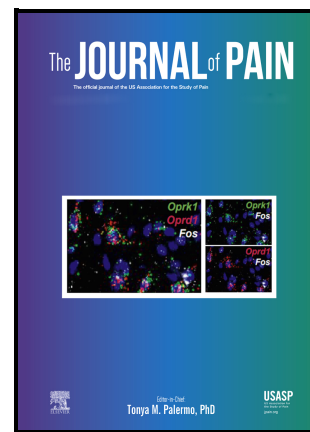


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## No evidence for cognitive impairment in an experimental rat model of knee osteoarthritis and associated chronic pain

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### ABSTRACT

Although chronic pain states have been associated with impaired cognitive functions, including memory and cognitive flexibility, the cognitive effects of osteoarthritis (OA) pain remain to be clarified. The aim of this study was to measure cognitive function in the mono-iodoacetate (MIA) rat model of chronic OA-like knee pain. We used young adult male Lister hooded rats, which are well suited for cognitive testing. Rats received either a unilateral knee injection of MIA (3mg/50µL) or saline as control. Joint pain at rest was assessed for up to 12 weeks, using weight-bearing asymmetry, and referred pain at a distal site, using determination of hindpaw withdrawal thresholds (PWT). The watermaze delayed-matching-to-place (DMP) test of rapid place learning, novel object recognition (NOR) memory assay and an operant response-shift and -reversal task were used to measure memory and behavioural flexibility. Open field locomotor activity, startle response, and prepulse inhibition (PPI) were also measured for comparison. MIA-injected rats showed markedly reduced weight-bearing on the injured limb, as well as pronounced cartilage damage and synovitis, but interestingly no changes in PWT. Rearing was reduced, but otherwise locomotor activity was normal and no changes in startle and PPI were detected. MIA-injected rats

had intact watermaze DMP performance, suggesting no substantial change in hippocampal function, and there were no changes in NOR memory or performance on the operant task of behavioural flexibility. Our finding that OA-like pain does not alter hippocampal function, unlike other chronic pain conditions, is consistent with human neuroimaging findings.

**Perspective:** Young adult rats with osteoarthritis-like knee pain showed no impairments in hippocampal memory function and behavioural flexibility, suggesting that osteoarthritis pain impacts cognitive functions less than other chronic pain conditions. In patients, osteoarthritis pain may interact with other factors (e.g., age, socio-economic factors and medication) to impair cognition.

**Key words:** Chronic pain, Osteoarthritis, Cognitive functions, Memory, Behavioural flexibility.

## INTRODUCTION

Chronic pain can impair cognitive function, in a way that appears to depend on the pain condition, possibly reflecting differences in pain intensity or different pathophysiological mechanisms<sup>3,49,62</sup>. It has been suggested that chronic pain reduces cognitive resources by diverting them to pain-related stimuli or by impairing structure and function of key brain regions, including the hippocampus and prefrontal cortex (PFC)<sup>50,62</sup>. Although additional factors, including pain medication and comorbidities, are also likely to contribute to cognitive impairment associated with chronic pain in patients<sup>49,62</sup>, experimental studies have shown impairments in cognitive functions associated with hippocampus and PFC in rodent models of neuropathic<sup>39,41,50</sup> and inflammatory pain<sup>46,55,56</sup>.

Osteoarthritis (OA) is estimated to affect 250 million people worldwide, with knee OA having the highest prevalence<sup>29</sup>. Recent longitudinal studies show that OA pain is associated with cognitive impairments, reflected in measures of verbal recall and fluency, subtraction ability, and attention<sup>32,35</sup>. Moreover, grey matter changes<sup>6</sup> and functional reorganisation<sup>7,31</sup> across cortical and subcortical regions have been identified in OA patients. However, unlike chronic back pain and complex regional pain syndrome, OA patients do not have a significant loss of hippocampal volume<sup>50</sup>. Experimental models of OA have been widely used to study the mechanisms of OA pain behaviour (see refs in<sup>25</sup>). Although one study reported impaired novel object recognition (NOR) memory in the murine mono-iodoacetate (MIA) model of OA pain<sup>52</sup>, the impact of rodent models of OA pain on distinct cognitive functions has yet to be addressed. Such rodent models make it

possible to study the impact of chronic pain separately from confounding factors, including comorbidities or pain medication.

The aim of this study was to measure a range of cognitive functions, which have been linked to the hippocampus and PFC, in the MIA rat model of OA-like knee pain. We used Lister Hooded rats as these are more suitable for cognitive testing than the albino rat strains commonly used in pain research<sup>28,37,77</sup>. To assess distinct cognitive functions, study 1 used the watermaze delayed-matching-to-place (DMP) task of rapid place learning, which is highly sensitive to hippocampal dysfunction<sup>9,14,72</sup>, and study 2 used the NOR memory test, which depends on medial temporal lobe structures, including hippocampus and perirhinal cortex<sup>16,76</sup>, and an operant task of behavioural flexibility, which depends on PFC function<sup>13</sup>. Cognitive testing was complemented by measurements of pain behaviour (weight-bearing asymmetry and von Frey test of paw-withdrawal threshold (PWT)) and of sensorimotor processes (acoustic startle response and its prepulse inhibition [PPI] and open-field locomotor activity).

## METHODS

### *Rats*

We ran two studies, using two separate cohorts of young adult male Lister hooded rats (250-275g, about 8 weeks old upon arrival, Charles River, UK): study 1 (watermaze DMP test of rapid place learning) used a total of 16 rats, and study 2 (NOR memory test and operant test of behavioural flexibility) used 32 rats (Figure 1). We only included male rats, because the parameters of the MIA model and of the behavioural assays were chosen based on previous studies in males. Also, in our hands, female rats have shown less robust performance on the watermaze DMP task than males (Charlotte Taylor and Tobias Bast, unpublished observations). Nevertheless, we recognise that our focus on male rats is an important limitation.

Rats were group housed, 4 per cage in individually ventilated cages ("double decker" cages - 462 x 403 x 404 mm; Tecniplast, UK), in temperature- and humidity-controlled conditions ( $21 \pm 1.5^\circ\text{C}$ ,  $50 \pm 8\%$ ) and with an alternating 12/12 h light/dark cycle (lights on at 7 A.M). Rats had food and water available *ad libitum*, except when testing on the food-reinforced operant task in study 2. Following arrival in the animal unit, rats had a period of acclimatisation (3-7 days), during which they were habituated to handling by the experimenter for a few days, before any experimental procedures started. All behavioural testing was carried out during the light phase. Studies had the approval of the University of Nottingham's Animal Welfare and Ethical Review Board (AWERB) and were conducted in accordance with the requirements of the UK Home Office Animals Scientific

Procedures Act (1986) and the International Association for the Study of Pain. Procedures are reported in compliance with the ARRIVE guidelines<sup>57</sup>.

### ***Induction of the monosodium iodoacetate (MIA) model of osteoarthritis pain***

Rats received a single intra-articular injection of either 3 mg/50  $\mu$ L MIA (Sigma, UK) or 50  $\mu$ L 0.9% saline (control group) through the infra-patellar ligament of the left knee, using a 30-gauge 8-mm hypodermic needle, under brief isoflurane anaesthesia (isoflurane 2.5 – 3% in 1L/min O<sub>2</sub>)<sup>40</sup>. Health and welfare checks were performed immediately after anaesthetic recovery, daily for 5 days, and weekly thereafter. The MIA dose was selected based on previous studies conducted in albino rats (see refs in<sup>25</sup>) and a pilot study in young adult male Lister hooded rats. In our pilot study, young adult Lister hooded rats injected into the knee with a dose of 1 mg/50  $\mu$ L MIA did not show robust pain behaviour and had only weak OA-like knee pathology, whereas 3 mg/50  $\mu$ L of MIA produced marked pain behaviour and knee pathology (pilot data not shown). Initially, rats were allocated randomly to the treatment groups using an online randomiser, blocked by cage (half of the rats in each cage received MIA treatment and the other half saline). Following baseline behaviour measurements and before model induction, group allocation was slightly adjusted based on the baseline measurements in order to match groups for their baseline measurements as far as possible, while still ensuring that half of the rats in each cage received MIA treatment and the other half saline. The experimenter was blinded to the treatment allocations throughout data collection and analysis.

### ***Pain behaviour and sensorimotor testing***

In both study 1 and 2, baseline pain and sensorimotor behaviours were assessed prior to model induction and then at several subsequent time points (Figure 1). Pain behaviour was assessed using two behavioural tests: joint pain at rest was assessed by weight-bearing asymmetry using an incapacitance tester (Linton Instrumentation, Diss, UK)<sup>12</sup>; and referred pain at a site distal to the injured joint was assessed via determination of mechanical PWT using the up/down method for both ipsilateral and contralateral hind paws<sup>15,18</sup>. Weight-bearing asymmetry was calculated as [ipsilateral g/(ipsilateral g + contralateral g)]. As the intervals between successive von Frey hairs are logarithmic, PWTs were reported as log(von Frey hair values in g)<sup>47</sup>. For both tests, rats were first habituated to the apparatus for 3 days before testing, to reduce stress and exploratory activity on test days. To habituate the rats to the weight-bearing apparatus, rats were placed in the Perspex box for a few minutes per day. To habituate the rats to the von-Frey test apparatus, on the first two days of

habituation, rats were placed in the chambers for approximately 30 min and, on the third day of habituation, rats were first placed into the chambers for 30 min, after which the 6-g filament was applied to the plantar surface of the rats' hind paws to familiarise the rats with the presence of the stimuli.

