



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 (3 mg/50 μ L) 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. The watermaze delayed-matching-to-place test of rapid place learning, novel object recognition memory assay, and an operant response-shift and -reversal task were used to measure memory and behavioral flexibility. Open-field locomotor activity, startle response, and prepulse inhibition 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 paw withdrawal threshold. Rearing was reduced, but otherwise, locomotor activity was normal and no changes in startle and prepulse inhibition were detected. MIA-injected rats had intact watermaze delayed-matching-to-place performance, suggesting no substantial change in hippocampal function, and there were no changes in novel object recognition memory or performance on the operant task of behavioral 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 OA-like knee pain showed no impairments in hippocampal memory function and behavioral flexibility, suggesting that OA pain impacts cognitive functions less than other chronic pain conditions. In patients, OA pain may interact with other factors (e.g., age, socio-economic factors, and medication) to impair cognition.

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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.¹⁻³ It has been suggested that chronic pain reduces cognitive resources by diverting them to pain-related stimuli or by impairing the structure and function of key brain regions, including the hippocampus and prefrontal cortex (PFC).^{3,4} Although additional factors, including pain medication and co-morbidities, are also likely to contribute to cognitive impairment associated with chronic pain in patients,^{2,3} experimental studies have shown impairments in cognitive functions associated with hippocampus and PFC in rodent models of neuro-pathic⁴⁻⁶ and inflammatory pain.⁷⁻⁹

Osteoarthritis (OA) is estimated to affect 250 million people worldwide, with knee OA having the highest prevalence.¹⁰ Recent longitudinal studies show that OA pain is associated with cognitive impairments, reflected in measures of verbal recall and fluency, subtraction ability, and attention.^{11,12} Moreover, gray matter changes¹³ and functional reorganization^{14,15} 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.⁴ Experimental models of OA have been widely used to study the mechanisms of OA pain behavior (see refs in Ref 16). Although 1 study reported impaired novel object recognition (NOR) memory in the murine mono-iodoacetate (MIA) model of OA pain,¹⁷ 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.¹⁸⁻²⁰ 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,²¹⁻²³ and study 2 used the NOR memory test, which depends on medial temporal lobe structures, including the hippocampus and perirhinal cortex,^{24,25} and an operant task of behavioral flexibility, which depends on PFC function.²⁶ Cognitive testing was complemented by measurements of pain behavior (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 2 studies, using 2 separate cohorts of young adult male Lister hooded rats (250–275 g, 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 behavioral flexibility) used 32 rats (Fig 1). We only included male rats because the parameters of the MIA model and of the behavioral 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 recognize 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 × 403 × 404 mm; Tecniplast, UK), in temperature- and humidity-controlled conditions (21 ± 1.5 °C, 50 ± 8%) and with an alternating 12/12 hours light/dark cycle (lights on at 7 AM). 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 acclimatization (3–7 days), during which they were habituated to handling by the experimenter for a few days, before any experimental procedures started. All behavioral testing was carried out during the light phase. Studies had the approval of the University of Nottingham's Animal Welfare and Ethical Review Board 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 (Animal Research: Reporting of In Vivo Experiments) guidelines.²⁷

Induction of the MIA Model of OA Pain

Rats received a single intra-articular injection of either 3 mg/50 µL MIA (Sigma, UK) or 50 µL .9% saline (control group) through the infra-patellar ligament of the left knee, using a 30-gauge 8-mm hypodermic needle, under brief isoflurane anesthesia (isoflurane 2.5–3% in 1 L/min O₂).²⁸ Health and welfare checks were performed immediately after anesthetic recovery, daily for 5 days, and weekly thereafter. The MIA dose was selected based on previous studies conducted in albino rats (see refs in Ref 16) 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 µL MIA did not show robust pain behavior and had only weak OA-like knee pathology, whereas 3 mg/50 µL of MIA produced marked pain behavior and knee pathology (pilot data not shown). Initially, rats were allocated randomly to the treatment groups using an online randomizer, blocked by a cage (half of the rats in each cage received MIA treatment and the other half saline). Following baseline behavior 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 Behavior and Sensorimotor Testing

In studies 1 and 2, baseline pain and sensorimotor behaviors were assessed prior to model induction and then at several subsequent time points (Fig 1). Pain behavior was assessed using 2 behavioral tests: joint pain at rest was assessed by weight-bearing asymmetry using an incapitance tester (Linton Instrumentation, Diss, UK)²⁹; 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.^{30,31} 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).³² 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 2 days of habituation, rats were placed in the chambers for approximately 30 minutes and, on the third day of habituation, rats were first placed into the chambers for 30 minutes, after which the 6-g filament was applied to the plantar surface of the rats' hind paws to familiarize 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 the hippocampus and PFC.³³ 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 (39.5 cm × 23.5 cm × 24.5 cm) with two 4 × 8 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, USA), as previously described.³⁴ Two consecutive breaks of adjacent bottom photobeams generated a horizontal locomotor count, and breaks of photo beams of the top configuration generated rearing counts. Open field sessions lasted 30 minutes. 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, USA), as described previously.³⁴ Test sessions lasted a total of 23 minutes, throughout which rats were exposed to a 62-dB (A) background noise. After 5-minute 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 (10–20 seconds): pulse-alone trials and 4 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)] × 100.

