Colony housing promotes structural and functional changes during surgically induced osteoarthritis in rats

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SUMMARY

Objective: The aim of the study was to investigate how social housing with high locomotion activity affects experimental osteoarthritis (OA) in rats.

Design: Rats were housed either conventionally in type IV cages in pairs or in rat colony cages (RCC) on 4 levels interconnected by jump holes or staircase in groups of 48. OA was induced by anterior cruciate ligament transection and resection of the medial meniscus (ACLT + tMx), medial meniscal tear (MMT) or destabilization of the medial meniscus (DMM). Functional changes were characterized by continues tracking of individual activity and catwalk gait analysis. Cartilage volume and bone structure were investigated at week 20 after surgery by histology and micro-CT.

Results: In the RCC, healthy rats changed cage levels 82 ± 15 times daily, reduced by 30% after ACLT + tMx (p < 0.0001). In both housing systems, the order of severity of the investigated models was ACLT + tMx > MMT > DMM in all outcome measures. Compared to Type IV, RCC housed rats developed stronger gait disturbance symptoms (ACLT + tMx; 95% CI = -15.2; p < 0.004), the cartilage volume was reduced (ACLT + tMx; 95% CI = -0.1-0.5; p < 0.0001), serum levels of the cartilage remodeling marker AGNx1 were higher (MMT; 95% CI = -53-(-6); p = 0.001), bone was denser with increased volume (ACLT + tMx; 95% CI = 0.8-7.5; p = 0.004) and joints were less flexible (ACLT + tMx; 95% CI = 3.6-14; p < 0.0001).

Conclusion: Housing rats in an environment allowing increased locomotion and socialization promotes structural and functional alterations during joint instability-induced OA. This increases the assay window, improves the relevance for the human disease and enables to discriminate the models in structural and behavioral parameters.

1. Introduction

OA is the 2nd most common cause of years lived with disability worldwide and the life-time risk of symptomatic knee OA is 40% for male and 47% for females [1]. Major risk factors are genetic factors, traumatic events disturbing joint mechanics and obesity, often associated with low grade systemic inflammation [2]. It is well established that physical activities can impact OA progression. However, the protective or deleterious direction of this influence may depend on the respective quality, the strength and on the respective pathophysiological conditions during which locomotion activities are performed.

While peak forces and micro-traumata may function as initiator or trigger of OA, mechanical load from continuous physical activity often has a substantial protective impact on joint health [3] and ambulatory loading increases cartilage thickness [4]. Also in early stage OA, certain types of exercise has been shown to significantly reduce pain and improve function and quality of life in people with knee and hip OA [5]. Several mechanistic studies indicate that mechanical forces can protect and regenerate cartilage. Ex vivo, cyclic tensile strain attenuated IL-1β induced chondrocyte damage [6]. In vitro, cyclic hydrostatic pressure could stimulated the production of key matrix proteins collagen type II (Col 2) and aggrecan [7].

In contrast, severe mechanical stress to joint cartilage can be a substantial accelerator of pathologic catabolic metabolism [8]. In studies with obese patients or anterior cruciate ligament injury a relationship between kinematic changes during ambulation and the initiation of knee OA was found [8]. Especially after traumatic events when ligaments or the menisci lose parts of their function to distribute and buffer forces, mechanical loading during physical activities can exceed the tolerance of the articular surface and therefore cause
cartilage damage. In consequence, damage associated molecular patterns initiate inflammatory responses and oxidative stress which induce metalloproteinases causing an ongoing catabolic vicious circle [9]. Severe shear stress onto human articular cartilage samples causes chondrocyte death [10].