Locomotor activity and the acoustic startle response and its PPI are basic sensorimotor processes, modulated by forebrain regions, including hippocampus and PFC<sup>8</sup>. Altered performance in these tests may point to changes in these forebrain regions and would also need to be considered when interpreting any changes in performance on the cognitive tests (because gross sensorimotor effects may confound cognitive testing). Locomotor activity was assessed in open-field chambers (39.5cm x 23.5cm x 24.5cm) with two 4x8 photobeam configurations, one close to the bottom of the cage and the other one close to the top of the cage (Photobeam Activity System; San Diego Instruments, US), as previously described<sup>59</sup>. Two consecutive breaks of adjacent bottom photobeams generated a horizontal locomotor count, and breaks of photobeams of the top configuration generated rearing counts. Open field sessions lasted 30 min. Immediately after locomotor testing, acoustic startle response and PPI of the startle responses were measured, using the SR LAB Startle Response System (San Diego Instruments, US), as described previously<sup>59</sup>. Test sessions lasted a total of 23 min, throughout which rats were exposed to a 62-dB (A) background noise. After 5-min acclimatization, rats were exposed to a series of 10 startle pulses (40-ms, 120-dB (A) broad-band bursts) presented alone, so that the startle response could habituate to a relatively stable level of startle reactivity for the remainder of the session. In the second block, 50 trials were presented to measure PPI. There were 5 different trial types, each one presented 10 times in pseudo-random order and with a variable inter-trial interval (10-20s): pulse-alone trials and four types of prepulse-plus-pulse trials, in which a weak 20-ms prepulse [72, 76, 80, or 84 dB(A)] preceded the startle pulse by 100 ms. The percentage of PPI (%PPI) induced at each prepulse intensity was calculated as: %PPI = [(mean amplitude on pulse-alone trials – mean startle amplitude on prepulse-plus-pulse trials) / (mean startle amplitude on pulse-alone trial)] X 100.

### ***Measurements of learning and memory and of behavioural flexibility***

Detailed descriptions of the apparatus and procedures can be found in the Supplementary Material.

### ***Watermaze DMP task of rapid place learning***

The watermaze delayed-matching-to-place (DMP) task is highly sensitive to hippocampal dysfunction<sup>9,44,58,72</sup>, requiring rats to learn within one trial the daily changing place of a hidden

platform to escape efficiently from a circular pool of water, which is surrounded by prominent visual cues for spatial orientation. Rats perform four daily trials. During trial 1, rats rapidly learn the novel location of the hidden platform, and then on subsequent trials use the place memory to efficiently locate the hidden platform. Trial 2 is occasionally run as “probe trial”, during which the platform is removed to monitor the rats’ search preference for the zone containing the platform (correct zone). In study 1, rats were pre-trained on the watermaze DMP task, and their baseline performance was measured before MIA model induction. After model induction, watermaze DMP performance was evaluated at weeks 2, 4, 7 and 12 (Figure 1A). Methods were adapted from our previous studies<sup>44</sup>. Search preference for the platform area and its vicinity during probes was used as the main performance measure of rapid, 1-trial, place memory performance. Percentage of time searching the correct zone was calculated as: [time in correct zone (s)/total time in all 8 zones (s)] x 100%. By chance, i.e., if the rats were searching randomly, this value would be  $100\% / 8 = 12.5\%$ . Additionally, path lengths to reach the platform perimeter were recorded for all trials, with steep reduction from trial 1 to trial 2 indicating acquisition of 1-trial place memory. Swim speed was also measured during the 60-s probe trials, to examine potential motor impairments in the MIA-injected rats. For analysis, search preference and swim speed was averaged across the two probes at each testing time point (on day 6 and 8 for baseline testing and the two probes conducted as part of the 4-day testing blocks at each post-operative testing time point) and path length measures for trial 1 to 4 were averaged across all four days at each testing time point (days 4 to 8 of pretraining for baseline values and all four days of the 4-day test blocks at all post-operative testing time points).

### ***NOR memory test***

The NOR memory test exploits rats’ innate tendency, without use of training or reinforcement, to explore novel stimuli, including novel objects, more than familiar stimuli<sup>24,60</sup>. During the test, rats are presented with a novel object and a familiar object, i.e. an object that they had the opportunity to explore during a preceding sample phase. Rats will typically spend more time exploring the novel object, reflecting recognition memory of the familiar object explored during the sample phase. Brain areas required for simple NOR memory include medial temporal lobe regions, especially the perirhinal cortex<sup>76</sup>, and the hippocampus, particularly if the retention delay between sample phase and test is greater than 10 min<sup>16</sup>. In study 2, NOR memory was assessed before MIA model induction, and on day 29-31 after model induction (Figure 1B), using a procedure with a 24-h retention delay between sample phase and test, which was adapted from previous work<sup>60</sup>. Time exploring each object (direct contact with or active exploration of the object by directing the nose towards the object from a distance of less than 1 cm) was measured in both the sample phase (two identical objects) and the

test phase (one familiar and one novel object). The discrimination ratio was calculated as follows:  $D = \text{total time exploring novel object} / (\text{total time exploring novel object} + \text{total time exploring familiar object})$ .

### ***Operant test of behavioural flexibility***

Behavioural flexibility is the ability to switch stimulus-response patterns rapidly, as reflected by response shifts or reversals<sup>73</sup>, and can be assessed in rats using a food-reinforced operant test<sup>13</sup>. Shifts refer to a subject beginning to make responses to new or previously irrelevant stimuli (e.g., from pressing the lever indicated by a cue light to pressing either the left or right lever in an operant box), whereas reversals refer to changing a response from a previously rewarded to a previously non-rewarded stimulus within the same category (e.g., from left to right lever). Previous studies suggest that behavioural flexibility, as measured on the operant task, depends on the PFC and subcortical regions, including ventral striatum<sup>13</sup>. In study 2, following MIA model induction (from day 38 to 59), rats were trained first to acquire a lever press response to a cue light in order to receive food reward, before being tested for their ability to shift this response to a spatial response (pressing either the left or right lever to receive food) and then to reverse this spatial response (start to press the opposite lever to receive food reward) (Figure 1B). Operant testing procedures were adapted from previous studies<sup>13</sup>. The analysis of this test focused on trials to criterion, the percentage of correct responses, and percentage of omissions.

### ***Assessment of OA-like knee pathology***

At the end of study 1 (day 93 after model induction) and 2 (day 70 after model induction), rats were anaesthetised with an overdose of sodium pentobarbitone (Dolethal, Vetoquinol; 2mL intraperitoneal), and then transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.9% saline. Both knees were collected, decalcified, stained and histologically scored to confirm development of OA-like pathology via established methods<sup>33,42,64</sup>. Briefly, knee joints were stored in the fixative for 72h after collection, transferred to an ethylenediaminetetraacetic acid + 7.5% polyvinylpyrrolidone solution for decalcification (6-7 weeks), and then split in the frontal plane<sup>36</sup>. Trimmed joints were mounted in paraffin wax and 5µm sections cut using a microtome. Haematoxylin and Eosin<sup>70</sup> and Safranin-O-Fast Green<sup>70</sup> histological stains were used to confirm OA-like damage to the knee joints. Cartilage damage was scored on a scale of 1-5 for severity with a multiplier of 1-3 for area coverage, to give a composite score out of 15. Osteophytes were scored 1-3 based on size (1 = mild <40µm; 2 = moderate, 40-160µm, 3 = severe, >160µm)<sup>33,42,64</sup>.



### ***Sample sizes and statistical analysis***

The target sample size for both studies was 32 (n=16 per group), so group differences corresponding to an effect size of Cohen's  $d=1$  could be detected with a power of about 80%, using an independent t-test (2-tailed,  $p<0.05$ ). In study 1, we used a sequential design<sup>53</sup>. The first series of testing with half the target sample size revealed that there were no substantial group differences in the main memory measures, and indicated that completion of the second series to achieve the target sample size would not reveal significant group differences. Therefore, study 1 was terminated after completion of the first series<sup>53</sup>. Moreover, one MIA rat was excluded from study 1 due to a physiological abnormality (swelling in the non-injected right hind paw, possibly due to fighting), preventing completion of behavioural data collection. Therefore, in study 1, data analysis was based on n=8 saline and n=7 MIA rats. In study 2, 32 rats were tested in parallel, and no rats had to be excluded from data analysis, resulting in n=16 rats in both the saline and MIA group.

GraphPad Prism 8 and IBM SPSS Statistics 24 were used to prepare the graphs and perform the statistical analysis. All data were normality tested using D'Agostino-Pearson test prior to subsequent analyses. The different measurements were analysed, using analysis of variance (ANOVA) with group (MIA or saline) as between-subjects factor and testing day as repeated measures/within-subjects factor (2-way ANOVA) and with task block (open field and startle data), prepulse intensity (PPI data), trial (watermaze and operant data) and object (novel vs. familiar, NOR data) as within-subjects factors, as appropriate (3-way ANOVA). Baseline measures were not included in the ANOVA analysis, but were analysed separately, using independent t-tests or 2-way ANOVA (with prospective group as between-subjects factor and task block, prepulse intensity or trial as within-subjects factor), to ensure that there was no significant group difference at this stage. Trials-to-criterion measures from the operant task and knee pathology measures were compared between groups, using independent t-test or the Mann-Whitney U test, if the normality assumption was not met.  $P<0.05$  was accepted as significance threshold for all analyses.