Measurements of Learning and Memory and Behavioral Flexibility

Detailed descriptions of the apparatus and procedures can be found in the [Supplementary Material](#).

Watermaze DMP Task of Rapid Place Learning

The watermaze DMP task is highly sensitive to hippocampal dysfunction,^{21,23,35,36} requiring rats to learn within 1 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 4 daily trials. During trial 1, rats rapidly learn the novel location of the hidden platform, and then in 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 (Fig 1A). Methods were adapted from our previous studies.³⁵ 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. The percentage of time searching the correct zone was calculated as: (time in correct zone [s]/total time in all 8 zones [s]) × 100%. By chance, that is, 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 a steep reduction from trial 1 to trial 2 indicating the acquisition of 1-trial place memory. Swim speed was also measured during the 60-second probe trials, to examine potential motor impairments in the MIA-injected rats. For analysis, the search preference and swim speed was averaged across the 2 probes at each testing time point (on day 6 and 8 for baseline testing and the 2 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 4 days at each testing time point (days 4–8 of pretraining for baseline values and all 4 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 the use of training or reinforcement, to explore novel stimuli, including novel objects, more than familiar stimuli.^{37,38} During the test, rats are presented with a novel object and a familiar object, that is, 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,²⁵ and the hippocampus, particularly if the retention delay between the sample phase and the test is greater than 10 minutes.²⁴ In study 2, NOR memory was assessed before MIA model induction, and on days 29 to 31 after model induction (Fig 1B), using a procedure with a 24 hours retention delay between the sample phase and test, which was adapted from the previous work.³⁸ Time exploring each object (direct contact with or active exploration of the object by directing the nose toward the object from a distance of less than 1 cm) was measured in both the sample phase (2 identical objects) and the test phase (1 familiar and 1 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 Behavioral Flexibility

Behavioral flexibility is the ability to switch stimulus-response patterns rapidly, as reflected by response shifts or reversals,³⁹ and can be assessed in rats using a food-reinforced operant test.²⁶ Shifts refer to a subject beginning to make responses to new or previously irrelevant stimuli (eg, 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 response from a previously rewarded to a previously non-rewarded stimulus within the same category (eg, from left to right lever). Previous studies suggest that behavioral flexibility, as measured on the operant task, depends on the PFC and subcortical regions, including the ventral striatum.²⁶ In study 2, following MIA model induction (from days 38–59), rats were trained first to acquire a lever press response to a cue light in order to receive a 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) (Fig 1B). Operant testing procedures were adapted from previous studies.²⁶ The analysis of this test focused on trials to criterion, the percentage of correct responses, and the percentage of omissions.

Assessment of OA-Like Knee Pathology

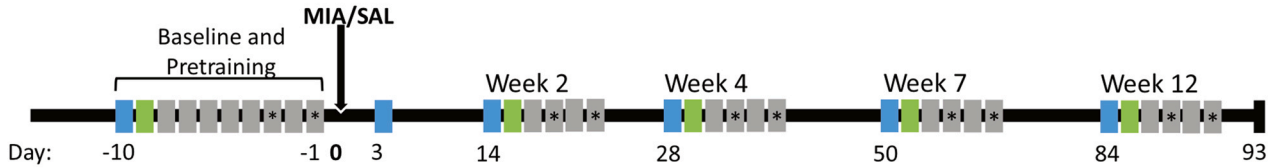
At the end of study 1 (day 93 after model induction) and 2 (day 70 after model induction), rats were anesthetized with an overdose of sodium pentobarbitone (Dolethal, Vetoquinol; 2 mL intraperitoneal), and then transcardially perfused with .9% saline followed by 4% paraformaldehyde in .9% saline. Both knees were collected, decalcified, stained, and histologically scored to confirm the development of OA-like pathology via established methods.^{40–42} Briefly, knee joints were stored in the fixative for 72 hours after collection, transferred to an ethylenediaminetetraacetic acid + 7.5% polyvinylpyrrolidone solution for decalcification (6–7 weeks), and then split in the frontal plane.⁴³ Trimmed joints were mounted in paraffin wax and 5 μ m sections were cut using a microtome. Haematoxylin and eosin⁴⁴ and Safranin-O-Fast Green⁴⁴ histological stains were used to confirm OA-like damage to the knee joints. Cartilage damage was scored on a scale of 1 to 5 for severity with a multiplier of 1 to 3 for area coverage, to give a composite score out of 15. Osteophytes were scored 1 to 3 based on size (1 = mild < 40 μ m; 2 = moderate, 40–160 μ m, 3 = severe, > 160 μ m).^{40–42}