Surgery-based animal OA models destabilize the knee joint by Anterior Cruciate Ligament Transection with total or partial resection of the medial Meniscus (ACLT + p/tMx), Medial Meniscus Tear (MMT) or Destabilization of the Medial Meniscus (DDM), all causing altered joint mechanics leading to a localized overload on the medial tibial and femoral plateaus [11–14]. Beyond this trigger of mechanical burden, subsequently a pro-catabolic cascade with protease driven cartilage matrix degradation and bone remodeling consequently leads to chronic OA which resembles many structural changes commonly observed in knee OA patients [10]. That load bearing is highly critical for triggering OA development in these models was clearly suggested by the finding that in the MMT model mid-tibial amputation completely prevented any cartilage damage [15]. However, moderate mechanical burden from short term gently treadmill walking suppressed cartilage degradation (OARSI score) and subchondral cyst growth after DMM surgery [13]. Finally, it remains to be elucidated how different types of spontaneous locomotion, which can produce mechanical challenge, contributes to disease progression in these models. In contrast to forced locomotion, e.g. via treadmill challenge, spontaneous locomotion is affected in turn by disease driven pain and expected to be in feedback interaction with different OA-severity stages [16].

Traditionally, rats are housed in small cages (e.g. 1820 cm² type IV macrolon cages) with 1–4 cage mates. In this environment, rats show a very limited degree of locomotion which is in striking contrast to what can be expected in rats in their natural habitats living in large communities in complex burrows [17]. We emphasize that the unnatural living environment in classical cages gives rats very limited possibilities for socially driven activities. This may however represent an important dimension especially in the context of OA. OA patients who suffer from pain may negatively adapt their spontaneous activities which are not essential for survival. These changes in activities can then, as described in the above sections, interact with disease progression in multiple ways.

Another dimension which can be important for animal model to human translatability can be the aspect of socialization during disease pathology. While the psychologic effect of well-being and feeling cared for is well known and described for OA and other painful diseases [18], it is mainly ignored in animal experimentation. Type IV cage housing appears to be restricting a physiological social and active rat life and may therefore interfere differently with OA progression and pain than housing in a more complex and social home cage.

To better reflect the relevant interaction between OA disease and locomotion, we recently developed the Rat Colony Cage (RCC) for a social and enriched housing. In this cage, 48 rats live in one colony on 22,246 cm² over 4 levels which are connected by jump holes and staircases. Previously we reported, that this environment fundamentally changes the general behavior of rats [19]. Already after a few weeks of housing in the RCC, rats were more active and curious, less stressed during handling and interventions and they habituated much faster to a novel test environment than rats in a cohort housed in type IV cages. Implementation of a Radio Frequency Identification (RFID) based tracking system allowed automated health monitoring based on individual activity deviations from the group average.

To better understand the contribution of spontaneous activity on OA of a traumatic etiology, we compared the outcome of ACLT + tMx, MMT and DMM surgery models, which cause a different quality and strength of joint instability, between rats housed in a novel rat colony cage (RCC) and rats housed in regular type IV cages. The study focuses on parameters of gait performance, cartilage destruction, bone remodeling and joint function which are described and observed clinically.

2. Methods

2.1. Animals and general housing conditions

Male Lister Hooded (CrI:LI) outbred SPF rats (8–9 weeks with a weight of 150–175 g) were purchased from Charles River Laboratories (Sulzfeld, Germany). At the breeder, rats were housed in conventional polycarbonate cages (595 mm × 380 mm × 200 mm high; floor area, 1820 cm²) in groups of 5 animals per cage. On arrival at our facility, half of the cohort was housed in the same type IV cages in pairs of 2 and the other half was directly transferred to colony housing cages. Here 48 Lister Hooded rats were housed together. Distribution ensured equal average weight of rats in different housing conditions. For both housing types, single animals represent the experimental unit. For detailed husbandry conditions see Brenneis et. al, 2017 [19]. Day night rhythm was inverted with red light during 6 am and 6 p.m. All procedures were approved by the animal protection authorities of the local district government (approval Da 4/1009, Regional Authorities of Hessen, Darmstadt, Germany).