## **RESULTS**

### ***Pain behaviour and sensorimotor measurements: MIA knee injections induced weight-bearing asymmetry and decreased rearing activity***

MIA-treated rats placed markedly less weight on the injured leg compared with saline-injected rats across all testing time points in study 1, resulting in significant weight-bearing asymmetry (group:

$F_{(1,13)}=66.22$ ,  $p<0.0001$ ; time:  $F_{(4,52)}=3.32$ ,  $p<0.0001$ ; time x group:  $F_{(4,52)}<1$ ) (Figure 2A). However, there were no differences in PWT between MIA-, and saline-injected rats across all testing time points in study 1 (group:  $F_{(1,13)}=1.0$ ,  $p=0.33$ ; time:  $F_{(4,52)}=1.66$ ,  $p=0.18$ ; time x group:  $F_{(4,52)}<1$ ) (Figure 2B). MIA-treated rats showed significantly less rearing than saline controls in study 1 (main effect of group:  $F_{(1,13)}=4.86$ ,  $p=0.046$ ; all interactions involving group:  $F<1.9$ ,  $p>0.18$ ) (Figure 2C). Similarly, in study 2 MIA-treated rats showed weight-bearing asymmetry (group:  $F_{(1,30)}=33.89$ ,  $p<0.0001$ ; time:  $F_{(3,90)}=0.71$ ,  $p=0.55$ ; group x time:  $F_{(3,90)}=0.21$ ,  $p=0.89$ ) (Figure 2D) and no differences in PWT compared with saline-injected rats (group:  $F_{(1,30)}=1.68$ ,  $p=0.20$ ; time:  $F_{(2,9,87.4)}=1.62$ ,  $p=0.19$ ; time x group:  $F_{(3,90)}<1$ ) (Figure 2E). Also, reduced rearing activity was observed in MIA-treated rats in study 2 (main effect of group:  $F_{(1,30)}=5.08$ ,  $p=0.03$ ; all interactions involving group:  $F<1$ ) (Figure 2F). MIA-treated rats did not show altered horizontal locomotor activity in study 1 (group:  $F_{(1,13)}<1$ , all interactions involving group:  $F<1$ ), but there was a trend for reduced locomotor activity in study 2 (group:  $F_{(1,30)}=3.65$ ,  $p=0.07$ , all interactions involving group:  $F<1.05$ ,  $p>0.3$ ) (Figure S1). MIA-treated rats did not exhibit altered startle reactivity or PPI (all main effects or interactions involving group:  $F<2.14$ ,  $p>0.15$ , except for a trend towards a main effect of group on startle response in study 2,  $F_{(1,30)}=2.91$ ;  $p=0.10$ , reflecting a numerical decrease in the startle response in rats injected with MIA, which was already present at baseline, i.e. before knee injections, and persisted across all testing time points) (Figures S2 and S3).

### ***MIA- induced OA-like knee pain pathology***

MIA-treated rats showed marked OA-like knee pathology (Figure S4). In both study 1 and 2, the degree of cartilage damage, synovitis, and osteophyte formation varied from mild to severe. It is important to note that individual rats may have shown a score of 0 for one feature of knee pathology, but that the sum of scores for all features examined was always  $> 0$ . Therefore, all MIA-treated rats presented with some features of OA-like knee pathology. MIA-treated rats had lateral and medial tibial plateau loss of cartilage integrity (study 1-  $t_{(13)}=4.63$ ,  $p=0.0005$ ; study 2-  $U=24$ ,  $p<0.0001$ ), as well as increased synovial inflammation (study 1-  $U=0$ ,  $p=0.0003$ ; study 2-  $U=48$ ,  $p=0.0001$ ) compared to saline-injected rats. There was a trend for MIA-injected rats to show a higher number of osteophytes in study 1 ( $U=14$ ,  $p=0.08$ ) and a significant increase in study 2 ( $t_{(29)}=2.51$ ,  $p=0.02$ ). Given the variability in knee pathology, we conducted a correlational analysis to examine the relationship between different knee pathology measures (cartilage integrity, synovitis) and pain-related behavioural measures (weight-bearing asymmetry /reduced PWT/ reduced rearing). There was no evidence for any correlation for any measure ( $R<0.69$ ,  $p>0.12$ ). Although there was a trend towards a positive relationship between cartilage integrity and weight bearing asymmetry in study 1 ( $R=0.69$ ,  $p=0.07$ ),

there was a trend towards a negative relationship in study 2 ( $R=-0.51$ ,  $p=0.06$ ). So, overall, there was no clear relationship between knee pathology and pain-related behavioural measures.

### ***Intact hippocampus-dependent rapid place learning performance after MIA model induction***

At baseline testing, both prospective groups showed similar marked pathlength reductions from trial 1 (encoding) to trial 2 (retrieval) (main effect of trial:  $F_{(3,90)}=12.5$ ;  $p<0.0001$ ; interaction prospective group X trial:  $F_{(3,90)}<1$ ), as well as similar ( $t_{(30)}<1$ ) and markedly above-chance ( $t_{(15)}>3.68$ ,  $p<0.002$ ) search preference for the correct zone when trial 2 was run as a probe (Figure 3). After MIA treatment, both groups showed virtually identical performance patterns in terms of path length across all testing time points (main effect of group and interaction involving group: all  $F<1$ ), with marked path length reductions from trial 1 to 2 (trial:  $F_{(3,84)}=80.19$ ;  $p<0.0001$ ) (Figure 3A). Both groups also showed similar search preference for the correct zone when trial 2 was run as a probe trial (main effect and interaction involving group:  $F<1.8$ ;  $p>0.20$ ), and search preference was highly above chance in both groups at all testing time points (saline:  $t_{15}>2.69$ ,  $p<0.018$ ; MIA:  $t_{15}>3.17$ ,  $p<0.006$ ) (Figure 3B). The only significant effect of group on performance measures was a significant interaction of group and time point in the ANOVA of swim speed data ( $F_{(3,84)}=8.87$ ;  $p<0.0001$ ), reflecting that MIA rats had slower swim speed than control rats at the last two testing time points (Figure 3C). More specifically, control rats slightly increased their swim speed from week 7, whereas the swim speed of MIA rats remained stable.

### ***Intact NOR memory after MIA model induction***

At baseline and after model induction, exploration time was similar in both groups ( $F_{(1,30)}<1$ ), and both groups showed a similar preference for the novel object (main effect of object:  $F_{(1,30)}>27$ ,  $p<0.0001$ ; no group X object interaction:  $F<1$ ) (Figure 4A). The discrimination ratio was not altered in the MIA-treated group (group:  $F_{(1,30)}=2.27$ ,  $p=0.14$ ), and was significantly different from chance in both groups at baseline and after model induction ( $t>2.41$ ,  $p<0.03$ ) (Figure 4B).

### ***Intact performance on the operant task of behavioural flexibility after MIA model induction***

Both MIA-treated and control rats completed the pretraining on the operant task efficiently. MIA treatment did not impair any aspect of the operant testing. Both groups learned the cue discrimination similarly well, showing similar trials to reach the criterion ( $t_{(30)}<1$ ; Figure 5A), % of correct responses ( $t_{(30)}=1.35$ ,  $p=0.2$ ; Figure 5B) and % of omissions (median, percentiles: saline,

0.5, 0-1.75; MIA, 0, 0-1;  $U=109.5$ ,  $p=0.46$ , data not shown). At the visual discrimination stage, 4 rats (3 saline, 1 MIA) failed to achieve the criterion of 10 successive correct responses within three 150-trial sessions (highlighted by the ellipses in Figure 5A) and, therefore, were excluded from further testing.

Shifting from visual cue discrimination to the spatial response rule was also not affected by MIA treatment. Both MIA- and saline-injected rats showed a similar number of trials to reach criterion on the first day (mean+SEM: saline,  $111.9\pm 12.4$ ; MIA,  $104.1\pm 4.3$ ;  $t_{(26)} < 1$ ) and similarly increased their % of correct responses across the 4 days of the shift phase (main effect of day:  $F_{(3,78)}=38.73$ ,  $p < 0.0001$ ; no main effect or interaction involving group:  $F_{(1,26)} < 1$  and  $F_{(3,78)} > 1.77$ ,  $p > 0.2$ , respectively; Figure 5C). Omissions were low ( $< 1\%$ ) and did not differ between groups (data not shown).

Finally, MIA treatment did not impair the ability to reverse the spatial response rule. Both groups showed a similar number of trials to reach criterion on the first day (mean+SEM: saline,  $111.2\pm 6.3$ ; MIA,  $114.9\pm 7.5$ ;  $t_{(26)} < 1$ ) and an increase in the % of correct responses across the 4 days of the reversal phase (time:  $F_{(3,78)}=130.5$ ,  $p < 0.0001$ ) (Figure 5D). However, for both measures, there was no main effect or interaction involving group ( $F_{(3,78)} < 1.67$ ,  $p > 0.2$ ). Omissions were never greater than 1 omission per session and did not differ between groups (data not shown).

