.[3] While OA is multifactorial, it also has been found to exhibit strong environmental determinants in laboratory animals, now known to include the social context.[4,5] Housing, for example, can greatly influence OA development; the number of animals per cage is also linked to both activity and stress levels.[6,7] However, the strength of any linkage between the social context and joint pathology, which culminates in the presumed pain-related changes in gait, has not yet been examined.

The social environment may indeed be a most pertinent consideration, even in animal models of OA; wild rats can live in very large colonies, establishing complex systems at high-density overlap and extensive social interactions. This contrasts with the housing of rats in most research facilities where animals are housed in small groups, with limited environmental enrichment, complexity, or prospect of exhibiting the breadth of natural behaviors. Studies that have examined the effects of rat number and environmental complexity on welfare and stress responses have described marked predilection for cages containing acquainted rats as opposed to extra space/environmental enrichment.[8] Moreover, the presence of cagemates has, through social buffering, also been found to modify pain sensitivity and stress.[9-11]

Gait disturbance is used to quantify OA pain sensitivity and is frequently used together with histopathological joint changes to assess OA severity[7,12,13]; both are used to characterize pathological changes of tissue structure treatment interventions.[14] OA severity can thus be measured using many parameters. Longitudinal "catwalk" tests have been used to investigate the emergence of multiple gait characteristics that evaluate basal support position, paw use and ground contact, and levels of symmetry.[15] Bone mass and shape changes can be extensively characterized by micro-CT scanning and cartilage volume is determined by histopathology.[16-18] These specific parameters can readily be related to traditional histological measures of cartilage lesion severity, as well as tidemark, subchondral bone, and chondro-osteophyte changes. The strength of the alignment between these multiple measures across diverse models of OA has been explored,[12,19] but an understanding of their sensitivity to changes in the social context is incomplete.

The strength of these alignments has indeed been questioned. For example, in a surgical destabilization OA model involving medial meniscal transection (MMT), symptomatic reduction in pain in response to nonsteroidal anti-inflammatory treatment (rofecoxib) or neuropathic pain drugs (gabapentin) is, as in human patients, disconnected from any structural alterations in the affected joint.[20-24] There has also been considerable and long debate regarding the relationship between the cartilage and bone changes in OA-affected joints.[25] Finally, the extent to which OA-related gait changes are attributable solely to pain or to structural joint deficits remains largely unclear. It nonetheless is clear that the few findings up to date have emerged almost entirely from the study of rodents housed in traditional small cages (termed Type IV herein) with only limited numbers of cagemates.

Herein, we seek to explore the utility of a recently developed, enriched social system (rat colony cage, RCC) for housing up to 48 rats, over four levels connected by jump holes and staircases.[26] This RCC system has previously been shown to fundamentally change rat behavior within a few weeks, increasing activity, curiosity, and the speed of habituation and decreasing stress related to novel test environments.[26] To better understand the impact of the social environment on OA development, three surgical destabilization OA models were performed in rats housed in either the RCC system or regular Type IV cages and outcomes of selected traditional OA measures investigated and compared.[27] The approach described herein addresses the hypothesis that colony housing exerts a social impact on locomotion behaviors to influence the interaction between symptomatic (gait) and structural (bone, cartilage) outcome measures. To address this, our study uses an unbiased, systematic, and statistical evaluation of the full spectrum of longitudinal changes in gait performance and has explored their relationship to multiple histological and morphological cartilage and bone OA severity markers. Our findings reveal that the social environment allows for the emergence of stronger links between bone imaging and histological outcomes and removes gait differences as a variable in the etiology of these structural changes in OA.

2 | MATERIALS AND METHODS

2.1 | Animals, housing conditions, and experimental design

Detail of the study design and data collection were as described previously[27] and all procedures were approved by the animal protection authorities of the local district government (Hessen Regional Authorities, Germany). Briefly, 96 male Lister Hooded (Crl:LIS) outbred SPF rats aged 8-9 weeks (weighing 150-175 g) from Charles River Laboratories (Sulzfeld, Germany) were stratified based on body weight and randomized to either be housed: (i) in pairs (24 pairs, 48 total) in conventional polycarbonate cages (595 × 380 × 200 mm, floor

area, 1820 cm^2, termed type IV housing in line with manufacture specification) or (ii) in one group of 48 in the rat colony cage (total of 22 246 cm^2 over four levels connected by two jump holes and a staircase, termed RCC housing (see[27]). In each housing system, rats were further randomized to one of three surgical models or no surgery control (n = 12/group), and maintained for 19 weeks thereafter, during which time 11 separate gait recordings were made (at weeks 0, 3, 5, 7, 9, 11, 12, 13, 14, 15, and 19), before sacrifice and evaluation of joint OA severity by standard histological grading and bone micro-CT analyses. Experimenters were blinded to surgery groups during the catwalk tests and were blinded to both housing systems and surgery groups for the micro-CT scanning and histology scoring. Eighteen rats (six rats from each of the three surgical models) in the RCC housing received anti-NGF treatment at 12 weeks postsurgery and their data were excluded from the analysis. Thus, data from a total of 78 rats were analyzed, with 48 in the type IV and 30 in the RCC systems, respectively.

2.2 | Joint instability surgery

Surgery was performed after 4 weeks of habituation to their respective caging system. Three surgical models were chosen in order to produce differing degrees of joint destabilization. Accordingly, surgery was performed by one of the following three procedures: (i) ACLT + tMx (anterior cruciate ligament transection with resection of the medial meniscus): a skin incision was made medial in parallel to the patella of the right joint. The patella was translocated proximately and the joint capsule was opened. Then, the anterior cruciate ligament was transected by using a sharp hook, the anterior meniscotibial tendon was dissected by using a scalpel and the medial meniscus was removed followed by a flush with sterile saline (0.9%). To avoid unwanted damages from the surgery procedure, some remains of the meniscus (~10%) was left in the joint. However, this is still substantially different to a more defined pMx procedure were ~50% of the meniscus is left in the joint; (ii) MMT (medial meniscus transection): a medial skin incision was made parallel to the patella of the right knee and a longitudinal section made between the quadriceps tendon and the medial collateral ligament. Next, the collateral ligament was loosened with forceps and ligated, close to the meniscus, and the medial meniscus dissected by a scissor cut to the collateral ligament (smallest area of the meniscus); DMM (destabilization of the medial meniscus): a skin incision was made from distal to the patella to a position proximal to the right tibial plateau. The muscle layer was opened with a scalpel with the knee in flexion and the medial meniscus tendon was ligated. No procedure was performed on control rats. In the type IV housing, the two rats housed together were always from the same surgery group. In the RCC system, rats from each of these surgery groups were housed together with rats from all other groups.