2.2. Joint instability surgery and outcome measures with statistics

To model chronic OA, joint instability has been induced in 12 rats per housing type by ACLT + tMx, MMT and DMM surgery (details see supplementary methods) and animals were followed up over 20 weeks. Animals were allocated to surgery groups in a random order. The catabolic aggrecan neoeptope biomarker AGN1 was measured in serum by ELISA on days 0 and 85 after surgery, joint diameters were measured with a caliper on days 0, 63, 85, 92, 140 after surgery, gait disturbance was determined by the catwalk test in week –1, 3, 5, 7, 9, 11 and 12 after surgery and spontaneous activity was tracked continuously via RFID reading antennas at jumping holes and stair case entries [19]. In week 12 after surgery, 50% of the RCC housed rats received treatment and were therefore excluded from the analysis (6 rats instead of 12 per surgery group). To keep for outcomes with higher variation the animal number per group sufficiently high, the catwalk and activity results were summarized only until week 12. After necropsy 20 weeks post-surgery, micro-CT and histological analysis of structural changes were performed and maximum joint flexibility angle was measured by an “angle meter”. One ACLT + tMx rat was euthanized prematurely and excluded from the analysis due to irreversible patella dislocation after surgery. Results were compared based on the core assumption that the 24 conventional cages used in this experiment were comparable in all aspects, such that the cage number had no effect on the outcome. Outliers were identified by the ROUT test (Q = 1%). For statistical analysis of locomotion data (Fig. 2), a linear mixed effect model with baseline adjustment was calculated by NOVAC in “R”. For all other data sets, the effect of surgery was analyzed by 1-way ANOVA followed by Dunnet posthoc testing for multiple comparison at a single time points and 2-way ANOVA followed by Sidak posthoc testing for multiple comparison over time. A 1-way ANOVA followed by a Tukey multiple comparison test was used to compare all groups at a single time point and identify housing related effects within same surgery procedures. Statistical significance was accepted for an alpha mistake of 5%. For more details about materials and methods used see supplementary methods.

3. Results

3.1. High level of spontaneous locomotion in the rat colony cage (RCC)

Upon delivery, rats were stratified based on equal weight into a Type IV cage and RCC housing cohort. In the RCC cage rats appeared socially interactive, curious and showed active locomotion throughout the study period as reported earlier [19]. After 3 weeks habituation, rats were adapted to the RCC cage, accessed all cage levels and spontaneously moved between levels in a very frequent manner. At all six antennas,
single healthy rats were detected via RFID in average 11,446 ± 2113 times during the 20-week period after surgeries and 82 ± 15 times daily [Fig. 1].

To estimate changes in locomotion due to surgery induced joint instability and progression of osteoarthritis, the number of detections of individual rats at all antennas was compared between different groups. To reduce variability based on disturbances by facility staff visits, only the detections between 3 pm and 7 am were included in the locomotion analysis. The time course shows that detections decreased in all groups over time [Fig. 2-(A)]. Group comparison revealed that all surgeries significantly reduced detections during the first week [Fig. 2-(B)]. This was most pronounced in the ACLT + tMx group. During the intermediate phase (week 2–8) there were only moderate and non-significant differences between groups [Fig. 2-(C)]. However, in the chronic phase (week 8–12) the overall activity was again lower in all surgery groups compared to healthy [Fig. 2-(B)]. It should be noted that OA rats were still able to perform 79% of the activity detected in healthy control rats. This indicates that mechanical challenge on joint structures is still given also during chronic disease and it can impact OA development in the RCC cage.

3.2. Effect of housing condition on gait disturbances in different joint instability models

By investigating gait parameter from spatial and temporal footprint pattern by Catwalk analysis, we found that the print length as percent of instability models

cage. Differences in gait parameters between housing conditions were
models when rats were housed in the RCC cage than if in the Type IV chronic phase of the models. This parameter was more affected in all
the contralateral hind paw, is the most strongly affected parameter in the

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3.3. Effect of RCC housing on cartilage volume determined by stereometric histology