## DISCUSSION

The MIA model was associated with OA-like knee pathology and knee pain, reflected by weight-bearing asymmetry, but no changes in PWTs, and reduced rearing behaviour in young adult male Lister hooded rats. MIA treated rats did not show any impairment in performance in memory tests (watermaze DMP test of rapid place learning; NOR test) dependent on the medial temporal lobe, including the hippocampus, or on an operant assay of behavioural flexibility, which has been associated with fronto-striatal mechanisms.

### *MIA-induced knee pathology and pain phenotype in young adult LH rats*

To our knowledge, this was the first study of the MIA model of OA pain in non-albino rats. We used Lister Hooded rats, because these are more suitable for cognitive assays than albino strains, which have been reported to perform poorly in watermaze and operant assays<sup>28,37,77</sup>, probably partly reflecting their diminished vision<sup>65</sup>. MIA treatment produced consistent pain behaviour and knee pathology in Lister Hooded rats, including robust weight-bearing asymmetry and knee pathology with a loss of cartilage integrity, synovitis and occasional osteophyte formation. Following MIA

model induction, rats also exhibited decreased rearing activity, whereas horizontal locomotor activity was not reliably reduced. Previous findings regarding the impact of MIA-induced knee pain in one hind leg on horizontal locomotor activity in rats have been somewhat variable, with reductions in horizontal locomotor activity reported in some studies<sup>40,51</sup>, but not in others<sup>23</sup>. Our finding that rearing in rats is more sensitive to the MIA model than horizontal locomotor activity is consistent with a previous study in Sprague-Dawley rats<sup>51</sup>. This probably reflects that horizontal locomotor activity involves the rats distributing their weight on all four legs, whereas rearing involves weight distribution on the two hind legs. The slower swim speed in the MIA-treated rats compared with saline controls during watermaze testing at week 7 and 12 after MIA treatment may also reflect knee pain, although the reason for the swim speed increase in the control group is not clear. There were no consistent MIA-induced changes in the acoustic startle response and its PPI.

In the present study, MIA treatment did not result in a change in PWT, contrasting with studies in Sprague Dawley rats<sup>26,43,68</sup>. Lowering of hindpaw withdrawal thresholds is often considered to indicate the presence of central sensitisation mechanisms, since it demonstrates enhanced mechanical sensitivity at a site distal to the injured joint<sup>45</sup>. Therefore, the absence of PWT reductions in Lister hooded rats in the present study may reflect a relative lack of central sensitisation, compared to SD rats. However, the sensitivity of the von Frey test to detect PWT reductions in Lister hooded rats is worth further consideration. In the present study, baseline PWTs were lower in Lister hooded rats (PWT log=0.8-0.9) than those we typically measure in SD rats (around PWT log=1.25<sup>40</sup>), and, therefore this measure of pain behaviour is more sensitive to a floor effect in Lister hooded rats. In addition, Lister hooded rats are very inquisitive, which although beneficial for cognitive testing, makes von-Frey testing challenging. In the present study, although Lister hooded rats took longer to habituate to the testing environment, we were still able to measure PWTs. Another recent study recently reported challenges in measuring PWTs in a model of neuropathic pain (partial saphenous nerve injury) in Lister hooded rats, but the authors obtained measurements in the majority of rats and reported reduced PWTs<sup>61</sup>. Interestingly, in this previous study a higher baseline PWT (PWT log = 1.2) in Lister hooded rats was reported, which was similar to baseline PWTs in Sprague Dawley rats. It is possible that different baseline PWTs in Lister hooded rats reflect that the rats came from different breeders (Charles River, UK, in our study, Harlan, UK, in [58]). Overall, the lack of effect of the MIA model on PWTs in Lister hooded rats probably reflects a relative lack of central sensitisation, alongside some technical limitations.

It is worth considering the translational relevance of PWT reductions in the MIA model. To our knowledge, a measure of mechanical hypersensitivity in the human foot, which would directly

correspond to PWT reductions in rodent models of knee OA, has not been used in participants with knee OA. However, a range of measures have been used to assess the construct of central sensitization in participants with knee OA, including questionnaire measures and Quantitative Sensory Testing, including reduced pain pressure threshold (PPT) at the knee and at 'distal' sites on the lower leg<sup>1,63</sup>; reduced PPT somewhat resembles reduced PWT in rats, although PPT are measured at or closely to the injured knee. A recent meta-analysis of studies using such surrogate measures of central sensitisation, suggested that, overall, 20% of people with knee OA show central sensitization, with substantial variability depending on the measures used. The prevalence of central sensitisation based on PPT reduction was 29% across studies, with distal PPT reductions being less pronounced than PPT reduction at the knee<sup>63</sup>. However, mechanical hypersensitivity at the foot, which would more closely correspond to PWT reductions in rats, has not yet been examined directly, but is likely to have lower prevalence than the reported PPT reductions.

The relationship between surrogate measures of central sensitisation and cognitive function in people with knee OA is not clear. To our knowledge only two studies directly examined measurements of cognitive function specifically in people with OA. These studies<sup>32,35</sup> showed that OA pain is associated with cognitive impairment, reflected in measures of verbal recall and fluency, subtraction ability, and attention, but did not address the influence of measures of central sensitisation. In another recent study, 98 out of 103 participants with OA and chronic knee pain reported that their pain had a 'cognitive impact' ('stopped them from concentrating on what they were doing'), and this self-reported cognitive impact was higher in participants with lower PPT (measured at the proximal tibia, a site distal to the painful knee)<sup>1</sup>. This supports a contribution of central sensitisation to subjective cognitive impairment, but the link between central sensitisation and objective measures of cognitive impairment in people with knee OA remains to be examined.

It is possible that a higher MIA dose would cause a more severe pain phenotype, including reductions in PWT, and also impairments on the cognitive assays. However, in the present study, Lister hooded rats showed marked OA-like knee pathology, including cartilage damage, synovitis, and osteophyte formation, similar to the knee pathology that we find in Sprague Dawley rats following MIA knee injections (1mg/50ul) and consistent with key features of human OA knee pathology<sup>43</sup>. Although increasing the MIA dose can increase the destruction of the knee joint cartilage, this was variable in a previous study and did not result in a greater pain phenotype<sup>54</sup>. Interestingly, our previous study found that similar knee pathology to the present study was associated with marked PWT reductions in Sprague Dawley rats<sup>43</sup>, whereas in the present study Lister hooded rats did not show PWT reductions. In the present study, there was variability in knee pathology in MIA-treated rats and no clear relationship between knee cartilage damage and pain-

related behavioural measures. These findings in the MIA rat model are consistent with findings in patients with knee OA, that similar knee pathology can be associated with different pain phenotypes and that the extent to which knee pathology predicts knee pain is limited<sup>2,11,27,71</sup>.

#### ***No effect of the MIA model on hippocampus-dependent memory***

In the present study, neither rapid place learning performance in the watermaze DMP task nor NOR memory was altered by the MIA model. Previously, impaired watermaze place learning performance and NOR memory has been reported in rodent models of neuropathic and inflammatory pain<sup>48,78</sup>. Impaired NOR memory was also reported in MIA treated mice<sup>52</sup>, contrasting with our finding of intact NOR memory in MIA treated rats. The difference between the MIA model of OA pain in Lister hooded rats and these other studies may be the level of pain intensity and/or the involvement of different brain regions, including hippocampal changes. Indeed, MIA-treated mice show markedly reduced PWTs<sup>52</sup>, which likely reflects higher pain intensity and stronger involvement of central sensitisation in the mouse model. Although hippocampal changes in the MIA model have not yet been examined, studies have reported changes in the hippocampus of Sprague-Dawley rats with models of neuropathic and inflammatory pain<sup>22,66,78</sup>. It is noteworthy that a study conducted in Wistar Han rats (3-, 10- and 22-months old) with spared nerve injury did not report impairments in watermaze place learning performance<sup>39</sup>, indicating that effects on this measure of hippocampal function are not always observed in neuropathic pain models.

Our findings of intact hippocampus-dependent memory in MIA-injected Lister hooded rats are consistent with the suggestion from human imaging studies that other pain conditions, including chronic back pain and complex regional pain syndrome, affect the hippocampus more than chronic pain associated with OA-related knee damage. More specifically, chronic back pain and complex regional pain syndrome, but not knee OA, were reported to be associated with reduced hippocampal volume<sup>50</sup>. In addition, relatively intact recognition memory in some chronic pain conditions, including chronic back pain and fibromyalgia, has been reported<sup>38,69</sup>, supporting limited susceptibility of recognition memory to some chronic pain conditions.

