

(lengthwise and crosswise) at 5-mm intervals, and the number of infrared rays blocked by the animal was counted. The total locomotor activity in every 30 min was shown graphically and used as a daily behavioral pattern. The total locomotor activity over 24 hours was measured as daily locomotor activity.

Measurement of tail surface temperature before and after moxibustion in each group: The tail surface temperature was measured using a thermograph in group I and II. To avoid the influence of haircoat, the tail temperature which is used as an indicator for peripheral circulation was measured. Before and after, after 15 min of acclimation to the environment, the tail surface temperature was measured in conscious animals in a windless room maintained at a temperature of $15\pm 1^\circ\text{C}$ and a relative humidity of $60\pm 10\%$. Temperature of the rat tails was measured at a distance of 1 m from the thermography device. Rats were acclimated before the experiment.

Results: Changes in locomotor activity before and after moxibustion stimulation: Before moxibustion stimulation, in group I, the pattern of locomotor activity was regular. In the group I, there was no clear difference in the pattern of locomotor activity between the active and resting phases and the pattern of locomotor activity was irregular. The daily level of locomotor activity at 24 hours was significantly higher in the group I than in group II. After moxibustion stimulation of the group I, the pattern of locomotor activity became diphasic with clear active and resting phases, similar to that observed in group II. Moreover, the daily level of locomotor activity at 24 hours in group I was significantly higher than that in group II. **Changes in tail surface temperature before and after moxibustion stimulation:** Before moxibustion stimulation, the tail surface temperature was significantly lower in group I than in group II. After moxibustion stimulation, there was no significant difference in the tail surface temperature between group I and group II.

Conclusions: The increased locomotor activity of rat AA was presumably ascribable to the removal of blood circulation reduced blood flow in the peripheral circulation rather than the induction of stress by moxibustion. These results demonstrate that moxibustion stimulation is effective for the treatment of pain relief.

53 TOWARDS AN ANIMAL MODEL FOR JOINT REGENERATION IN OSTEOARTHRITIS

R. Dinsler¹, M. Geyer¹, A. Wietelmann², C. Schreyöck¹, C. Fiehn³, C. Müller⁴, U. Müller-Ladner¹, T. Borchardt². ¹Kerckhoff-Klinik, Justus-Liebig-University of Giessen, Bad Nauheim, GERMANY, ²Max Planck Institute for Heart and Lung Research, Bad Nauheim, GERMANY, ³Centre for Rheumatic Diseases, Baden-Baden, GERMANY, ⁴Kerckhoff-Klinik, Bad Nauheim, GERMANY.

Purpose: Osteoarthritis is a disease of progressive joint failure, leading to pain and disability. Only symptomatic treatment options exist. A key problem is the absence of endogenous repair capacity of adult mammalian joint structures. Amphibians are able to regenerate lost limbs, thus we hypothesized that endogenous repair may be possible in amphibians after local joint injury.

Methods: Knee joints of adult newts (*Notopthalmus viridescens viridescens*) were treated intraarticularly with collagenase in analogy to murine models of osteoarthritis. The clinical and histological course was analyzed.

Results: Clear cut joint injury was observed by inspection, with a clinical score (incorporating swelling, spontaneous joint use, deformity, and range of motion), and with histologic analysis after treatment. The severity of joint damage increased over the first three weeks and then abated. Disruption of joint anatomy with cartilage loss was confirmed by magnetic resonance imaging. Histologically, loss of proteoglycan and collagen II staining was observed in addition to thinning of cartilage. Chondrocytes of the femoral and tibial joint underwent cell death. Beginning after three and five weeks, evidence for mesenchymal progenitor cell recruitment with chondrogenic differentiation was observed. Clinical use of joints was normal within five weeks, histological healing still continues after 12 weeks of observation. Currently, the observation period is extended and molecular factors involved are determined by RT-PCR of joint extracts.

Conclusions: Joint injury can be induced in the newt with approaches that lead to osteoarthritis in murine models. In contrast to murine models, newts are able to recruit progenitor cells in order to induce the regeneration of joint structures. Further studies will help to elucidate cellular and molecular mechanisms.

54 A NOVEL AGE- AND STRAIN-DEPENDENT IN VIVO MODEL OF ARTICULAR CARTILAGE HEALING IN MICE

N.M. Eltawil¹, C. De Bari², P. Achan¹, C. Pitzalis¹, F. Dell'Accio¹. ¹Barts and The London School of Medicine and Dentistry, London, UNITED KINGDOM, ²University of Aberdeen, Aberdeen, UNITED KINGDOM

Purpose: To optimize and validate an in vivo model of mechanical cartilage injury and regeneration in mice.