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, $P < .05$). In study 1, we used a sequential design.⁴⁵ 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 the completion of the first series.⁴⁵ Moreover, 1 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 the completion of behavioral 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 (GraphPad, Boston, MA, US) and IBM SPSS Statistics 24 (IBM, Portsmouth, UK) 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 analyzed, using analysis of variance (ANOVA) with a group (MIA or saline) as a 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

A) Study 1:



B) Study 2:

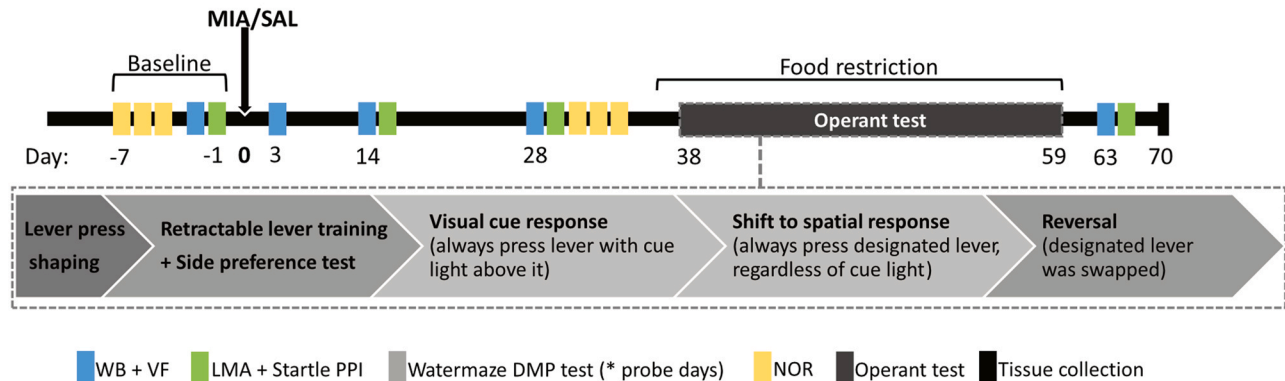


Figure 1. Overview of study 1 and study 2. (A) Timeline for study 1—watermaze DMP test of rapid place learning: Before MIA model induction, baseline pain behavior and sensorimotor measures were collected and pretraining and baseline measurements on the watermaze DMP task were completed. After model induction (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 behavior (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 euthanized on day 93, and knees were collected for post-mortem analysis. (B) Timeline for study 2—NOR memory test and operant test of behavioral flexibility: Before MIA model induction, baseline measures of NOR, pain behavior, and sensorimotor processes were collected. After model induction (day 0), NOR memory was re-assessed on days 29 to 31 after model induction. After this, rats were put on food restriction from days 35 to 59 and rats underwent testing on the food-reinforced operant task of behavioral flexibility from days 38 to 59. Pain behavior and sensorimotor processes were measured at several time points following model induction, before and after NOR, and operant testing. Rats were euthanized on day 70, and knees were collected for post-mortem analysis. Abbreviations: SAL, saline; WB, weight-bearing; VF, Von-Frey; LMA, locomotor activity.

analyzed separately, using independent t-tests or 2-way ANOVA (with the prospective group as a between-subjects factor and task block, prepulse intensity or trial as a 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 an independent t-test or the Mann-Whitney U test, if the normality assumption was unmet. $P < .05$ was accepted as the significance threshold for all analyses.