#### ***No effect of the MIA model on the operant task of behavioural flexibility***

In the present study, MIA treatment did not impair operant performance and operant measures of behavioural flexibility, including rule shifting and reversal learning. Previous studies in rat models of neuropathic pain reported impaired operant performance, including slower adaptation to optimal choices<sup>19</sup>, and impaired spatial reversal learning in the watermaze<sup>39</sup>. These measures do appear to have translational relevance as chronic pain patients with fibromyalgia and primary

Sjögren syndrome with small fibre neuropathy show impaired behavioural flexibility on the Wisconsin Card Sorting Test<sup>30,74</sup>. In addition, in older adults with mixed pain conditions, pain severity (measured by questionnaire) was associated with impaired mental flexibility measured with the Trail Making Test<sup>34</sup>. The absence of impairments in operant performance and behavioural flexibility in MIA-injected Lister hooded rats, which contrasts with impairments in some chronic pain conditions reported by previous clinical and pre-clinical studies, may reflect lower severity of or more limited involvement of central mechanisms in the chronic pain associated with OA-like knee pathology in the present study compared to the other chronic pain conditions.

### ***Chronic OA pain and cognitive deficits: translational relevance***

In the present study, we used an established model of OA pain in a rat strain well-suited for the investigation of cognitive function. In our study, OA-like pain behaviour was not associated with cognitive impairment in LH rats, unlike other experimental chronic pain conditions. The absence of impairments in hippocampal learning and memory function in the present study is consistent with human neuroimaging findings, suggesting that knee OA impacts the hippocampus less than other chronic pain conditions<sup>50</sup>. There is, however, evidence that chronic pain caused by knee OA causes forebrain changes<sup>6,7,31</sup>, and recent studies in human participants support that OA pain may contribute to cognitive impairments, reflected in measures of verbal recall and fluency, subtraction ability and attention<sup>32,35</sup>. These measures may partly depend on similar neurocognitive mechanisms as those assessed in the present study, although it remains to be examined if human participants with chronic OA pain are impaired on translational behavioural assays that correspond more directly to the ones used in the present study, such as a virtual DMP test<sup>14</sup> and CANTAB assays of executive functions, including behavioural flexibility<sup>5</sup>. There are several factors that may interact with chronic pain to impair cognitive function in patients, and which impact upon the translational relevance of the experimental models. First, ageing is a key contributor to cognitive decline<sup>21</sup>. This is of relevance as OA is an age-associated disorder<sup>17</sup>, and the average age of participants in the two recent studies showing a negative impact of chronic OA pain on cognition in human participants was > 60 years at the start of data collection<sup>32,35</sup>. Therefore, ageing may interact with chronic OA pain to impair cognitive function. In support of this possibility, impairments in behavioural flexibility associated with chronic pain have been reported to be age-dependent in both rodent<sup>39</sup> and human studies<sup>75</sup>. Moreover, recent neuroimaging evidence suggests that ageing may increase the vulnerability of a brain circuit including PFC and hippocampus to chronic lower back pain<sup>4</sup>, suggesting that the cognitive functions associated with these brain regions may also become more vulnerable to chronic pain with age. Second, social deprivation and education have recently been reported to interact with chronic pain to affect

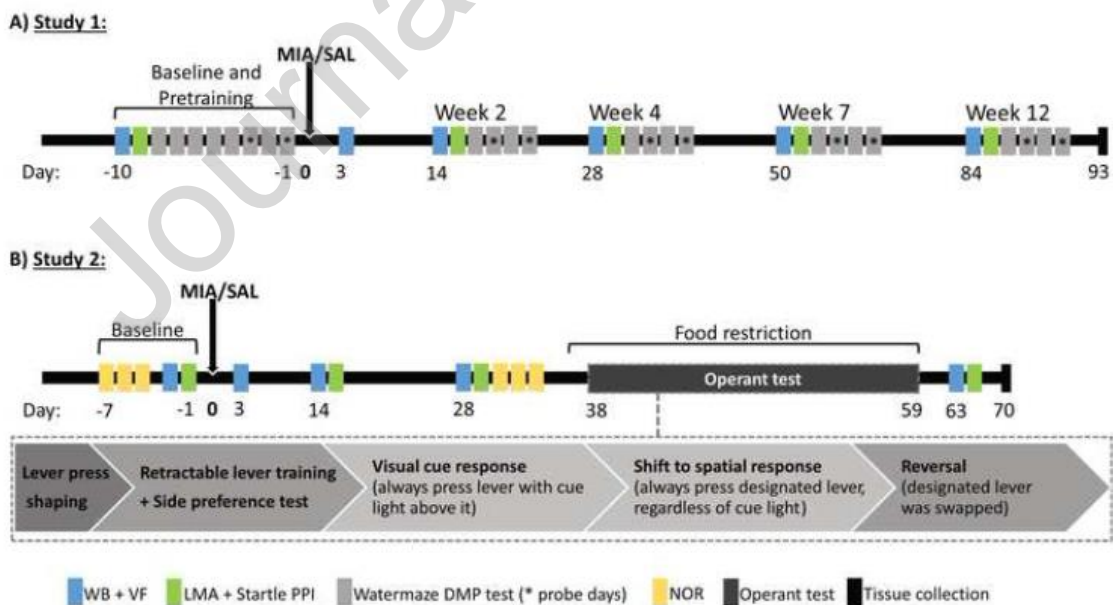


cognitive ability in people with OA <sup>35</sup>. Third, prescribed OA pain medication may contribute to cognitive impairments <sup>49</sup>. In particular, a negative impact of long-term opioid treatment on cognitive task performance has been reported in patients with chronic pain <sup>67,69</sup>. Examining the impact of age and pain medication on cognitive task performance in the MIA rat model offers the opportunity to test directly how these factors interact with chronic pain caused by OA-like knee damage to cause cognitive impairment, whereas the impact of socio-economic factors cannot be modelled experimentally in animals.

## ACKNOWLEDGMENTS

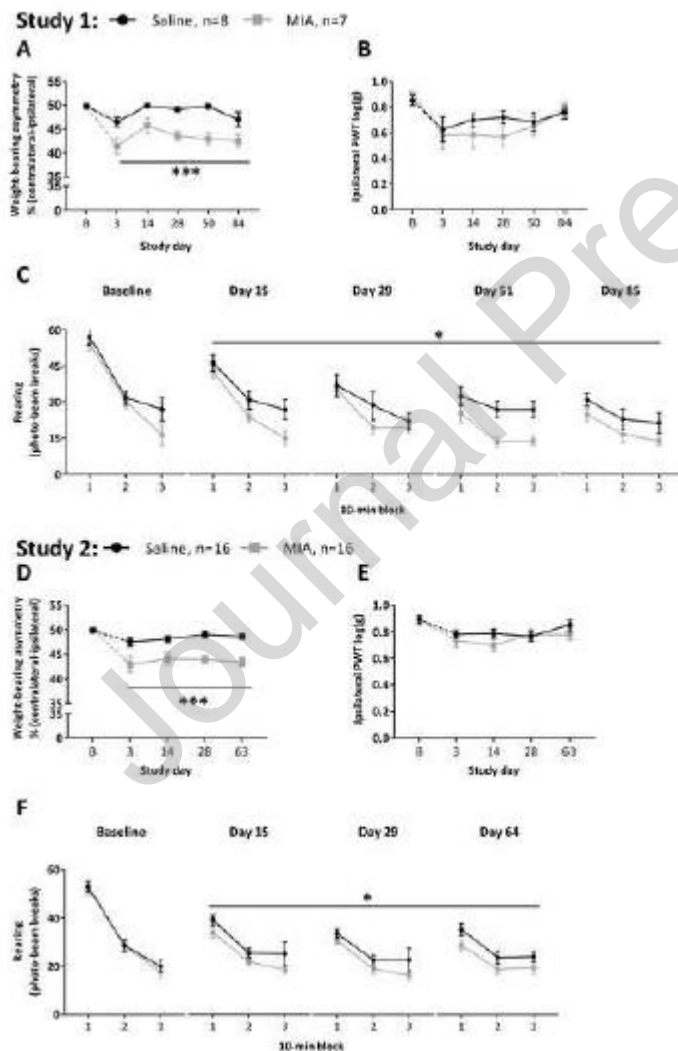
The authors thank the following people for their contributions to this article: Seyed Shahtaheri for helping with knee pathology scoring. Author contributions: Study concept and design: S.G., G.J.H., S.G.W., V.C., and T.B.; Data acquisition: S.G.; Data analysis: S.G., G.J.H., V.C., and T.B; Manuscript-original: S.G., V.C. and T.B. Manuscript-review and editing: G.J.H. and S.G.W.