To investigate whether housing conditions can impact the serum levels of cartilage matrix degradation products, AGN1 is an epitope of an aggrecan fragment which is exclusively generated from cleavage by “a disintegrin and metalloproteinase with thrombospondin motifs 4 and 5 (ADAMTS-4 and 5)” [21], the major catabolic enzymes involved in cartilage matrix degradation during osteoarthritis [22]. In serum samples we found (prior to surgery but already for 3 weeks in different cage conditions) higher AGN1 levels in naïve rats housed in the RCC condition compared to naïve rats housed in the Type IV cage (Fig. 5 (A); p = 0007). 12 weeks later, all groups, including healthy and in both housing conditions, exhibited reduced serum AGN1 levels probably due to aging and skeletal maturation (Fig. 5-(B + C)). When calculating the % reduction of the AGN1 of individual rats, these age-related changes in AGN1 levels were found less in the surgery groups compared to healthy. Importantly, this surgery effect on relative AGN1 levels more pronounced and significant after MMT when rats were housed in the RCC. Altogether, the data suggest that RCC housing with high locomotion activity increases serum marker of extracellular matrix remodeling already in healthy conditions and even more after joint instability surgeries.

3.5. Effect of housing conditions on bone structure determined by μCT ex vivo

By micro-CT analysis in week 20, we found bony spurs (osteophytes) formed along the joint margins of the tibia plateau causing enlarged bone

Figure 1. Rat colony cage (RCC) housing and activity tracking. A) Image of the RCC. The cage was enriched with toys at level 4, shelter at level 2, food and water was supplied in level 1, water in body weigh stations of level 4 and nest material in level 1. Passages between levels were detected in individual rats by RFID technology at 6 antenna B) Table of total number of detections of individual rats at different antenna. Total number of detections within last 20 weeks and daily average. Mean ± SD of 12 healthy rats.
areas in rats of both housing types. This was more severe and enabled
everity grading of OA-models in RCC-housing (Fig. 6(A)). Subchondral
bone thickness measured down to the growth plate in the epiphyseal
region of the tibia was reduced in RCC. The surgery effect on this
parameter was completely absent in Type IV housing (Fig. 6(B)). Further,
trabecular bone analysis of the medial epiphyseal region in the tibia
revealed increased bone volume (Fig. 6 (C)) and trabecular thickness
(Fig. 6(D)) in OA-rats from both housing conditions compared to healthy
animals. However, changes in these parameters were more pronounced
in OA-rats housed in RCCs compared to corresponding animals from Type
IV housing. Furthermore, increased bone volume and high trabecular
thickness appeared to be strongest in moderate models of OA
(ACLT + tMx) rather than in mild ones (DMM).

3.6. Effects of RCC housing on joint broadening and stiffness

Loss of cartilage, effusions and bone remodeling can result in pain
and functional impairment like joint stiffness [23,24]. This can go along
with joint broadening and be reflected in reduced flexibility while hind
limb stretching. We measured knee joint diameter with an electronic
caliper and found that it is increased at the ipsi-compared to the
limb stretching. We measured knee joint diameter with an electronic
caliper and found that it is increased at the ipsi-compared to the

Figure 2. Spontaneous locomotion in
different surgery groups during RCC
housing: A) Time course. Daily detections
between 3 pm and 7 am (time when rats
were not disturbed by staff) as percentage of
baseline activity. B-D) Locomotion classified
in time segments after surgery. Average
percentage of data shown in A. One outlier
was removed in the DMM group, as identi-

ified by the ROUT test (Q = 1%). Shown is
the mean ± SEM of 23–24 rats/group in two
independent experiments. ANOVA Multi-
ple Comparisons of Means with Tukey Con-
trasts; *p < 0.05; ***p < 0.0001 by One-
way ANOVA with Dunnett’s post test.

4. Discussion

OA is a musculoskeletal disease that is in strong interaction with the
actual usage of the affected joint. The degree of OA progression and the
functionality of joint mechanics can have an important impact on the
translation of physical activity into mechanical burden to the cartilage
and how the joint responds to it with structural remodeling.