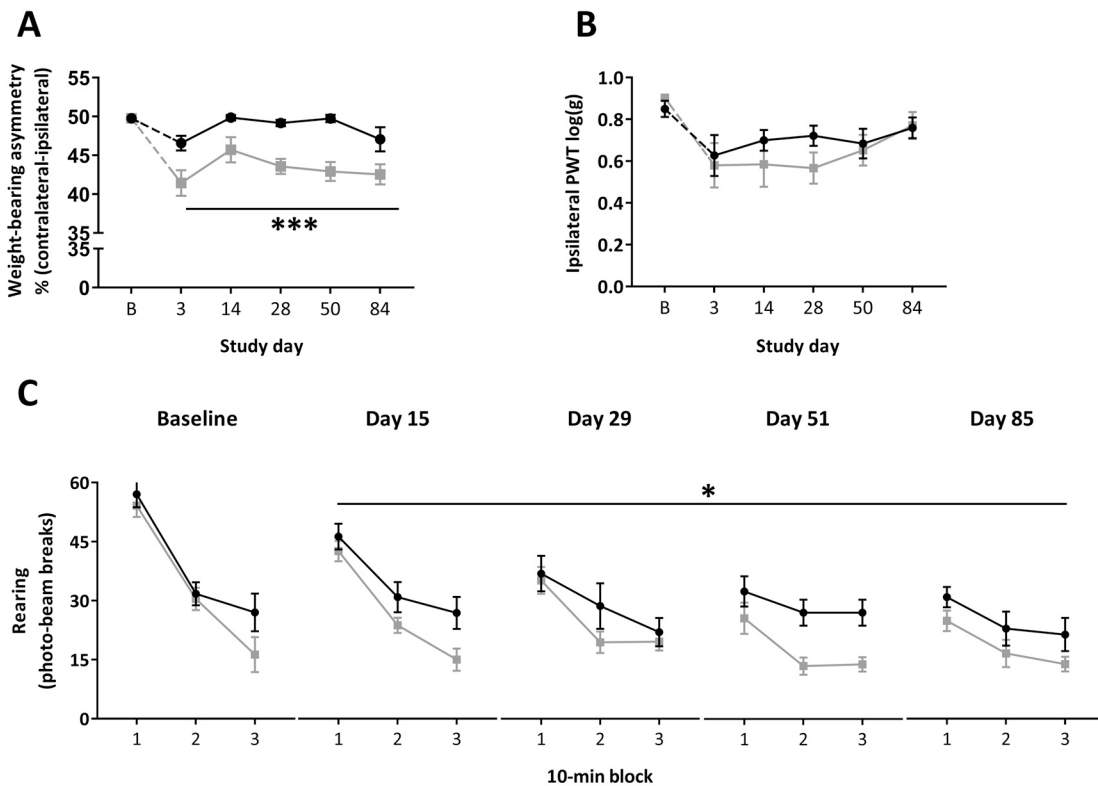
Results

Pain Behavior and Sensorimotor Measurements: MIA Knee Injections Induced Weight-Bearing Asymmetry and Decreased Rearing Activity

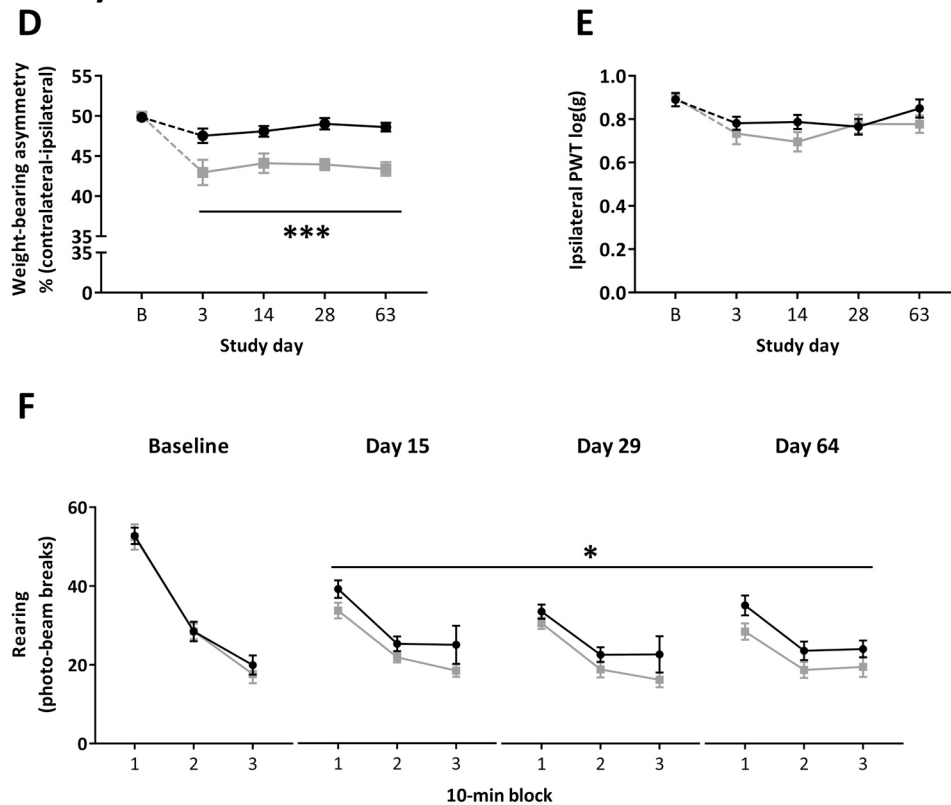
MIA-treated rats placed markedly less weight on the injured leg compared with saline-injected rats across all testing time points in study 1, resulting in significant weight-bearing asymmetry (group: $F_{(1,13)} = 66.22$, $P < .0001$; time: $F_{(4,52)} = 3.32$, $P < .0001$; time \times group: $F_{(4,52)} < 1$) (Fig 2A). However, there were no differences