## Legends:



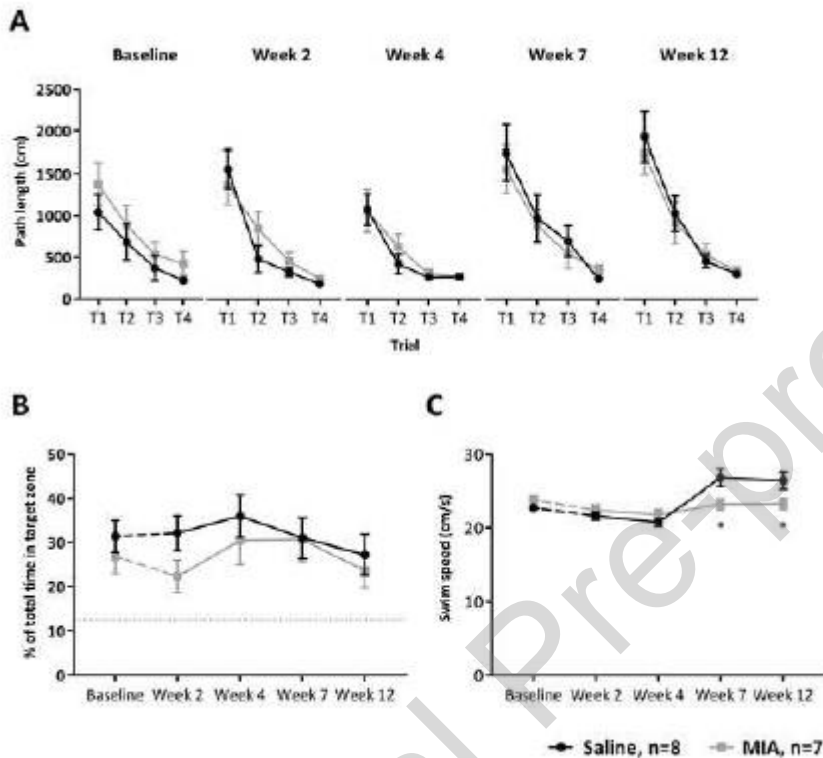
**Figure 1 – Overview of study 1 and study 2. A)** Timeline for study 1 – watermaze DMP test of rapid place learning: Before MIA model induction, baseline pain behaviour and sensorimotor measures were collected and

pre-training and baseline measurements on the watermaze DMP task were completed. After model induction (day 0), rats were tested on the watermaze DMP task in weeks 2, 4, 7 and 12 to longitudinally evaluate the impact of OA-like chronic knee pain on hippocampus-dependent rapid place learning performance. Pain behaviour (weight-bearing and Von-Frey tests) and sensorimotor processes (locomotor activity and startle and its PPI) were measured in the same weeks, before watermaze DMP testing. Rats were euthanised on day 93, and knees were collected for post mortem analysis. **B)** Timeline for study 2 – NOR memory test and operant test of behavioural flexibility: Before MIA model induction, baseline measures of NOR, pain behaviour and sensorimotor processes were collected. After model induction (day 0), NOR memory was re-assessed on day 29-31 after model induction. After this, rats were put on food restriction from day 35 to 59 and rats underwent testing on the food-reinforced operant task of behavioural flexibility from day 38 to 59. Pain behaviour and sensorimotor processes were measured at several time points following model induction, before and after NOR and operant testing. Rats were euthanised on day 70, and knees were collected for post mortem analysis. DMP, delayed-matching-to-place; LMA, locomotor activity; MIA, mono-iodoacetate; NOR, novel object recognition; PPI, prepulse inhibition; VF, Von-Frey; WB, weight-bearing.

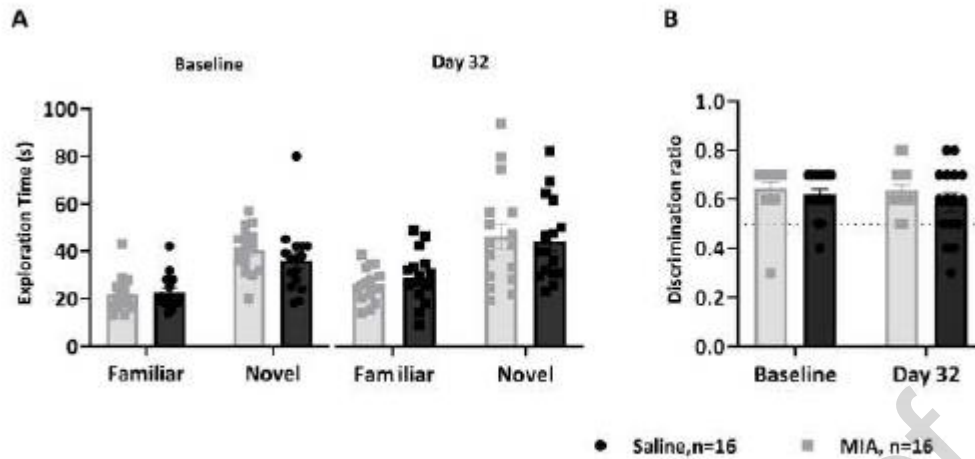


**Figure 2 – MIA-induced knee pain in Lister hooded rats: weight bearing asymmetry and reduced rearing, but no significant change in paw withdrawal threshold (study 1 and 2).** Rats were injected with either 3mg/50 $\mu$ l MIA ( $\square$ , grey squares) or 50 $\mu$ l saline ( $\bullet$ , black dots) into the left knee. In both studies, MIA injection induced marked weight bearing asymmetry across all testing time points after model induction (study 1, **A**; study 2, **D**), whereas no changes were observed in the von-Frey test of paw withdrawal threshold (study 1, **B**; study 2, **E**).

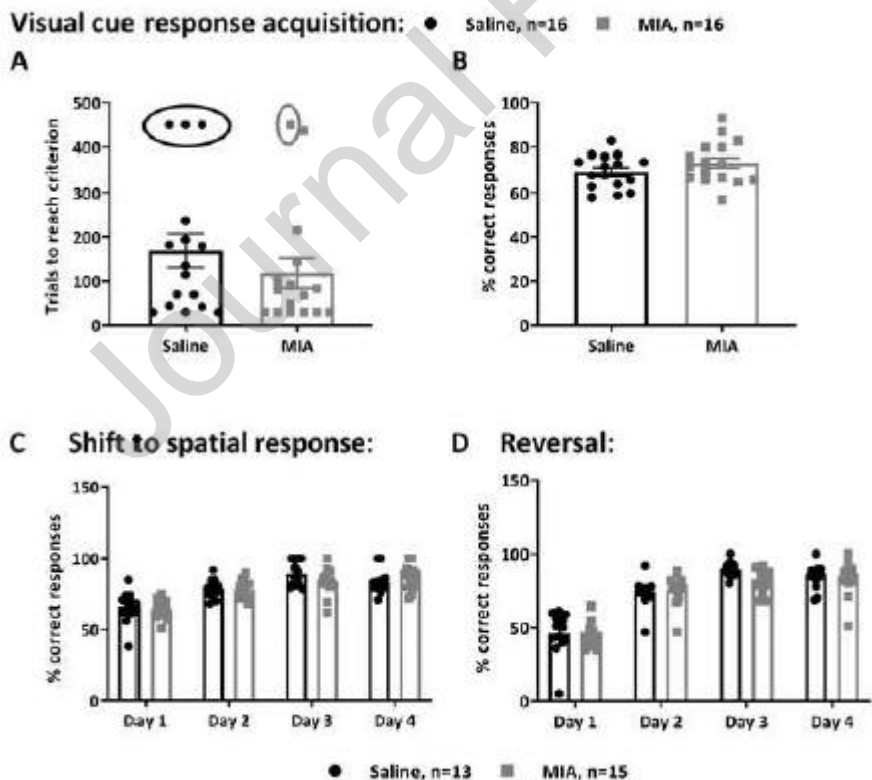
Rearing was significantly reduced in MIA-injected rats compared with saline controls across the complete 30-min open field test sessions and across all testing time points after model induction (study 1, C; study 2, F). Data are presented as mean±SEM. \* and \*\*\* indicate significant ANOVA main effect of group across all testing time points after model induction (2-way ANOVA,  $p < 0.05$  and  $p < 0.0001$ , respectively). B, baseline; MIA, mono-iodoacetate; PWT, paw withdrawal threshold.



**Figure 3 – Intact rapid place learning performance on the watermaze Delayed-Matching-to-Place (DMP) task following MIA model induction.** Rats were injected with either 50 $\mu$ l of 3mg of MIA (\*, grey squares) or 50 $\mu$ l saline (●, black dots) in the left knee. They were tested on the watermaze DMP task at baseline, i.e. before the knee injections, and then during weeks 2, 4, 7 and 12 after the knee injections. **A)** Path lengths to reach the platform across the four daily trials (T1 to T4) to a novel location show marked reduction across trials, including from trial 1 to 2, reflecting rapid place learning, which was not affected by MIA injection. **B)** Search preference for the target zone when trial 2 to the daily new goal location was run as probe, with the escape platform unavailable, was markedly above chance (12.5%, stippled line) across all testing time points, reflecting 1-trial place learning; search preference was unaffected by MIA injection. **C)** Swim speed was similar in both groups during baseline and in week 2 and 4 following injection, but was increased in saline compared to MIA-injected rats in week 7 and 12 following knee injection. Data are presented as mean±SEM. \* $p < 0.05$  MIA vs. saline, following group X interaction in 2-way ANOVA.



**Figure 4 – Intact novel object recognition (NOR) memory following MIA model induction.** Rats were injected with either 50 $\mu$ l of 3mg of MIA (■, grey squares) or 50 $\mu$ l saline (●, black dots) in the left knee. They were tested on the NOR memory task at baseline, i.e. before the knee injections, and 32 days after the knee injections. Both groups showed similarly marked recognition memory for the familiar object at both testing time points. This was reflected by increased **A**) exploration time for the novel, compared to the familiar, object and **B**) an above-chance (0.5, stippled line) discrimination ratio. Data are presented as mean $\pm$ SEM.