2.3 | Gait analysis

Catwalk tests (CatWalk XT 10.0 system, Wageningen, The Netherlands) were performed in all rats to evaluate the evolution of gait characteristics before and at three time periods after surgical induction of joint instability (0 week, 3-7 weeks, 9-12 weeks, and 13-19 weeks). Where appropriate, interrogation of gait changes involved subdivision of the 135 specific measured parameters into four categories, based upon previous reviews[28,29] which related to: (i) run characteristics, speed, base of support, and print position, (ii) temporal paw statistics, (iii) spatial paw statistics, or (iv) phase dispersion/coupling.

2.4 | Bone characterization by micro-CT scanning

After necropsy, knee joints were collected and scanned using a micro-CT system (SkyScan 1176; Bruker, Kontich, Belgium). Cross-sectional slices were generated with NRecon software (version 1.6.9.4, Bruker), and all data sets were adjusted to anatomical markers with DataViewer software (Bruker). Three-dimensional analysis was performed using CTAn software (Bruker). Detail scanning parameters regarding image acquisition, image processing, and the 3D volume of interest locations[30] were as described previously[27] and also provided in Supplementary document 1D. These analysis generates a total of 312 measurements from within each joint[27,30] and these are divided by virtue of their tibial or femoral location and whether they are made in the medial or lateral joint compartment; they include joint space and subchondral thickness, osteophytes, and bone sclerosis score for both lateral and medial joint compartments as well as tissue volume, bone volume, percent bone volume, surface, surface/volume ratio, surface density, trabecular thickness and separation, trabecular number, number and volume of closed pores, connectivity, mean total cross-sectional tibia area, and bone mineral density analyzed as gray scale index. To aid interrogation of micro-CT measurements, they were integrated to generate a total of nine spatially defined parameters: (i) total score, (ii) tibia score, (iii) medial joint space, (iv) medial tibia, (v) medial femur, (vi) lateral joint space, (vii) lateral tibia, (viii) lateral femur, or (ix) tibia maximum intensity projection (MIP).

2.5 | Cartilage volume determination by histopathology and general histology scoring

Cartilage volume was measured using a randomly placed point grid overlay by image analysis software ("Stereology" microDimension GmbH) after appropriate image importation, alignment and manual outlining of an identical region

of interest in seven sections, systematically, from each joint. Microscopic scoring of the cartilage was performed by two observers and included assessment of cartilage lesion severity, intensity of matrix staining, cellularity and tidemark, subchondral bone abnormalities, chondro-osteophyte changes as well as cartilage degradation in both the medial and lateral aspects of the tibia articulation of the rat knee joint (modified from[31]). Consecutive sections displaying the most severe lesion within the weight-bearing area of each joint from each animal were analyzed. In keeping with OARSI histological scoring system,[32] a total of 22 histological parameters (including volume) were presented for both medial and lateral tibia compartments.

2.6 | Statistics

All micro-CT and histology data from each rat joint were standardized prior to analysis by subtracting the mean, and then, dividing by the standard deviation of the values found in the control, nonoperated rats in the Type IV housing system (referred to as *z*-scores). Gait data were also standardized against the Catwalk data from the control rats in the Type IV system at each of the recording weeks, and then, averaged within the four time points (0, 3-7, 9-12, and 13-19 weeks). If a data point has a *z*-score of 0, then, its score is identical to the mean; a z-score of 1.0 (or −1.0) would indicate that the value is one standard deviation above (or below) the mean. Heatmaps based on these standardized z-score measures were used to visually explore the similarity/differences between housing systems and surgery groups. Spearman's correlations were calculated among gait (at 13-19 weeks), micro-CT and histology measurements, and correlation coefficients were presented as heatmaps for each of the housing systems.

A dimension reduction technique, namely principal component analysis (PCA) was used to summarize the variations in gait, micro-CT or histology across all rats. The first two principal components (PCs) that explained the most variation in the data were extracted for further analysis. Contributions (loadings) of each particular parameter to the principal components were presented as a heatmap. Linear mixed effects model-based analysis was employed to evaluate the effects of the housing system, surgery, and time on the PCs of gait; two separate two-way ANOVA models were used to assess the effects of the housing system and the surgery on the PCs of micro-CT or on the PCs of histology. Finally, multivariable linear regressions were used to evaluate the influence of gait PCs and micro-CT PCs on the histology and also the effects of gait PCs on micro-CT PCs. Proportions of variation explaining (R^2) by the models were summarized.

There were total 78 rats included in this analysis, and two rats had missing observations on gait, micro-CT, and/or histology measures. One ACLT + tMx rat in the RCC housing was excluded from the study and analysis due to injury after week 1 (reason unrelated to the surgery), and general histology scoring was not available for one DMM rat in the type IV system. This resulted in varying sample size (n = 76-78) for different analyses.

3 | RESULTS

3.1 | Social housing elevates levels of gait disturbance, yet, harmonizes variation and neutralizes the deviation attributed to OA models

Overall our Catwalk analyses showed that gait was significantly modified by the housing system, with marked deviation even between control rats in RCC or Type IV systems. This housing-related distinction in gait was evident throughout the study duration, with clear segregation in gait behavior in rats maintained in the RCC system, even before surgical OA induction (Figure 1A). Control RCC-housed rats indeed showed similar coupling/phase dispersion changes to surgical OA rats in the Type IV system at 3-7 weeks (Figure 1A), suggesting that RCC-housed controls mimic the gait of rats with surgically induced OA. These data are consistent with a generalized harmonization of gait between groups in the RCC system, which neutralizes most of the deviation attributed to OA models.

In line with prior data from Type IV housing, we observed gait changes in each of the three surgery groups compared with their respective control rats; clear differences in heatmap patterns were found between controls and each of the ACLT, DMM, or MMT surgery groups from as early as 3-7 weeks and all later time-points. In contrast, such surgery-related gait divergence was effectively neutralized in the RCC system (Figure 1). Indeed, similar surgery-related gait changes were observed at all later time-points in all three OA groups (in both systems) as well as in control rats maintained in the RCC system. Our analysis revealed that there are also specific, additional housing-related gait characteristics observed in all groups of rats in the RCC system, indicating that modifications in gait are at least partly the product of the housing system. These data are consistent with greater gait similarity and a marked behavioral convergence across all groups in the RCC system.