in PWT between MIA-, and saline-injected rats across all testing time points in study 1 (group: $F_{(1,13)} = 1.0$, $P = .33$; time: $F_{(4,52)} = 1.66$, $P = .18$; time \times group: $F_{(4,52)} < 1$) (Fig 2B). MIA-treated rats showed significantly less rearing than saline controls in study 1 (main effect of group: $F_{(1,13)} = 4.86$, $P = .046$; all interactions involving group: $F < 1.9$, $P > .18$) (Fig 2C). Similarly, in study 2 MIA-treated rats showed weight-bearing asymmetry (group: $F_{(1,30)} = 33.89$, $P < .0001$; time: $F_{(3,90)} = .71$, $P = .55$; group \times time: $F_{(3,90)} = .21$, $P = .89$) (Fig 2D) and no differences in PWT compared with saline-injected rats (group: $F_{(1,30)} = 1.68$, $P = .20$; time: $F_{(2,9,87.4)} = 1.62$, $P = .19$; time \times group: $F_{(3,90)} < 1$) (Fig 2E). Also, reduced rearing activity was observed in MIA-treated rats in study 2 (main effect of group: $F_{(1,30)} = 5.08$, $P = .03$; all interactions involving group: $F < 1$) (Fig 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: $F_{(1,30)} = 3.65$, $P = .07$, all interactions involving group: $F < 1.05$, $P > .3$) (Supplementary Fig 1). MIA-treated rats did not exhibit altered startle reactivity or PPI (all main effects or interactions involving group: $F < 2.14$, $P > .15$, except for a trend toward a main effect of group on startle

Study 1: ● Saline, n=8 ■ MIA, n=7



Study 2: ● Saline, n=16 ■ MIA, n=16



(caption on next page)

Figure 2. MIA-induced knee pain in Lister hooded rats: weight-bearing asymmetry and reduced rearing, but no significant change in paw withdrawal threshold (PWT) (study 1 and 2). Rats were injected with either 3 mg/50 μ L MIA (■, gray squares) or 50 μ L saline (●, 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-minute open field test sessions and across all testing time points after model induction (study 1, **C**; study 2, **F**). Data are presented as mean \pm SEM. * and *** indicate significant ANOVA main effect of the group across all testing time points after model induction (2-way ANOVA, $P < .05$ and $P < .0001$, respectively). Abbreviation: B, baseline.

response in study 2, $F_{(1,30)} = 2.91$; $P = .10$, reflecting a numerical decrease in the startle response in rats injected with MIA, which was already present at baseline, that is, before knee injections, and persisted across all testing time points) (Supplementary Figs 2 and 3).

MIA-Induced OA-Like Knee Pain Pathology

MIA-treated rats showed marked OA-like knee pathology (Supplementary Fig 4). In both studies 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 1 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— $t_{(13)} = 4.63$, $P = .0005$; study 2— $U = 24$, $P < .0001$), as well as increased synovial inflammation (study 1— $U = 0$, $P = .0003$; study 2— $U = 48$, $P = .0001$) compared to saline-injected rats. There was a trend for MIA-injected rats to show a higher number of osteophytes in study 1 ($U = 14$, $P = .08$) and a significant increase in study 2 ($t_{(29)} = 2.51$, $P = .02$). 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 behavioral measures (weight-bearing asymmetry/reduced PWT/reduced rearing). There was no evidence for any correlation for any measure ($R < .69$, $P > .12$). Although there was a trend toward a positive relationship between cartilage integrity and weight-bearing asymmetry in study 1 ($R = .69$, $P = .07$), there was a trend toward a negative relationship in study 2 ($R = -.51$, $P = .06$). So, overall, there was no clear relationship between knee pathology and pain-related behavioral measures.

Intact Hippocampus-Dependent Rapid Place Learning Performance After MIA Model Induction

At baseline testing, both prospective groups showed similar marked path length reductions from trial 1 (encoding) to trial 2 (retrieval) (main effect of trial: $F_{(3,90)} = 12.5$; $P < .0001$; interaction prospective group \times trial: $F_{(3,90)} < 1$), as well as similar ($t_{(30)} < 1$) and markedly above-chance ($t_{(15)} > 3.68$, $P < .002$) search preference for the correct zone when trial 2 was run as a probe (Fig 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 trials 1 to 2 (trial: $F_{(3,84)} = 80.19$; $P < .0001$) (Fig 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: $F < 1.8$; $P > .20$), and search preference was highly above chance in both groups at all testing time points (saline: $t_{15} > 2.69$, $P < .018$; MIA: $t_{15} > 3.17$, $P < .006$) (Fig 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 ($F_{(3,84)} = 8.87$; $P < .0001$), reflecting that MIA rats had slower swim speed than control rats at the last 2 testing time points (Fig 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: $F_{(1,30)} > 27$, $P < .0001$; no group \times object interaction: $F < 1$) (Fig 4A). The discrimination ratio was not altered in the MIA-treated group (group: $F_{(1,30)} = 2.27$, $P = .14$), and was significantly different from chance in both groups at baseline and after model induction ($t > 2.41$, $P < .03$) (Fig 4B).