Despite of the knowledge on the relationship between OA and
physical activity, all rodent models of OA are typically executed in type
IV cages, which exhibit very low possibilities for spontaneous locomo-
tion. With the development of the RCC [19], we realized a cage system in
which rats are offered multiple enrichment parts to achieve different
functional areas, socialization and vertical movement options to stimu-
late spontaneous load bearing activities. Since all rats, including those
with chronic OA, frequently explored all levels voluntarily with 82 ± 15
daily jumps and stair walks, we expect that RRC housing largely pro-
motes mechanical burden on joints in destabilized joints compared to
type IV cage housing. This enable studying the contribution of sponta-
neous activity on OA development by comparing functional and struc-
tural alterations between RCC and Type IV housed rats in 3 models of
surgically induced OA which were described to exhibit differences in
surgery procedure is unlikely since no differences between healthy and
surgical rats in synovitis score were observed in week 20 (Supplementary
Fig. 1). However, with respect to this limitation, it should clearly be
emphasized that this study aims to investigate the different joint insta-

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bility models as tools to induce clinically relevant aspects of OA for proof
of concept investigations in drug development. It does not aim to dissect
out the exclusive consequences of certain injuries at different sites, which
would make the use of sham controls necessary. Further, this study was
exclusively done with male rats. Recent investigations reveal that also
female rats (e.g. Sprague Dawley) can be socially housed in the RCC. They demonstrate even more spontaneous activity than males and the induction of OA works as well (data not shown). However, a systematic comparison to male rats and between cage types has yet not been performed.

We found that RCC housing increased the severity of investigated endpoints in different models. Catwalk tests revealed that RCC rats developed transiently and most pronounced in the early phase of the ACLT+HmX model a stronger gait disturbance. Gait disturbances has been shown to be a correlate for pain since rofecoxib, gabapentin or tanezumab was previously shown to improve hind paw weight distribution during walking in the MMT model [12,15]. Pain can potentially arise in OA models after cartilage proteolysis which creates DAMPs that cause transient inflammatory response leading to synovitis, a common driver of OA pain [25–27]. In the chronic time course until week 12, the catabolic events may attenuate once the cartilage or certain matrix molecules like aggrecan are mostly gone at the affected medial location [20].

Rats housed in RCC but not in Type IV cages showed a significant reduction in cartilage volume compared to non-operated controls and here a differentiation in severity between different models was possible. One reason that accounts for the enlarged assay window on changes in cartilage structure is that healthy rats showed a trend for more knee joint cartilage when living in the RCC. This indicates that the changed live style which includes e.g. frequent jumping etc. Can potentially strengthen the cartilage in a use dependent manner. This observation is in line with the fact that also in healthy subjects cartilage thickness increases with high ambulatory loads [4].

In vitro and ex vivo it was found that moderate mechanical pressure stimulates bone marrow-derived mesenchymal progenitor cells to express chondrogenic factor [6,7]. All this points to a relationship between use of joint and the need for protection of the bone against mechanical forces and may contribute to an improved animal to human translatability of pharmacological results. Further, our data suggest that the positive effect of extensive movement in the RCC on the musculoskeletal condition seemed to be reversed once

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Figure 3. Housing effects on gait performance. A-C) Time course after different types of surgery. Shown is the print length of the ipsilateral as percentage of the contralateral hind paw determined by the catwalk test. Mean ± SEM of 12 rats/group. *p = 0.004 by Two-Way ANOVA with Sidak’s multiple comparison test. D) Area above the curve. E) % of contralateral in week 19. *p = 0.02; **p = 0.008 by One-Way ANOVA with Dunnett’s post test.
Figure 4. Cartilage degradation: A) Representative images of joint sections of healthy or ACLT + tMx rats in RCC or Type IV housing. Shown is the ipsilateral medial area next to the damaged meniscus. Arrows indicate lack of cartilage. B) Cartilage volume. Cartilage volume was determined stereometrically. Shown are the results of the medial tibia, which showed the strongest damage in all models. Shown is the mean ± SEM of 6 rats for the RCC groups and 12 rats for the type IV cage groups. C) Cartilage volume, combined surgery groups. Statistics were made by One-Way ANOVA with Tukey’s multiple comparison test. *p = 0.01; ***p < 0.001; ****p < 0.0001 compared to healthy with the same housing condition. #p = 0.01 between ACLT pMx and DMM in the same housing condition.

there are kinematic changes during locomotion by surgery induced joint instability and cartilage erosion at the medial site was increased. This effect was, compared to ACLT + tMx, weaker after MMT and only minimal after DMM, indicating that the degree of mechanical and anatomical alterations after surgical joint destabilization and the resulting kinematic changes are critical for the movement related increase in cartilage erosion. This in turn emphasizes that RCC housing makes it not only possible to investigate structural differences based on the readout cartilage volume, it further enables to discriminate between different severity grades by different surgeries.