Evaluation of the principal components (PC) summarizing all Catwalk measurements (135 variables, standardized against healthy rats in Type IV housing, indicated by the z-score) shows that PC1 explains 25% of the gait variation across the entire study (contribution/loading of each of the measurements to the first two PCs are given in Figure S1). PC1 effectively captures the marked difference between the alternatively housed control groups of rats, as well as the

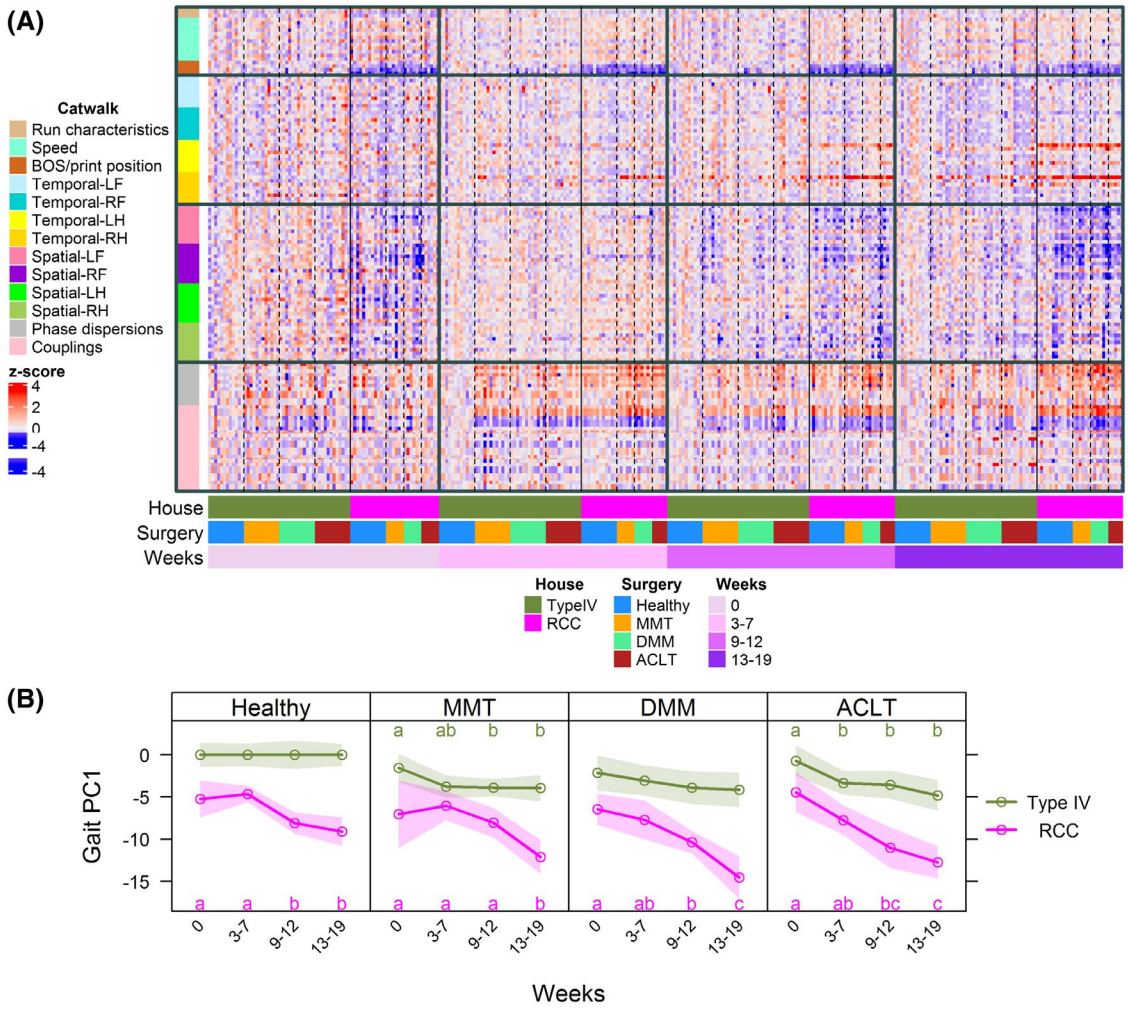

FIGURE 1 A, Heatmaps of the standardized 135 catwalk measurements (rows) across 77-78 rats (columns). Rats were grouped according to housing types and surgery groups for the four time periods (0 week, 3-7 weeks, 9-12 weeks, and 13-19 weeks); all measurements were standardized based on healthy rats under Type IV housing as indicated by z-scores (deeper red indicated higher readout and deeper blue indicated lower readout). Temporal and spatial measures of the four limbs were indicated as LF (left front), RF (right front), LH (left hind), or RH (right hind). B, Principal components analysis of the 135 Catwalk measurements (standardized based on healthy rats under Type IV house) where the first principal components explained 25% of total variation in gait across the four stages; mean and 95% confidence intervals of the means (shading along the mean lines) for PC1 were depicted across the four stages (type IV housing in burly wood and RCC in aquamarine). Significance between time points within the surgery group were indicated by different letters at the top (Type IV) and bottom (RCC) of the graph, time points sharing the same letter do not reach levels of statistical significance. For example, in the type IV housing MMT group, there was no difference between week 0 and weeks 3-7 (shared letter "a"), and there was no difference among weeks 3-7, 9-12, and 13-19 (shared letter "b"); however, there was a significant difference between week 0 and weeks 9-12 & 13-19 (no common letter)

changes in gait in each of the three OA models (Table 1). Overall, across the four time-points studied, 31%, 29%, 52%, and 58% of variation attributed to PC1 are explained solely by the housing system. Statistical evaluation of the scale of gait change induced by surgery (vs. control) demonstrates that this is more marked in Type IV than in RCC (Figure 1B). We found significant differences in gait PC1 between control and each OA group at all time-points postsurgery in Type IV (P ranged from $< .0001$ to 0.010). In contrast, OA groups in the RCC system differed from their respective controls in only DMM ($P = .029$) and ACLT ($P = .048$) groups at 3-7 weeks, ACLT at 9-12 weeks ($P = .06$), and in all three surgery groups at 13-19 weeks (P ranged from $< .0001$ to .018). These data (summarized by PC1) also indicate stronger gait disturbance within the surgery groups over time in RCC than in the Type IV system. Nonetheless, neither system allowed the statistical differentiation of gait changes between any of the three OA models (Figure 1B).

Deeper interrogation of the specific gait parameters reveals that they are best summarized upon subdivision of gait measures into four categories, namely: (i) run characteristics, speed, base of support, and print position, (ii) temporal paw statistics, (iii) spatial paw statistics, or (iv) phase dispersion/coupling. Excluding data from control rats in

RCC, such subdivision reveals divergent housing-related, surgery-related, and temporally related gait modifications in the two systems. For instance, we find that RCC imposes specific housing-related gait divergence in base of support and print position measures at all time periods, which are unmodified by surgery. Consistent with surgery-related gait changes, phase dispersion/coupling measures in both RCC and Type IV systems show similar changes soon after surgery, at 3-7 weeks. On the contrary, temporal paw statistics are not particularly modified by housing system or surgery; except hind limb maximum contact (measure of transition from braking to propulsion) which showed marked deviation at late periods in RCC. Similarly, spatial paw statistics also show convergence in both RCC and Type IV systems at 3-7 weeks, yet, intriguingly only RCC-housed rats show marked divergence in front paw contact lengths/areas with OA progression at later times. These data suggest that RCC exaggerates the trends in gait change through a combined effect of both the housing system and surgery.