Intact Performance on the Operant Task of Behavioral 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 ($t_{(30)} < 1$; Fig 5A), % of correct responses ($t_{(30)} = 1.35$, $P = .2$; Fig 5B) and % of omissions (median, percentiles: saline, .5, 0–1.75; MIA, 0, 0–1; $U = 109.5$, $P = .46$, 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 Fig 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 the criterion on the first day

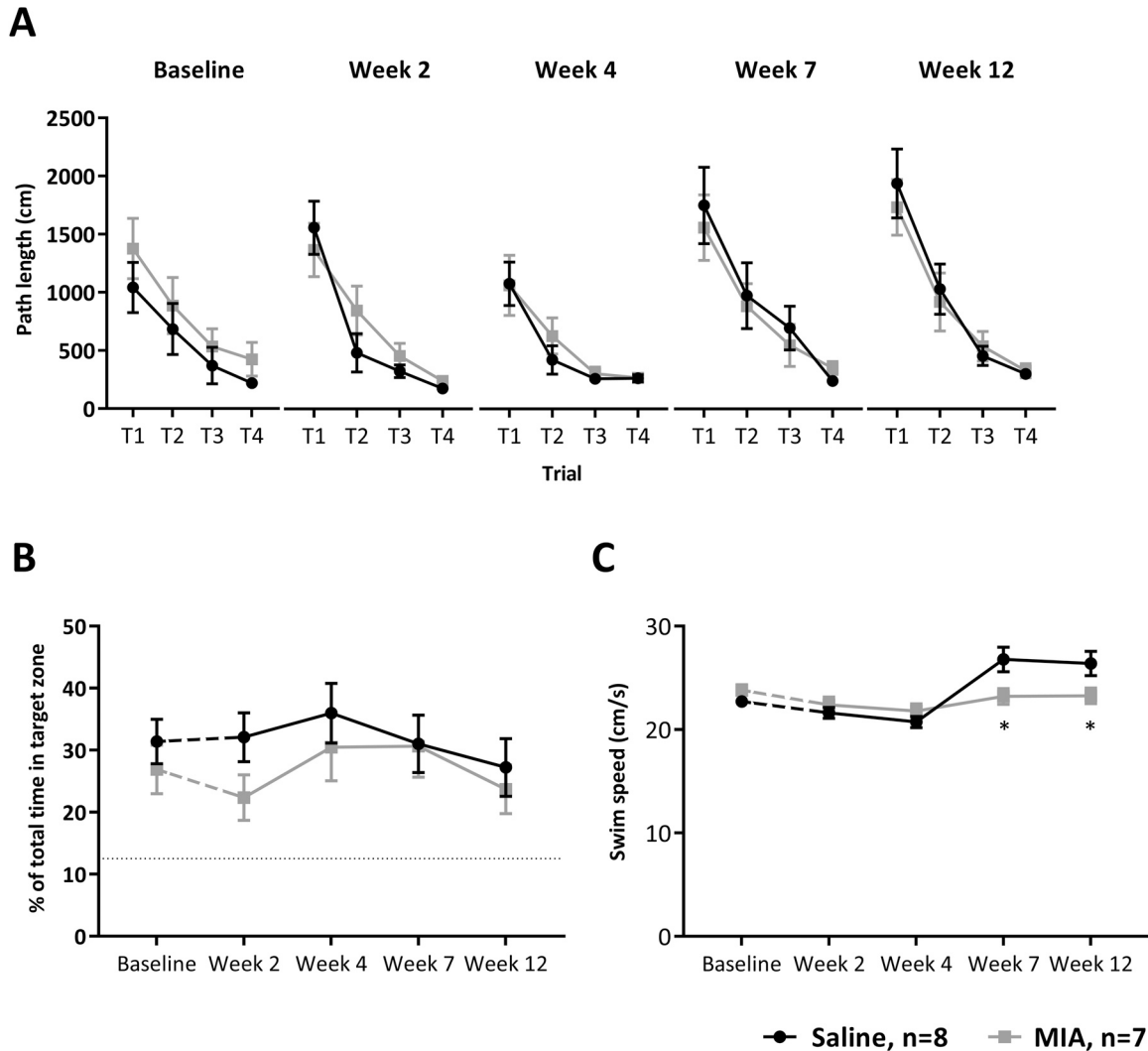


Figure 3. Intact rapid place learning performance on the watermaze Delayed-Matching-to-Place (DMP) task following MIA model induction. Rats were injected with either 50 μ L of 3 mg of MIA (■, gray squares) or 50 μ L saline (●, black dots) in the left knee. They were tested on the watermaze DMP task at baseline, that is, 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 4 daily trials (T1–T4) to a novel location show marked reduction across trials, including from trials 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 a 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 weeks 2 and 4 following injection, but was increased in saline compared to MIA-injected rats in weeks 7 and 12 following knee injection. Data are presented as mean \pm SEM. * $P < .05$ MIA versus saline, following group \times time point interaction in 2-way ANOVA.