RCC housing is expected to have multiple effects on many if not all joints of the body. We here found that systemic serum levels of AGNxl, an epitope of an aggrecan degradation product and cartilage remodeling marker [28], were significantly higher in RCC versus type IV housed rats. Besides during cartilage degradation, catabolic matrix turnover can also be a necessity for cartilage growth. Some empty space within the matrix can be necessary for efficient hypertrophy of the cartilage due to mechanoadaptation [28,29].

This could explain both findings in healthy rats, the trend for an increase in cartilage volume and an elevation of AGNxl, because of matrix due to anabolic processes. At twelve weeks after surgery, when cartilage degradation was potentially more advanced in social housing, a further increase in the systemic AGNxl level was found which now may reflect aggrecanase activity due to catabolic processes at the cartilage knee joint. Together, the AGNxl data supports the hypothesis that RCC housing with increased spontaneous locomotion elevates cartilage remodeling, in a healthy situation leading to the strengthening of the cartilage. However, during joint instability, catabolic processes appear to be accelerated in the RCC, leading to more cartilage loss and increases in systemic AGNxl levels.

Loss in cartilage can arise from its transformation into bone by enchondral ossification [30]. Micro-CT analysis revealed alteration of several parameters during OA development in destabilized rat joints. In the VOI chosen, the transversal plan view of the proximal tibia of rats with ACLT + tMx surgery had reduced diameters but were wider in surface. To quantify the enlargement of the tibia plateau as a typical reaction to the destabilization surgery, Maximum Intensity Projections (MIP) of the plan view were made and total bone volumes compared. Such projections include expansion of all adjacent bone including also osteophytes. As more osteophytes were counted in RCC housed rats, also MIP analysis of tibia enlargement was increased in RCC over type IV housed rats. In the analyzed VOI, trabeculae were thicker and more bone volume per tissue volume (BV/TV) was measured in rats that were housed in RCC compared to rats housed in type IV cages.

The analyzed data indicate that bone remodeling in destabilized knee joints was more evident in RCC housing compared to Type IV housing. Compensative mechanisms against the surgically induced instability increased osteophyte development and therefore enlargement of the tibial plateau [31]. Bone microarchitecture was significantly impaired exemplified by higher trabecular thickness and bone volume indicating features of late-stage OA like subchondral sclerosis [32]. As the increase in bone area, trabecular thickness and bone volume depended on the surgical OA-model, μCT allowed a severity graduation of the surgical models from mild (DMM) to moderate (MMT and ACLT + tMx). Interestingly, especially in the more severe ACLT + tMx model the differences between RCC and type IV housing became most evident. In operated RCC but not Type IV housed rats the subchondral bone thickness was significantly reduced compared to healthy rats housed under the same conditions. These findings hint for a clear link between the degree of joint destabilization, bone remodeling and the possibility to load and use the joint which is obviously higher in the RCC compared to type IV housing. Prospectively, also longitudinal measurements of disease progression in ACLT + tMx rats housed in RCC or type IV cage could add information about the association between joint loading and movement and pathophysiological progression in destabilized joints.

Finally, the determination of joint flexibility revealed that RCC housing promoted also a reduction in functionality from a resultant stiffness. This was, in accordance with the gait analysis, μCT and histology results, found to be most pronounced in the ACLT + tMx. It shows that altered joint structures in the RCC housing can translate into symptomatic changes like more pain and stiffness, when combined with a more severe type of joint instability.