3.1.1 | Social housing increases histological and micro-CT-based OA severity

Our analyses revealed that rats in all three OA surgery groups exhibit significant modification in histological scores and micro-CT bone changes in both systems (versus respective controls). Surprisingly, maintaining control rats in the RCC was enough to evoke differences in joint histology and micro-CT bone changes, compared to controls in the Type IV system. Heatmaps reveal that RCC-housed controls show patterns of histological and micro-CT change that mirror those in rats in the surgery groups (Figures 2A and 3A).

PCA showed significant housing-related and surgery-related difference in both histology and micro-CT measurements (Table 2, Figures 2B and 3B), with PC1 and PC2 together accounting for 86% of the variation in histological score (71% and 15%, respectively) and 76% of variation in micro-CT data (64% and 12%, respectively). Contributions of each of the measurements to the first 2 PCs for histological score and for micro-CT are given in Figure S1. Histology variables in PC1 that allowed most robust discrimination between ACLT, DMM, and MMT across the housing systems included medial tibia cartilage volume/OA score, revealing that medial changes were dominant. Histology PC2 variables encompassed the contrast in OA-related changes in medial versus lateral tibia cartilage. Micro-CT PCA similarly found the dominant variables to include trabecular changes in the medial tibia epiphysis and tibial MIP, which differed markedly between control and surgery groups in both RCC and Type IV systems. In agreement with previous studies, the medial tibia was most markedly affected in the OA models in both systems. Histological OA scores in the lateral tibia following ACLT are higher in the RCC system than in any other surgery groups, in either system.

TABLE 1 Linear mixed effects model assessing effects of house, surgery, time points, and their interactions on the first Catwalk principal component. (n = 77, n = 78 for catwalk at 0 week)

Gait PC1	*P*-value
House	**<.0001**
Surgery	**<.0001**
Time point	**<.0001**
Interaction House*Surgery	.60
Interaction House*Time point	**<.0001**
Interaction Surgery*Time point	**.0074**
Interaction House*Surgery*Time point	.41

Bold indicates $P < .05$.

It is likely important that the pattern of micro-CT change is more generalized across most joint compartments in control rats in RCC than in the Type IV system. Thus, micro-CT changes predominate in the medial tibia in all OA groups and extend to medial femur and lateral compartments of both femur and tibia only in OA groups in the RCC system; supporting interaction between surgery and RCC system housing to exaggerate the impact of OA across the entire joint (Figures 2A and 3A).

We also evaluated the contribution of housing-related and surgery-related variation in histology and micro-CT to find that the housing system accounted for only 1% and 12% of variation in PC1 and PC2 for histology, while surgery accounted for 59% and 4% variation. In contrast, housing system alone accounted for 19% and 63% of variation in micro-CT PC1 and PC2 while surgery explained 41% and 30% variation. These data indicate that the histological scores are primarily influenced by surgery, while micro-CT changes represent an interaction between surgery and the housing system.

3.1.2 | Social housing strengthens links between histological and micro-CT-based assessment of OA severity

Having explored if histology and micro-CT discriminates OA groups from controls, we subsequently examined the extent of the correlation between these OA severity measures in each system. We found stronger association between histology and micro-CT among OA rats in RCC than in the Type IV system (Figure 4). Thus, within the medial tibia region, 27 and 16 of the correlations had magnitude in absolute values greater than 0.5 for RCC and Type IV, respectively; 136 of the correlations had magnitude in absolute values greater than 0.5 in the lateral tibia region for RCC system and none for type IV housing.