(mean \pm 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: $F_{(3,78)} = 38.73$, $P < .0001$; no main effect or interaction involving group: $F_{(1,26)} < 1$ and $F_{(3,78)} > 1.77$, $P > .2$, respectively; Fig 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 \pm 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: $F_{(3,78)} = 130.5$, $P < .0001$) (Fig 5D). However, for both measures, there was no main effect or interaction involving group ($F_{(3,78)} < 1.67$, $P > .2$). 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 behavior 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 behavioral flexibility, which has been associated with frontostriatal mechanisms.

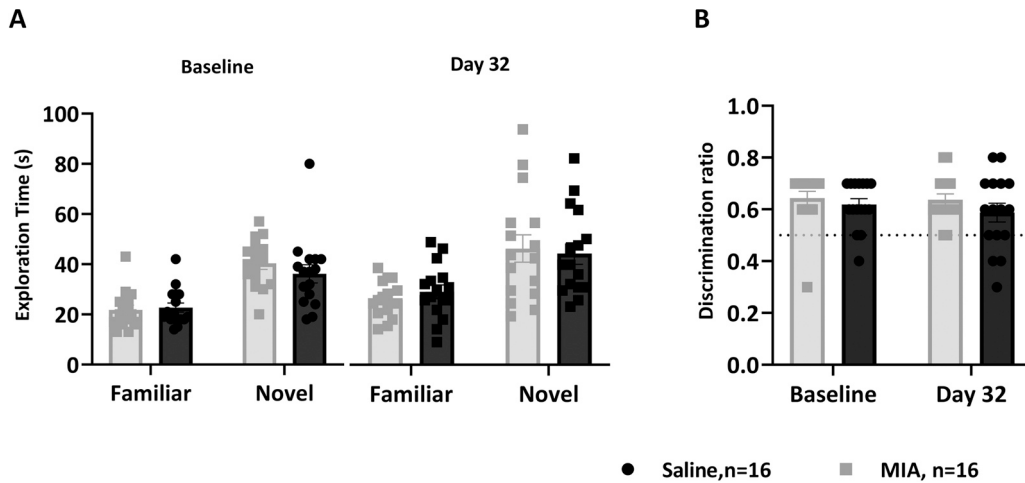


Figure 4. Intact novel object recognition (NOR) memory following MIA model induction. Rats were injected with either 50 μ L of 3 mg of MIA (■, gray squares) or 50 μ L saline (●, black dots) in the left knee. They were tested on the NOR memory task at baseline, that is, before the knee injections, and 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 (.5, stippled line) discrimination ratio. Data are presented as mean \pm SEM.

MIA-Induced Knee Pathology and Pain Phenotype in Young Adult Lister Hooded 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,¹⁸⁻²⁰ probably partly reflecting their diminished vision.⁴⁶ MIA treatment produced consistent pain behavior 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 1 hind leg on horizontal locomotor activity in rats have been somewhat variable, with reductions in horizontal locomotor activity reported in some studies,^{28,47} but not in others.⁴⁸ 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.⁴⁷ This probably reflects that horizontal locomotor activity involves the rats distributing their weight on all 4 legs, whereas rearing involves weight distribution on the 2 hind legs. The slower swim speed in the MIA-treated rats compared with saline controls during watermaze testing at weeks 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.⁴⁹⁻⁵¹ Lowering of PWTs is often considered to indicate the presence of central sensitization mechanisms since it demonstrates enhanced mechanical

sensitivity at a site distal to the injured joint.⁵² Therefore, the absence of PWT reductions in Lister hooded rats in the present study may reflect a relative lack of central sensitization, compared to Sprague Dawley 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 = .8–.9) than those we typically measure in Sprague Dawley rats (around PWT log = 1.25²⁸), and, therefore this measure of pain behavior 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.⁵³ 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 [53]). Overall, the lack of effect of the MIA model on PWTs in Lister hooded rats probably reflects a relative lack of central sensitization, 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