In summary, the results of this study suggest that an enlarged and enriched colony housing system improves the translation ability of results from rodent models of osteoarthritis. The here established outcome measure “spontaneous activity” consisting of the sum of the numbers of stair walks” and “jumps” between home cage levels represents a novel symptomatic characterization in rat models of OA. The results indicate that the spontaneous impulse to walk stairs in the RCC is reduced during...
Figure 5. Serum levels of the cartilage remodeling marker AGN\textsuperscript{x}1 (a disintegrin and metalloproteinase with thrombospondin motifs 4+5 (ADAMTS4+5) cleavage product). A) AGN\textsuperscript{x}1 serum levels after differential housing. 7 days prior to surgery but already for 3 weeks in different cage conditions. \(N = 48\). Student's t-test; \***p < 0.0007. B + C) Surgery effect on AGN\textsuperscript{x}1 in RCC and Type IV. Reduction of serum AGN\textsuperscript{x}1 levels from baseline (as in A) until 12 weeks after different surgery procedures. One-Way ANOVA with Dunnett's multiple comparison test. *p = 0.01. Shown is the mean + SEM of the percentage reductions (n = 12).

Figure 6. Bone structure. Bone structure was determined by micro-CT in week 20 after surgery. A) Mean total cross-sectional tibia area. B) Subchondral bone thickness at medial tibia. C) Trabecular thickness in medial tibia. D) Bone volume medial tibia. Shown is the mean + SEM of the percentage reductions (n = 12). One-Way ANOVA and Tukey multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared to healthy in the same housing condition, # compares to same model but in different housing type.

Figure 7. Joint broadening and stiffness. A) Time course of joint broadening. Joint diameter was measured with an electronic caliper. Shown is the mean ± SEM of the change in the ipsilateral compared to the contralateral knee joint (Shown is the mean ± SEM of 6 rats for the RCC groups and 12 rats for the type IV cage groups). Two-Way ANOVA for repeated measures and Tukey multiple comparison test. ##p < 0.01 for complete time course and comparison of ACLT + tMx groups between housing types. B + C) Reduction in maximal stretching angle of contralateral (B) or ipsilateral (C) joint. One-Way ANOVA and Tukey multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared to healthy within the same housing condition, # compares to same model but in different housing type.
surgery induced OA. Since e.g. pain during stair walking are questions addressed in clinical trials, this represents a novel readout with a potential for high “face validity” [33]. Importantly, RCC housing increases the assay window, with more absolute differences between healthy and diseased rats and steady variability for most structural and symptomatic endpoints. When using the model for preclinical Prove Of Concept (pPOC) investigations of novel drug candidates, this can reduce the number of animals needed to detect a certain effect and refines the housing conditions. We usually set a benefit of 30% over placebo as threshold for a meaningful effect size. When using cartilage volume as an example structural endpoint, a biometric group size calculation (two sided test for interference of means with power = 0.8) reveals that with the ACLT + tMx model in type IV cages 159 rats/group and in the RCC only 66 rats/group are necessary to detect a difference of 30% with p < 0.05. Despite this dramatic reduction in animal numbers, the time and costs for animal husbandry are comparable between the cage systems [19]. This example calculation clearly illustrates 3R-principle achievements for animal welfare [34].

Author’s contributions

Christian Brennies planned and designed the experiments, supervised the investigating laboratory, performed data analysis and wrote the manuscript. Stephanie Menges performed the μCT analysis and analyzed data. Andreas Westhof performed the in-life experiment and analyzed data. Sven Lindemann performed the histological investigation and its analysis. Christian Thudium performed the AgNoX1 measurement and its analysis. Kerstin Kleinschmidt-Doer developed the idea of colony housing in OA research and contributed to the design of the study and data analysis. All authors reviewed the manuscript.

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Declaration of competing interest

The authors Christian Brennies, Stephanie Menges, Andreas Westhof, Sven Lindemann and Kerstin Kleinschmidt-Doerr are employees of Merck Healthcare KGaA. The author Christian Thudium is an employee of Nordic Bioscience.

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Appendix A. Supplementary data

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