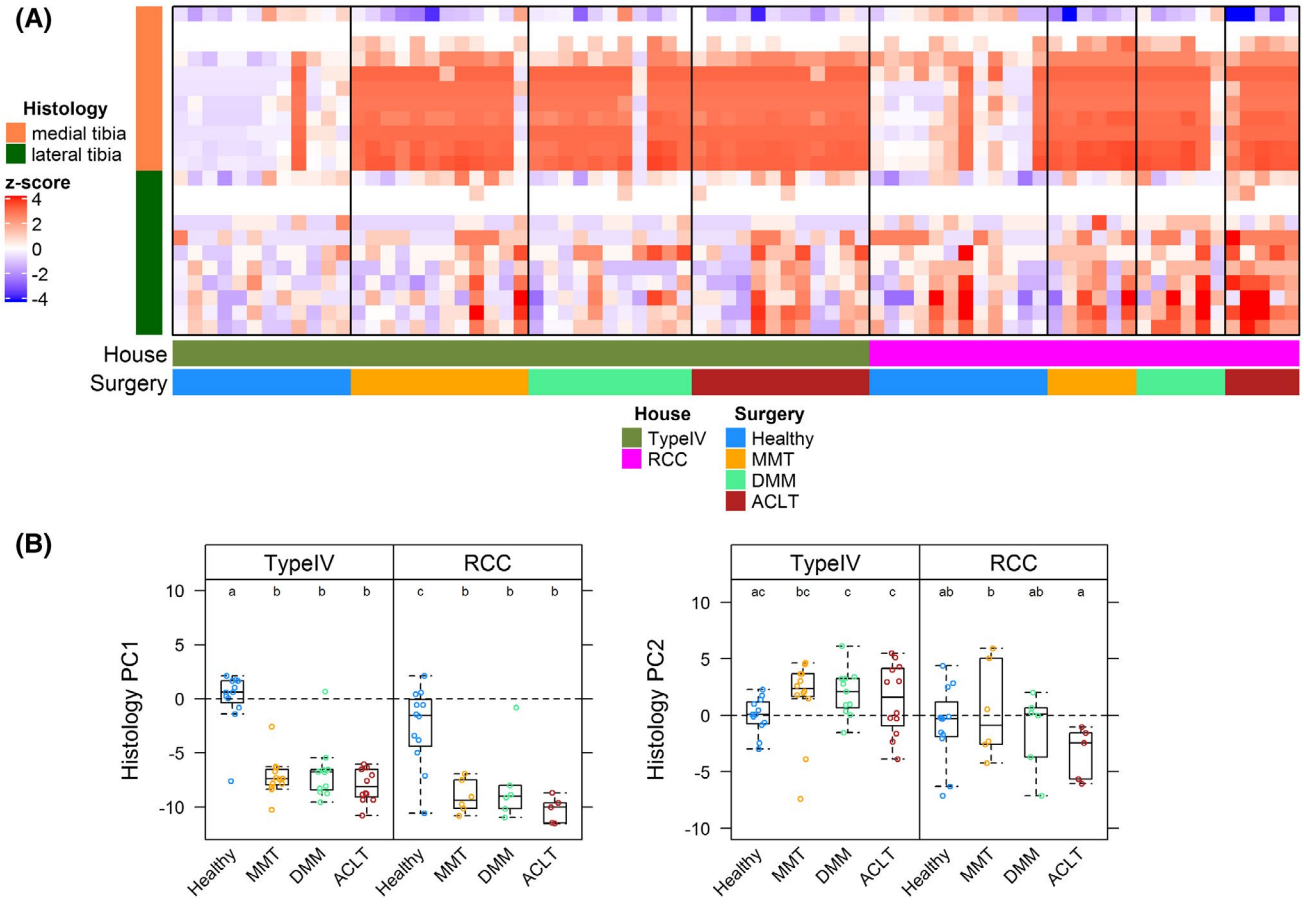

FIGURE 2 A, Heatmap of the standardized 22 histology measurements (rows) across 76 rats (columns) and rats were grouped according to housing types and surgery groups; all measurements were standardized based on healthy rats under Type IV house as indicated by *z*-scores (deeper red indicated higher readout and deeper blue indicated lower readout). B, Principal components analysis of the histology measurements where the first two principal components explained 71% and 15% of total variation in the data. Significance between surgery groups within the housing system and difference among rats receiving the same surgery but housed differently was indicated by different letters at the top the graph, groups sharing the same letter do not reach levels of statistical significance. Principal component analysis indicated that PC1 provided the overall severity across both medial and lateral compartments with similar loading across all measurements; PC2 seem to provide the severity of medial over lateral compartments as it had high positive loading for medial and high negative loading for lateral tibia. When combining the three surgery groups, there is significant difference between the two housing systems (PC1, $P = .03$; PC2, $P = .002$)

Additionally, 9 and 28 of the correlations had P-value $< .01$ in the medial tibia region under the RCC and type IV systems, respectively; 35 of the correlations had P-value $< .01$ in the lateral tibia region under the RCC system and none were significant at 1% level for type IV housing. Indeed, micro-CT and histological OA scoring in both medial and lateral tibia, but not femur compartments showed much stronger correlation in the RCC than in the Type IV system.

Inclusion of gait data showed additional positive correlation between medial tibia histology score and front paw area measures, specifically at 13-19 weeks postsurgery in RCC housing (Figure S2). However, gait data explain less than 5% of variation in histological OA severity when control rats in the RCC system are excluded (Table 3). A higher overall proportion of variation in histological OA grading was linked to micro-CT in the RCC than in the Type IV housing system (Table 3). Specifically, 43% and 13% of the variation in histology (PC1 and PC2) is accounted for by micro-CT (PC1 + PC2) in RCC system, whist in contrast these, respectively, account for only 9% and 4% of variation in the Type IV system. Gait changes can account for some variation in histology and micro-CT in both housing systems when all controls are included, but again this linkage is diminished upon their exclusion.

Together these data indicate that rats in standard Type IV housing allow some segregation between the three surgical OA models based on gait modifications which are, however, only poorly associated with micro-CT or histological assessment of severity, when healthy controls are excluded. In contrast, rats housed in a "social" RCC system develop marked uniformity in gait, yet, yield significantly greater scope to discriminate between the OA models based on traditional histological or micro-CT measurements, which are more strongly linked.

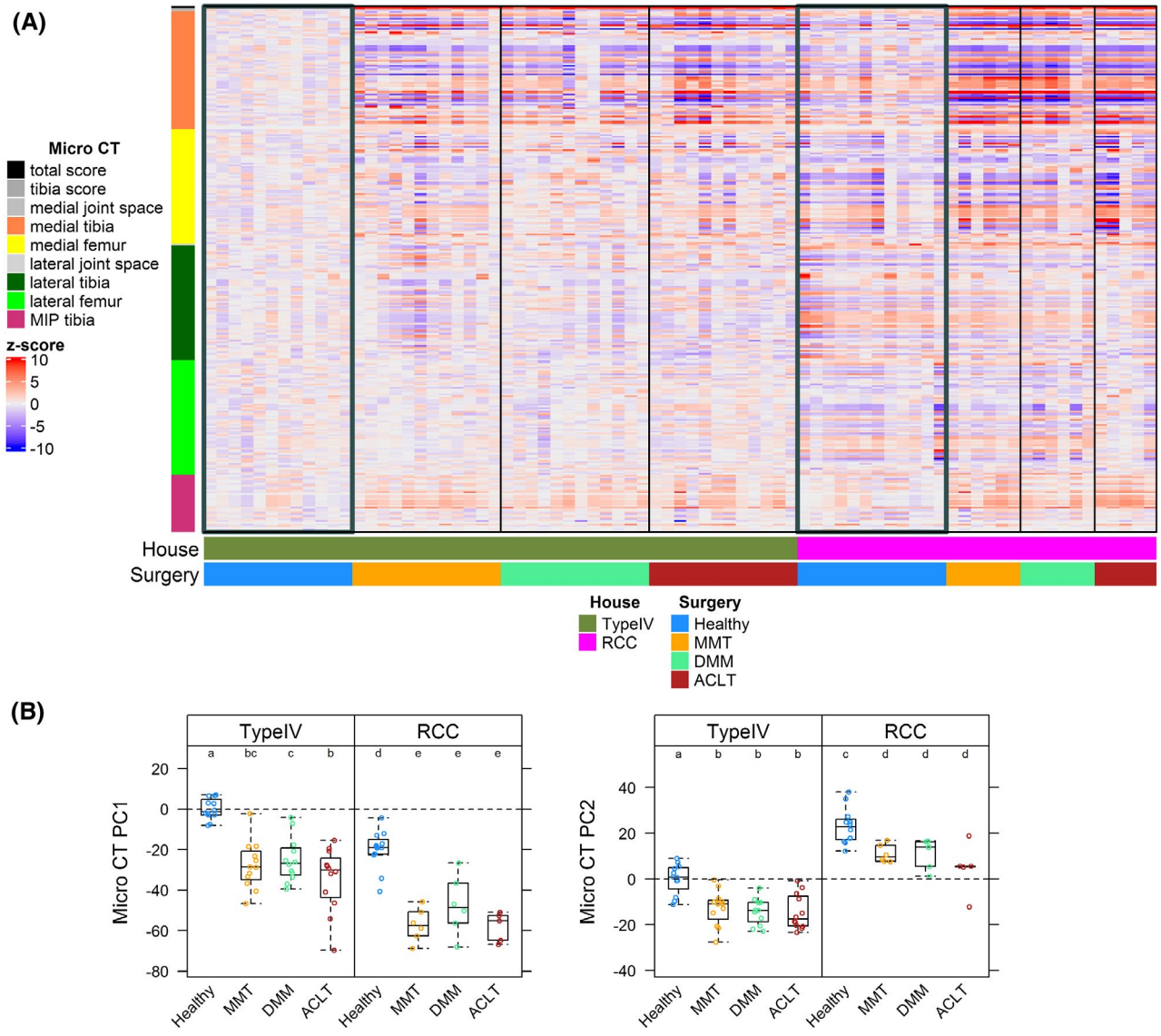

FIGURE 3 A, Heatmap of the standardized 312 micro-CT measurements (rows) across 77 rats (columns). Rats were grouped according to housing types and surgery groups; all measurements were standardized based on healthy rats under Type IV house as indicated by z-scores (deeper red indicated higher readout and deeper blue indicated lower readout). B, Principal components analysis of the 312 micro-CT measurements where the first two principal components explained 64% and 12% of total variation in the data. Significance between surgery groups within the housing system and difference among rats receiving the same surgery but housed differently were indicated by different letters at the top the graph, groups sharing the same letter do not reach levels of statistical significance. Loadings of the principal component analysis suggested that measurements from medial tibia contributed most to PC1, and measurements from lateral tibia, lateral femur, and medial femur contributed most to PC2. When combining the three surgery groups, there is significant difference between the two housing systems ($P < .001$ for both PC1 & PC2)