Chondral injuries are frequent, and can either improve spontaneously or acquire a chronic symptomatic course, which may require surgical intervention, and may lead to OA. Research in the molecular mechanisms controlling joint surface healing or evolution into OA has been so far limited by the lack of mouse models of joint surface regeneration which would allow the use of mouse genetics to study molecular function. In this study we have validated a mouse model of joint surface injury and repair with a strain and age-dependent outcome.

Methods: Full thickness defects were generated in the patellar groove of adult C57BL/6 and DBA/1 mice by microsurgery. Control knees were either sham-operated or non operated. Outcome was evaluated by histological scoring systems. Apoptosis and proliferation were studied using TUNEL and Phospho-Histone H3 staining. Type II collagen neodeposition and degradation were evaluated by immunostaining using antibodies to the CII telopeptide and C1,2C (Col2-3/4Cshort) respectively. Aggrecanase and MMPs activity were assessed by immunostaining for TEGE373 and VDIPEN neopeptide.

Results: Eight weeks following surgery, adult eight weeks old DBA/1 mice displayed consistent repair of the defects with safranin-O positive cartilage tissue. Age matched C57BL/6 mice repaired poorly and developed osteoarthritic (OA) features. Cartilage injury induced apoptosis and matrix remodeling in both strains. However, compared to C57BL/6, DBA/1 mice displayed a progressive decline of chondrocyte apoptosis, persistent cell proliferation within the repair tissue, persistent type II collagen neodeposition, less type II collagen degradation, less aggrecanase-induced aggrecan degradation, and more MMP-induced aggrecan degradation. Aged eight months old DBA/1 mice failed to repair, but, contrarily to age-matched C57BL/6 mice, developed no signs of OA.

Conclusions: We have generated a murine model of cartilage regeneration in which the outcome of joint surface injury is strain and age dependent. This model will allow testing the function of different molecules in the context of joint surface regeneration in adult mammals using genetic models.

55 VERY RAPID CLEARANCE AFTER A JOINT BLEED KNEE CAN NOT PREVENT ADVERSE EFFECTS ON CARTILAGE AND SYNOVIAL TISSUE; A CANINE IN VIVO STUDY

N.W. Jansen¹, G. Roosendaal¹, M.J. Wenting¹, J.W. Bijlsma¹, H.A. Hazewinkel², F.P. Lafaber¹. ¹University Medical Center Utrecht, Utrecht, NETHERLANDS, ²Utrecht University Veterinary Medicine, Utrecht, NETHERLANDS

Purpose: Joint bleeds lead to joint destruction. *In vitro* exposure of human and canine cartilage to blood results in long lasting severe adverse changes in cartilage. An *in vivo* joint haemorrhage in the canine knee joint demonstrates similar adverse effects although less outspoken and long-lasting. We investigated the clearance rate of blood from canine knee joints as a possible explanation for this discrepancy.

Methods: Blood was injected into the knee joint of Beagle dogs, either 48 h, 24 h or 15 m before termination. The amount of red and white blood cells present in the joint cavity was determined. Chondrocyte activity and cartilage matrix integrity as well as cartilage destructive activity of synovial tissue were determined biochemically. Additionally, synovial tissue was analyzed by use of histochemistry.

Results: Fifteen minutes after the injection of autologous blood, the red blood cell count was $5.7 \times 10^{12}/\text{L}$, comparable to the amount present in whole blood, and gradually decreased ($1.6 \times 10^{12}/\text{L}$ at 24 hours) to $0.2 \times 10^{12}/\text{L}$ within 48 hours (less than 5%). The amount of white blood cells increased in the first 24 hours, and was still increased after 48 hours, although less than after 24 hours.

The proteoglycan synthesis rate and -release were adversely affected already within 24 hours (-22% and +24% respectively), and these effects were more severe 48 hours post-injection (-34% and +53% resp.). Synovial tissue culture supernatants demonstrate cartilage destructive properties as expressed by an increased release, a decreased synthesis rate, and decreased content of cartilage proteoglycans; increasing with time after the experimental haemorrhage (+207%/+247%; -58%/-62%; -8%/-28% respectively, for 24/48 hours).