**Figure 5 – Intact performance on the operant task of behavioural flexibility after MIA model induction.** Rats were injected with either 50 $\mu$ l of 3mg of MIA (■, grey squares) or 50 $\mu$ l saline (●, black dots) in the left knee. Rats were tested on this task 38 days after model induction. Acquisition of the initial cue discrimination task,

as reflected by **A)** trials to criterion and **B)** % of correct responses, was not affected by MIA injection compared with controls. Rats in the ellipses did not reach criterion on the 3 days of testing and, therefore, did not continue to the further testing phases. MIA- and saline-injected rats shifted similarly well from the cue response rule to a spatial rule, as reflected by both groups showing similar **C)** % of correct responses across the 4 days of the shift phase. Similarly, both groups showed similarly efficient response reversals, as reflected by both groups showing similar **D)** % of correct responses. Data are presented as mean±SEM.

## REFERENCES

1. Akin-Akinyosoye K, Frowd N, Marshall L, Stocks J, Fernandes GS, Valdes A, McWilliams DF, Zhang W, Doherty M, Ferguson E, Walsh DA: Traits associated with central pain augmentation in the Knee Pain In the Community (KPIC) cohort. *Pain* 159:1035–44, 2018
2. Arendt-Nielsen L, Skou ST, Nielsen TA, Petersen KK: Altered Central Sensitization and Pain Modulation in the CNS in Chronic Joint Pain. *Curr Osteoporos Rep* 13:225–34, 2015
3. Baliki MN, Apkarian AV: Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* 87:474–91, 2015
4. Baran TM, Lin F V., Geha P: Functional brain mapping in patients with chronic back pain shows age-related differences. *Pain* 163:e917–26, 2022
5. Barnett JH, Robbins TW, Leeson VC, Sahakian BJ, Joyce EM, Blackwell AD: Assessing cognitive function in clinical trials of schizophrenia. *Neurosci Biobehav Rev* 34:1161–77, 2010
6. Barroso J, Vigotsky AD, Branco P, Reis AM, Schnitzer TJ, Galhardo V, Apkarian AV: Brain gray matter abnormalities in osteoarthritis pain: a cross-sectional evaluation. *Pain* 161:2167–78, 2020
7. Barroso J, Wakaizumi K, Reis AM, Baliki M, Schnitzer TJ, Galhardo V, Apkarian AV: Reorganization of functional brain network architecture in chronic osteoarthritis pain. *Hum Brain Mapp* 42:1206–22, 2021
8. Bast T, Feldon J: Hippocampal modulation of sensorimotor processes. *Prog Neurobiol* 70:319–45, 2003
9. Bast T, Wilson IA, Witter MP, Morris RGM: From Rapid Place Learning to Behavioral Performance: A Key Role for the Intermediate Hippocampus. Rugg MD, editor. *PLoS Biol* 7:e1000089, 2009
10. Bauer M, Buckley MG, Bast T: Individual differences in theta-band oscillations in a spatial

- memory network revealed by EEG predict rapid place learning. *bioRxiv* :2020.06.05.134346, 2020
11. Beswick AD, Wylde V, Gooberman-Hill R, Blom A, Dieppe P: What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. *BMJ Open* 2:e000435, 2012
  12. Bove S, Calcaterra S, Brooker R, Huber C, Guzman R, Juneau P, Schrier D, Kilgore K: Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. *Osteoarthr Cartil* 11:821–30, 2003
  13. Brady AM, Floresco SB: Operant Procedures for Assessing Behavioral Flexibility in Rats. *J Vis Exp* 1–13, 2015
  14. Buckley MG, Bast T: A new human delayed-matching-to-place test in a virtual environment reverse-translated from the rodent watermaze paradigm: Characterization of performance measures and sex differences. *Hippocampus* 28:796–812, 2018
  15. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 53:55–63, 1994
  16. Cohen SJ, Stackman Jr. RW: Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav Brain Res* 285:105–17, 2015
  17. Collaborators D and II and P: Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388:1545–602, 2016
  18. Combe R, Bramwell S, Field MJ: The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? *Neurosci Lett* 370:236–40, 2004
  19. Cowen SL, Phelps CE, Navratilova E, McKinzie DL, Okun A, Husain O, Gleason SD, Witkin JM, Porreca F: Chronic pain impairs cognitive flexibility and engages novel learning strategies in rats. *Pain* 159:1403–12, 2018
  20. da Silva BM, Bast T, Morris RGM: Spatial memory: behavioral determinants of persistence in the watermaze delayed matching-to-place task. *Learn Mem* 21:28–36, 2014
  21. Deary IJ, Corley J, Gow AJ, Harris SE, Houlihan LM, Marioni RE, Penke L, Rafnsson SB, Starr JM: Age-associated cognitive decline. *Br Med Bull* 92:135–52, 2009
  22. del Rey A, Yau H-J, Randolph A, Centeno M V., Wildmann J, Martina M, Besedovsky HO, Apkarian

- VA: Chronic neuropathic pain-like behavior correlates with IL-1 $\beta$  expression and disrupts cytokine interactions in the hippocampus. *Pain* 152:2827–35, 2011
23. Devonshire IM, Burston JJ, Xu L, Lillywhite A, Prior MJ, Watson DJG, Greenspon CM, Iwabuchi SJ, Auer DP, Chapman V: Manganese-enhanced magnetic resonance imaging depicts brain activity in models of acute and chronic pain: A new window to study experimental spontaneous pain? *Neuroimage* 157:500–10, 2017
  24. Ennaceur A, Delacour J: A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 31:47–59, 1988
  25. Gonçalves S, Gowler PRW, Woodhams SG, Turnbull J, Hathway G, Chapman V: The challenges of treating osteoarthritis pain and opportunities for novel peripherally directed therapeutic strategies. *Neuropharmacology* 213:109075, 2022
  26. Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P: Mono-iodoacetate-induced experimental osteoarthritis. A dose-response study of loss of mobility, morphology, and biochemistry. *Arthritis Rheum* 40:1670–9, 1997
  27. Hannan MT, Felson DT, Pincus T: Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. *J Rheumatol* 27:1513–7, 2000
  28. Higgins GA, Grzelak ME, Pond AJ, Cohen-Williams ME, Hodgson RA, Varty GB: The effect of caffeine to increase reaction time in the rat during a test of attention is mediated through antagonism of adenosine A2A receptors. *Behav Brain Res* 185:32–42, 2007
  29. Hunter DJ, Bierma-Zeinstra S: Osteoarthritis. *Lancet* 393:1745–59, 2019
  30. Indart S, Hugon J, Guillausseau PJ, Gilbert A, Dumurgier J, Paquet C, Sène D: Impact of pain on cognitive functions in primary Sjögren syndrome with small fiber neuropathy. *Medicine* 96:e6384, 2017
  31. Iwabuchi SJ, Xing Y, Cottam WJ, Drabek MM, Tadjibaev A, Fernandes GS, Petersen KK, Arendt-Nielsen L, Graven-Nielsen T, Valdes AM, Zhang W, Doherty M, Walsh D, Auer DP: Brain perfusion patterns are altered in chronic knee pain: a spatial covariance analysis of arterial spin labelling MRI. *Pain* 161:1255–63, 2020
  32. James RJE, Ferguson E: The dynamic relationship between pain, depression and cognitive function in a sample of newly diagnosed arthritic adults: a cross-lagged panel model. *Psychol Med* 50:1663–71, 2020

33. Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA: Induction of osteoarthritis in the rat by surgical tear of the meniscus: Inhibition of joint damage by a matrix metalloproteinase inhibitor. *Osteoarthr Cartil* 10:785–91, 2002
34. Karp JF, Reynolds CF, Butters MA, Dew MA, Mazumdar S, Begley AE, Lenze E, Weiner DK: The Relationship Between Pain and Mental Flexibility in Older Adult Pain Clinic Patients. *Pain Med* 7:444–52, 2006
35. Kouraki A, Bast T, Ferguson E, Valdes AM: The association of socio-economic and psychological factors with limitations in day-to-day activity over 7 years in newly diagnosed osteoarthritis patients. *Sci Rep Nature* 12:943, 2022
36. Kraus VB, Huebner JL, DeGroot J, Bendele A: The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the guinea pig. *Osteoarthr Cartil* 18:S35–52, 2010
37. Kumar G, Talpos J, Steckler T: Strain-dependent effects on acquisition and reversal of visual and spatial tasks in a rat touchscreen battery of cognition. *Physiol Behav* 144:26–36, 2015
38. Lee DM, Pendleton N, Tajar A, O’Neill TW, O’Connor DB, Bartfai G, Boonen S, Casanueva FF, Finn JD, Forti G, Giwercman A, Han TS, Huhtaniemi IT, Kula K, Lean MEJ, Punab M, Silman AJ, Vanderschueren D, Moseley CM, Wu FCW, McBeth J: Chronic widespread pain is associated with slower cognitive processing speed in middle-aged and older European men. *Pain* 151:30–6, 2010
39. Leite-Almeida H, Almeida-Torres L, Mesquita AR, Pertovaara A, Sousa N, Cerqueira JJ, Almeida A: The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats. *Pain* 144:57–65, 2009
40. Lillywhite A, Woodhams SG, Gonçalves S V., Watson DJG, Li L, Burston JJ, Gowler PRW, Canals M, Walsh DA, Hathway GJ, Chapman V: Anxiety enhances pain in a model of osteoarthritis and is associated with altered endogenous opioid function and reduced opioid analgesia. *PAIN Reports* 6:e956, 2021
41. Low LA, Millicamps M, Seminowicz DA, Naso L, Thompson SJ, Stone LS, Bushnell MC: Nerve injury causes long-term attentional deficits in rats. *Neurosci Lett* 529:103–7, 2012
42. Mapp PI, Avery PS, McWilliams DF, Bowyer J, Day C, Moores S, Webster R, Walsh DA: Angiogenesis in two animal models of osteoarthritis. *Osteoarthr Cartil* 16:61–9, 2008
43. Mapp PI, Sagar DR, Ashraf S, Burston JJ, Suri S, Chapman V, Walsh DA: Differences in structural