Components		House	Surgery	House*Surgery interaction
22 Histology measurements	PC1 (71%)	0.14	**<0.0001**	0.86
	PC2 (15%)	**0.0018**	0.49	0.20
312 Micro-CT measurements	PC1 (64%)	**<0.0001**	**<0.0001**	0.63
	PC2 (12%)	**<0.0001**	**<0.0001**	0.70

Bold indicates $P < .05$.

TABLE 2 Two-way ANOVA assessing effects of house and surgery and their interaction on histological principal components and micro-CT principal components (n = 77)

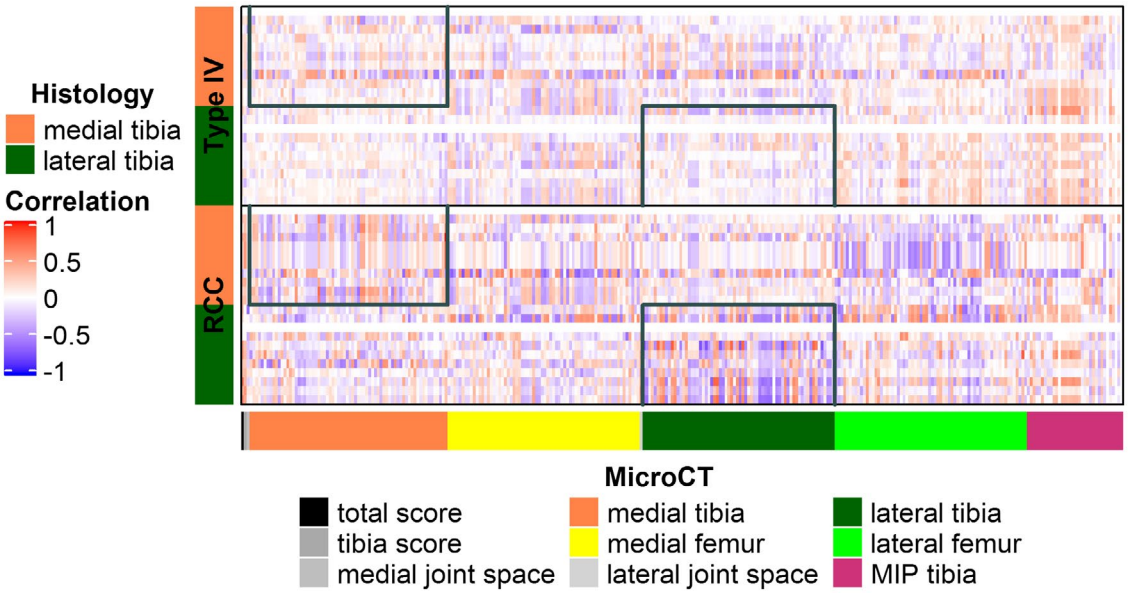

FIGURE 4 Heatmap showing Spearman's correlations between 312 micro-CT measurements and 22 histology measurements among the surgery induced OA rats in the two housing systems. Darker red (blue) indicated stronger positive (negative) correlations between measurements

TABLE 3 Proportion of variation (multiple *R*-squared) in the histology principal components explained by the Catwalk principal component (during weeks 13-19) and micro CT principal components, and proportion of variation in the micro-CT components explained by the Catwalk components based on multivariable linear regression analysis

	RCC		Type IV	
Including healthy controls	**Histology PC1**	**Histology PC2**	**Histology PC1**	**Histology PC2**
Gait PC1	23%	3%	22%	9%
Micro-CT (PC1 + PC2)	61%	2%	54%	10%
Gait PC1 & Micro-CT (PC1 + PC2)	62%	4%	55%	12%
Excluding healthy controls				
Gait PC1	1%	8%	0%	3%
Micro-CT (PC1 + PC2)	43%	13%	9%	4%
Gait PC1 & Micro-CT (PC1 + PC2)	45%	30%	9%	6%
Including healthy controls	**Micro-CT PC1**	**Micro-CT PC2**	**Micro-CT PC1**	**Micro-CT PC2**
Gait PC1	24%	12%	12%	29%
Excluding healthy controls				
Gait PC1	1%	9%	2%	8%

4 | DISCUSSION

Our findings indicate that group housing: (i) elevates yet harmonizes gait disturbance, neutralizing deviations traditionally attributed to OA and; (ii) increases the severity, and strengthens the links between histological and micro-CT-based joint changes. These data suggest that social housing minimizes inter-group behavioral variation and, hence, the impact of this constraint on traditional experimental outcomes. Our studies also show that socially housed rats exhibit stronger alignment in the structural consequences of surgically induced OA to enhance model utility. A most surprising finding was the acquisition of OA-like gait disturbances and joint changes in nonoperated controls.

Gait analyses are used extensively to evaluate pain changes in preclinical arthritis models.[28,29] However, due to the diversity in the gait descriptors reported, discrepancies between studies using this surrogate pain measure remain. These gait descriptors are also often correlated, and thus, alteration in a single descriptor is unlikely to provide the complete picture of any specific gait abnormality. To effectively understand these relationships and any compensatory pattern of modified gait, simultaneous and longitudinal analysis of these highly correlated measures is optimal. Our longitudinal PCA gait analysis

has provided clear evidence that changes in gait patterns can be attributed both to surgery and to the housing system.

We find that gait differences are not only a direct product of the surgical OA but are significantly impacted by social housing. For example, we show throughout the study duration that rats in the RCC system conserve the narrower average width between both the front and hind paw pairs (base of support) and print position, which was exhibited before surgery; not the case in rats maintained in the Type IV system. On the contrary, changes in inter-paw coordination appear to be primarily the product of surgery. The "traditional" separation of control and OA rat groups in the Type IV system is characterized by the emergence of differences in phase dispersion/coupling as early as 3-7 weeks post-surgery. While similar surgery-related changes in phase dispersion/coupling were observed in all three OA groups in the RCC system, they were also apparent in control rats housed socially. Indeed, acquisition of these OA-like gait disturbances was extensive in RCC-housed control rats, where even subtle modification in gait, involving the timing of brake-propulsion transition subphases of stance, emerged during our study period. The comparison between OA rats and the nonoperated controls within the same housing system could be confounded by the transient inflammation and pain induced by the surgery. However, it should be noted that the data used in our analysis (from experiments by Brenneis et al[27]) was principally aimed at comparing the different joint instability models including the transient postsurgical aspects as they are used for proof of concept investigations in drug development. The use of sham controls may be dispensable for ethical consideration in this context. It is also relevant to consider the differences between the nonoperated control groups of rats in the two housing systems, which are perhaps more relevant than if such differences had emerged in sham-operated groups of rats.

Temporal evaluation reveals that these "OA-like" gait characteristics were observed in RCC-housed control rats, only after surgery had been performed in their housemates, indicating that their acquisition is linked by cohabitation.[5] This adaptive behavioral convergence across all groups in the RCC system was also evident in the PCA-based analysis of gait data. It is likely that this adaptation in gait in control rats is due to the social "pressure" derived via cohabitation with the higher numbers of rats which had undergone surgery (versus control). Indeed, the marked similarity in the gait asymmetries in all groups is consistent with control rats "learning" behaviors, while being housed together, and retaining them even when isolated from each other for gait evaluation. This can easily be assessed using different ratios of surgery versus control rats in the RCC system. Testing whether random allocation of equal rat numbers to either right or left limb surgery, neutralizes the adaptive acquisition of gait disturbance in control rats would also be important.

Excluding consideration of control rats, PCA also disclosed a stronger, postsurgery disturbance in gait in the RCC system during later time-points. Thus, more marked deviations in temporal inter-paw coordination (phase dispersions/couplings) were evident in rats subjected to surgical OA and housed in the RCC system. This aligns with specific spatial gait disturbance measures, such as print length (% contralateral hind paw) that are also more pronounced in the RCC.[27]

Housing conditions, stress levels and activity can all influence OA development.[4,5] Our assessment of histological and micro-CT changes has shown significant housing-related and surgery-related differences. Rats in RCC showed greater structural joint changes than those in the Type IV system. Although histology and micro-CT are both structural readouts, micro-CT uncovers changes in the entire joint while histology only scores the tibia. This allowed greater details to be revealed by the micro-CT evaluation. PCA of these bone and joint changes disclosed that histological scores were more markedly impacted by surgery and that housing-related differences were mainly focused to the lateral tibia compartment, while micro-CT changes represented a combined influence from both surgery and the housing system.

Results of our systematic data analysis align with the study,[27] which was focused on specific, clinically relevant parameters of cartilage destruction, bone remodeling, and joint function. Both studies find increased severity for structural outcomes in rats housed in the RCC system, demonstrating that social housing is enough to exaggerate the range of OA impact across the entire joint. Regardless, our systematic PCA approach was still unable to differentiate any OA severity differences between the distinct surgical groups at 20 weeks, perhaps due to small sample size ($n = 6$ per group). A post hoc analysis revealed that to distinguish between the three surgery groups on the basis of histology scores alone, would require >16 rats/group in the RCC system (80% power with 5% type I error rate) which contrasts with the need for drastically larger sample sizes (54/group) for the traditional Type IV system. Similarly, distinguishing between the three OA groups by micro-CT alone requires fewer rats in the RCC than in Type IV system (>21 vs 31 rats/group to reach 80% power with 5% type I error rate). Together, this demonstrates a significant advantage in the RCC system to discriminate OA severity based on the conventional histological or micro-CT-based evaluation of OA.

Another advantage of the RCC system is that it produces closer linkage between histology and micro-CT OA measures than observed in Type IV housing. Specifically, stronger correlation was seen in both medial and lateral tibia compartments in the RCC system. Indeed, we found that a much higher proportions of variation in OA joint histology scores in OA groups were explainable by micro-CT changes in the RCC than in the Type IV system. Given that micro-CT can allow noninvasive and longitudinal evaluation of bone changes, the RCC system may render more plausible the earlier and more reliable detection of pathological OA joint changes.

The strength of this link among the OA structural readouts contrasted, however, with their general disconnection from the gait measures considered symptomatic of OA pain. Despite ~20% of variation in histology and micro-CT being explained by gait (PC1, 13-19 weeks), this link diminished completely, if control rats in both housing systems were excluded. Nonetheless, positive correlation was evident between histological OA scores in the medial tibia compartment and both front paw area measures in rats housed in the RCC but intriguingly not in the Type IV system. It is tempting to speculate that social housing affords a wider and more extensive range of movement which contributes to the generation of more OA joint changes and pain in the operated hind paw joint that, in turn, prompts rats to redistribute their weight toward their front paws to a greater extent than is seen in the Type IV system. Gait PC1 accounted for only 25% of total variation in longitudinal data and there was only limited variation in histological OA scores and micro-CT changes among the three surgery models. Thus, even though they were explicitly selected to engender differing degrees of destabilization and OA, this may explain the poor connection between symptoms and structure changes in our analyses.

Our unbiased and systematic evaluation of longitudinal gait changes and of multiple histological and morphological cartilage and bone OA severity markers has revealed a strong impact of the social environment on OA development. Gait adaptation, histology, and micro-CT changes were observed even in RCC-housed controls, indicating that these modifications are at least partly due to social housing alone. Although neither system allows sufficient statistical differentiation of symptoms or structure changes between the three surgical destabilization OA models in our study, sample size calculation suggests significant reduction in required numbers in RCC if OA were evaluated by either histology or micro-CT changes. Importantly, social housing is enough to induce stronger gait disturbance, exaggerate traditional, histological measures of OA, and allow stronger links between these and micro-CT changes in bone in their etiology.

ACKNOWLEDGMENTS

The analytical work from Ms Jamila Marsh-Dougherty and Mr Adam Callison's summer studentships have contributed to this study.

CONFLICT OF INTEREST

The authors Christian Brenneis, Stephanie Menges, Andreas Westhof, and Kerstin Kleinschmidt-Doerr are employees of Merck Healthcare KGaA.

AUTHOR CONTRIBUTIONS

Y.-M. Chang planned and performed data analysis and wrote the paper; S. Menges performed the μCT analysis; A. Westhof performed the in-life experiment; K. Kleinschmidt-Doerr developed the idea of colony housing in OA research and contributed to the design of the study; C. Brenneis planned and designed the experiments, supervised the investigating laboratory, and initiated and managed the collaboration with RVC London; A. Pitsillides supervised and guided the data analysis, discussed the results and interpretations, and wrote the paper. All authors reviewed the manuscript.