- and pain phenotypes in the sodium monoiodoacetate and meniscal transection models of osteoarthritis. *Osteoarthr Cartil* 21:1336–45, 2013
44. McGarrity S, Mason R, Fone KC, Pezze M, Bast T: Hippocampal Neural Disinhibition Causes Attentional and Memory Deficits. *Cereb Cortex* 27:4447–62, 2017
  45. Merksey H, Bogduk N: Classification of chronic pain. 2nd ed. Merksey H, Bogduk N, editors. IASP Press. Seattle: IASP Press; 1994
  46. Millecamps M, Etienne M, Jourdan D, Eschaliere A, Ardid D: Decrease in non-selective, non-sustained attention induced by a chronic visceral inflammatory state as a new pain evaluation in rats. *Pain* 109:214–24, 2004
  47. Mills C, LeBlond D, Joshi S, Zhu C, Hsieh G, Jacobson P, Meyer M, Decker M: Estimating Efficacy and Drug ED50's Using von Frey Thresholds: Impact of Weber's Law and Log Transformation. *J Pain* 13:519–23, 2012
  48. Moriarty O, Gorman CL, McGowan F, Ford GK, Roche M, Thompson K, Dockery P, McGuire BE, Finn DP: Impaired recognition memory and cognitive flexibility in the rat L5–L6 spinal nerve ligation model of neuropathic pain. *Scand J Pain* 10:61–73, 2016
  49. Moriarty O, McGuire BE, Finn DP: The effect of pain on cognitive function: A review of clinical and preclinical research. *Prog Neurobiol* 93:385–404, 2011
  50. Mutso AA, Radzicki D, Baliki MN, Huang L, Banisadr G, Centeno M V., Radulovic J, Martina M, Miller RJ, Apkarian A V.: Abnormalities in Hippocampal Functioning with Persistent Pain. *J Neurosci* 32:5747–56, 2012
  51. Nagase H, Kumakura S, Shimada K: Establishment of a novel objective and quantitative method to assess pain-related behavior in monosodium iodoacetate-induced osteoarthritis in rat knee. *J Pharmacol Toxicol Methods* 65:29–36, 2012
  52. Negrete R, García Gutiérrez MS, Manzanares J, Maldonado R: Involvement of the dynorphin/KOR system on the nociceptive, emotional and cognitive manifestations of joint pain in mice. *Neuropharmacology* 116:315–27, 2017
  53. Neumann K, Grittner U, Piper SK, Rex A, Florez-Vargas O, Karystianis G, Schneider A, Wellwood I, Siegerink B, Ioannidis JPA, Kimmelman J, Dirnagl U: Increasing efficiency of preclinical research by group sequential designs. *PLOS Biol* 15:e2001307, 2017
  54. Okun A, Liu P, Davis P, Ren J, Remeniuk B, Brion T, Ossipov MH, Xie J, Dussor GO, King T:

- Afferent Drive Elicits Ongoing Pain in a Model of Advanced Osteoarthritis. *Pain* 153:924–33, 2012.
55. Pais-Vieira M, Lima D, Galhardo V: Sustained attention deficits in rats with chronic inflammatory pain. *Neurosci Lett* 463:98–102, 2009
  56. Pais-Vieira M, Mendes-Pinto MM, Lima D, Galhardo V: Cognitive impairment of prefrontal-dependent decision-making in rats after the onset of chronic pain. *Neuroscience* 161:671–9, 2009
  57. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Boutron I, editor. *PLOS Biol* 18:e3000410, 2020
  58. Pezze M, Bast T: Dopaminergic modulation of hippocampus-dependent learning: Blockade of hippocampal D1-class receptors during learning impairs 1-trial place memory at a 30-min retention delay. *Neuropharmacology* 63:710–8, 2012
  59. Pezze M, McGarrity S, Mason R, Fone KC, Bast T: Too Little and Too Much: Hypoactivation and Disinhibition of Medial Prefrontal Cortex Cause Attentional Deficits. *J Neurosci* 34:7931–46, 2014
  60. Pezze MA, Marshall HJ, Fone KCF, Cassaday HJ: Dopamine D1 receptor stimulation modulates the formation and retrieval of novel object recognition memory: Role of the prelimbic cortex. *Eur Neuropsychopharmacol* 25:2145–56, 2015
  61. Phelps CE, Lumb BM, Donaldson LF, Robinson ES: The partial saphenous nerve injury model of pain impairs reward-related learning but not reward sensitivity or motivation. *Pain* 162:956–66, 2021
  62. Phelps CE, Navratilova E, Porreca F: Cognition in the Chronic Pain Experience: Preclinical Insights. *Trends Cogn Sci* 1–12, 2021
  63. Previtali D, Capone G, Marchettini P, Candrian C, Zaffagnini S, Filardo G: High Prevalence of Pain Sensitization in Knee Osteoarthritis: A Meta-Analysis with Meta-Regression. *Cartilage* 13:194760352210876, 2022
  64. Pritzker KPH, Gay S, Jimenez SA, Ostergaard K, Pelletier J-P, Revell PA, Salter D, van den Berg WB: Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthr Cartil* 14:13–29,

- 2006
65. Prusky GT, Harker KT, Douglas RM, Whishaw IQ: Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behav Brain Res* 136:339–48, 2002
  66. Ren W-J, Liu Y, Zhou L-J, Li W, Zhong Y, Pang R-P, Xin W-J, Wei X-H, Wang J, Zhu H-Q, Wu C-Y, Qin Z-H, Liu G, Liu X-G: Peripheral Nerve Injury Leads to Working Memory Deficits and Dysfunction of the Hippocampus by Upregulation of TNF- $\alpha$  in Rodents. *Neuropsychopharmacology* 36:979–92, 2011
  67. Richards GC, Lluca LJ, Smith MT, Haslam C, Moore B, O’Callaghan J, Strong J: Effects of long-term opioid analgesics on cognitive performance and plasma cytokine concentrations in patients with chronic low back pain. *PAIN Reports* 3:e669, 2018
  68. Sagar DR, Burston JJ, Hathway GJ, Woodhams SG, Pearson RG, Bennett AJ, Kendall DA, Scammell BE, Chapman V: The Contribution of Spinal Glial Cells to Chronic Pain Behaviour in the Monosodium Iodoacetate Model of Osteoarthritic Pain. *Mol Pain* 7:1744-8069-7–88, 2011
  69. Schiltenswolf M, Akbar M, Neubauer E, Gantz S, Flor H, Hug A, Wang H: The cognitive impact of chronic low back pain: Positive effect of multidisciplinary pain therapy. *Scand J Pain* 17:273–8, 2017
  70. Schmitz N, Lavery S, Kraus VB, Aigner T: Basic methods in histopathology of joint tissues. *Osteoarthr Cartil* 18:S113–6, 2010
  71. Skou ST, Thomsen H, Simonsen OH: The value of routine radiography in patients with knee osteoarthritis consulting primary health care: a study of agreement. *Eur J Gen Pract* 20:10–6, 2014
  72. Steele RJ, Morris RGM: Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. *Hippocampus* 9:118–36, 1999
  73. Tait DS, Bowman EM, Neuwirth LS, Brown VJ: Assessment of intradimensional/extradimensional attentional set-shifting in rats. *Neurosci Biobehav Rev* 89:72–84, 2018
  74. Verdejo-Garcia A, Lopez-Torrecillas F, Calandre EP, Delgado-Rodriguez A, Bechara A: Executive Function and Decision-Making in Women with Fibromyalgia. *Arch Clin Neuropsychol* 24:113–22, 2009
  75. Weiner DK, Rudy TE, Morrow L, Slaboda J, Lieber S: The Relationship Between Pain,

- Neuropsychological Performance, and Physical Function in Community-Dwelling Older Adults with Chronic Low Back Pain. *Pain Med* 7:60–70, 2006
76. Winters BD, Saksida LM, Bussey TJ: Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci Biobehav Rev* 32:1055–70, 2008
77. Yau JLW, Morris RGM, Seckl JR: Hippocampal corticosteroid receptor mRNA expression and spatial learning in the aged Wistar rat. *Brain Res* 657:59–64, 1994
78. You Z, Zhang S, Shen S, Yang J, Ding W, Yang L, Lim G, Doheny JT, Tate S, Chen L, Mao J: Cognitive impairment in a rat model of neuropathic pain. *Pain* 159:1518–28, 2018

#### HIGHLIGHTS

- MIA model of osteoarthritis-like knee pain in Lister hooded rats
- Marked weight-bearing asymmetry and reduced rearing activity
- Marked knee pathology (cartilage damage, synovitis, occasional osteophytes)
- No memory impairment (watermaze delayed-matching-to-place test, object recognition)
- No impairment in behavioural flexibility (operant rule shifts and reversal learning)