ORCID

Yu-Mei Chang https://orcid.org/0000-0001-6388-9626

REFERENCES

1. Murphy L, Schwartz TA, Helmick CG, et al. Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Rheum*. 2008;59:1207-1213.
2. U.S. Department of Health and Human Services Food and Drug Administration. *Osteoarthritis: Structural Endpoints for the Development of Drugs, Devices, and Biological Products for Treatment Guidance for Industry*. 2018. https://www.fda.gov/media/71132/download.
3. Suri P, Morgenroth DC, Hunter DJ. Epidemiology of osteoarthritis and associated comorbidities. *PM R*. 2012;4:S10-S19.
4. Salvarrey-Strati A, Watson L, Blanchet T, Lu N, Glasson SS. The influence of enrichment devices on development of osteoarthritis in a surgically induced murine model. *ILAR J*. 2008;49:23-30.
5. van der Kraan PM. Factors that influence outcome in experimental osteoarthritis. *Osteoarthritis Cartilage*. 2017;25:369-375.
6. Liu X, Wu R, Tai F, et al. Effects of group housing on stress induced emotional and neuroendocrine alterations. *Brain Res*. 2013;1502:71-80.
7. Meakin LB, Sugiyama T, Galea GL, Browne WJ, Lanyon LE, Price JS. Male mice housed in groups engage in frequent fighting and show a lower response to additional bone loading than females or individually housed males that do not fight. *Bone*. 2013;54:113-117.
8. Patterson-Kane EGHM, Harper D. Rats demand social contact. *Anim Welf*. 2002;11:327-332.
9. Berman D, Rodin BE. The influence of housing condition on autotomy following dorsal rhizotomy in rats. *Pain*. 1982;13:307-311.
10. Huzard D, Mumby DG, Sandi C, Poirier GL, van der Kooij MA. The effects of extrinsic stress on somatic markers and behavior are dependent on animal housing conditions. *Physiol Behav*. 2015;151:238-245.
11. Martin LJ, Tuttle AH, Mogil JS. The interaction between pain and social behavior in humans and rodents. *Curr Top Behav Neurosci*. 2014;20:233-250.
12. Malfait AM, Little CB. On the predictive utility of animal models of osteoarthritis. *Arthritis Res Ther*. 2015;17. https://doi.org/10.1186/s13075-015-0747-6.
13. Shu CC, Zaki S, Ravi V, Schiavinato A, Smith MM, Little CB. The relationship between synovial inflammation, structural pathology, and pain in post-traumatic osteoarthritis: differential effect of stem cell and hyaluronan treatment. *Arthritis Res Ther*. 2020;22. https://doi.org/10.1186/s13075-020-2117-2
14. Poulet B, de Souza R, Kent AV, et al. Intermittent applied mechanical loading induces subchondral bone thickening that may be intensified locally by contiguous articular cartilage lesions. *Osteoarthritis Cartilage*. 2015;23:940-948.
15. Santangelo KS, Kaeding AC, Baker SA, Bertone AL. Quantitative gait analysis detects significant differences in movement between

osteoarthritic and nonosteoarthritic guinea pig strains before and after treatment with flunixin meglumine. *Arthritis*. 2014;2014:503519.
16. Adebayo OO, Ko FC, Wan PT, et al. Role of subchondral bone properties and changes in development of load-induced osteoarthritis in mice. *Osteoarthritis Cartilage*. 2017;25:2108-2118.
17. Javaheri B, Poulet B, Aljazzar A, et al. Stable sulforaphane protects against gait anomalies and modifies bone microarchitecture in the spontaneous STR/Ort model of osteoarthritis. *Bone*. 2017;103:308-317.
18. Lorenz J, Seebach E, Hackmayer G, et al. Melanocortin 1 receptor-signaling deficiency results in an articular cartilage phenotype and accelerates pathogenesis of surgically induced murine osteoarthritis. *PLoS ONE*. 2014;9:e105858.
19. Malfait AM, Little CB, McDougall JJ. A commentary on modelling osteoarthritis pain in small animals. *Osteoarthritis Cartilage*. 2013;21:1316-1326.
20. Bedson J, Croft PR. The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. *BMC Musculoskelet Disord*. 2008;9:116.
21. Bove SE, Laemont KD, Brooker RM, et al. Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia. *Osteoarthritis Cartilage*. 2006;14:1041-1048.
22. Huskisson EC, Berry H, Gishen P, Jubb RW, Whitehead J. Effects of antiinflammatory drugs on the progression of osteoarthritis of the knee. LINK Study Group. Longitudinal investigation of nonsteroidal antiinflammatory drugs in knee osteoarthritis. *J Rheumatol*. 1995;22:1941-1946.
23. Ohtori S, Inoue G, Orita S, et al. Efficacy of combination of meloxicam and pregabalin for pain in knee osteoarthritis. *Yonsei Med J*. 2013;54:1253-1258.
24. TenBroek EM, Yunker L, Nies MF, Bendele AM. Randomized controlled studies on the efficacy of antiarthritic agents in inhibiting cartilage degeneration and pain associated with progression of osteoarthritis in the rat. *Arthritis Res Ther*. 2016;18. https://doi.org/10.1186/s13075-016-0921-5.
25. Burr DB. The importance of subchondral bone in the progression of osteoarthritis. *J Rheumatol Suppl*. 2004;70:77-80.
26. Brenneis C, Westhof A, Holschbach J, Michaelis M, Guehring H, Kleinschmidt-Doerr K. Automated tracking of motion and body weight for objective monitoring of rats in colony housing. *J Am Assoc Lab Anim Sci*. 2017;56:18-31.
27. Brenneis CM, Menges S, Westhof A, Lindemann S, Thudium C, Kleinschmidt-Doerr K. Colony housing promotes structural and functional changes during surgically induced osteoarthritis in rats. *Osteoarthritis Cartilage Open*. 2020;2. https://doi.org/10.1016/j.ocarto.2020.100100.
28. Jacobs BY, Kloefkorn HE, Allen KD. Gait analysis methods for rodent models of osteoarthritis. *Curr Pain Headache Rep*. 2014;18:456.
29. Lakes EH, Allen KD. Gait analysis methods for rodent models of arthritic disorders: reviews and recommendations. *Osteoarthritis Cartilage*. 2016;24:1837-1849.
30. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Muller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res*. 2010;25:1468-1486.
31. Kraus VB, Huebner JL, DeGroot J, Bendele A. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the guinea pig. *Osteoarthritis Cartilage*. 2010;18(Suppl 3):S35-S52.
32. Gerwin N, Bendele AM, Glasson S, Carlson CS. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the rat. *Osteoarthritis Cartilage*. 2010;18(Suppl 3):S24-S34.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Chang Y-M, Menges S, Westhof A, Kleinschmidt-Doerr K, Brenneis C, Pitsillides AA. Systematic analysis reveals that colony housing aligns gait profiles and strengthens link between histological and micro-CT bone markers in rat models of osteoarthritis. *The FASEB Journal*. 2021;35:e21451. https://doi.org/10.1096/fj.202002009R