Visual cue response acquisition: ● Saline, n=16 ■ MIA, n=16

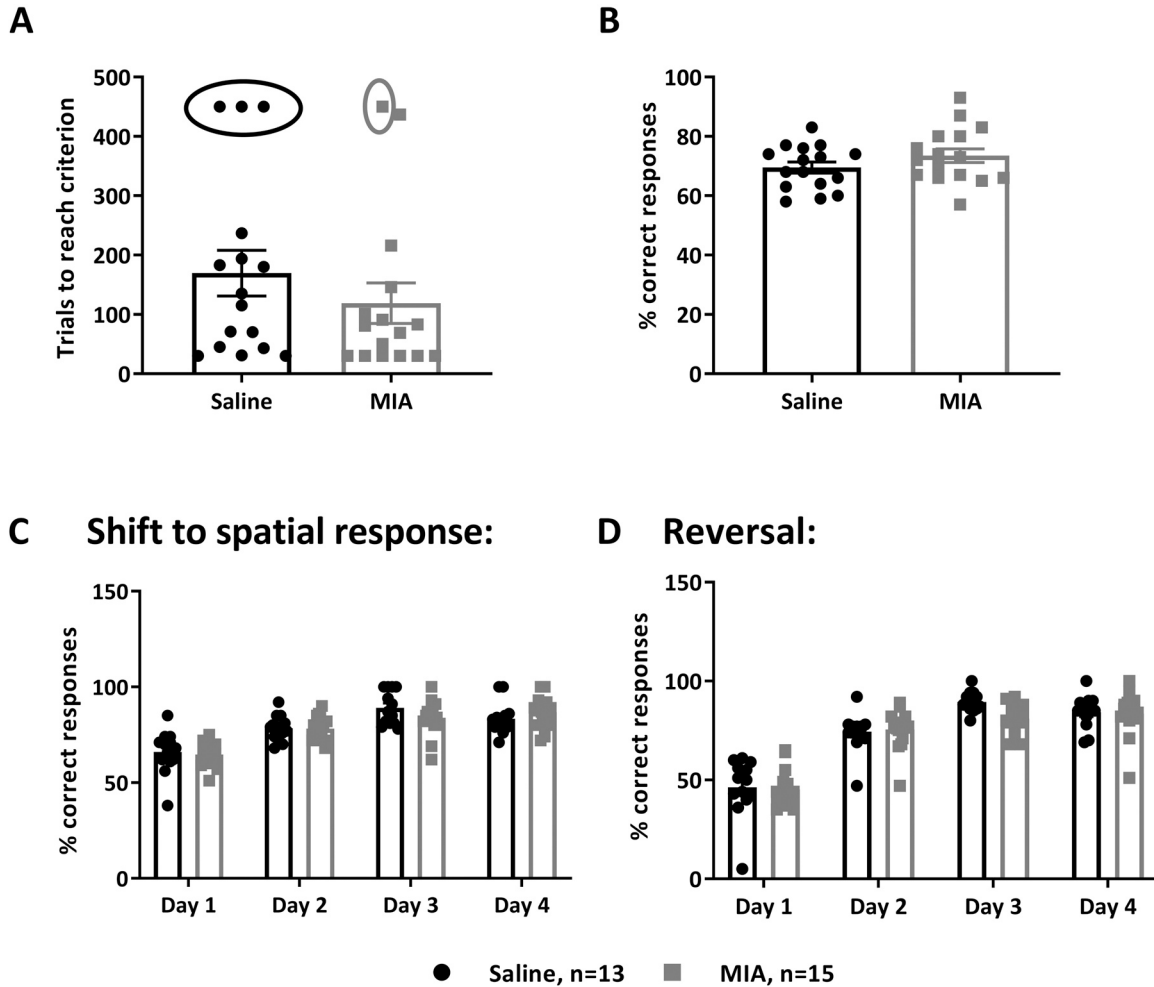


Figure 5. Intact performance on the operant task of behavioral flexibility after MIA model induction. Rats were injected with either 50 μ L of 3 mg of MIA (■, gray squares) or 50 μ L saline (●, black dots) in the left knee. Rats were tested on this task 38 days after model induction. Acquisition of the 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 the 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 \pm SEM.

and Quantitative Sensory Testing, including reduced pain pressure threshold (PPT) at the knee and at “distal” sites on the lower leg^{54,55}; reduced PPT somewhat resembles reduced PWT in rats, although PPT is measured at or close to the injured knee. A recent meta-analysis of studies using such surrogate measures of central sensitization suggested that, overall, 20% of people with knee OA show central sensitization, with substantial variability depending on the measures used. The prevalence of central sensitization based on PPT reduction was 29% across studies, with distal PPT reductions being less pronounced than PPT reduction at the knee.⁵⁵ 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 a lower prevalence than the reported PPT reductions.

The relationship between surrogate measures of central sensitization and cognitive function in people

with knee OA is not clear. To our knowledge, only 2 studies directly examined measurements of cognitive function specifically in people with OA. These studies^{11,12} 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 sensitization. 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).⁵⁴ This supports a contribution of central sensitization to subjective cognitive impairment, but the link between central sensitization 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 (1 mg/50 μ L) and consistent with key features of human OA knee pathology.⁵⁰ 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.⁵⁶ Interestingly, our previous study found that similar knee pathology to the present study was associated with marked PWT reductions in Sprague Dawley rats,⁵⁰ 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 behavioral 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.⁵⁷⁻⁶⁰

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.^{61,62} Impaired NOR memory was also reported in MIA-treated mice,¹⁷ 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,¹⁷ which likely reflects higher pain intensity and stronger involvement of central sensitization 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.⁶²⁻⁶⁴ 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,⁵ 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.⁴ In addition, relatively intact recognition memory in some chronic pain conditions, including chronic back pain and fibromyalgia, has been reported,^{65,66} supporting limited susceptibility of recognition memory to some chronic pain conditions.

No Effect of the MIA Model on the Operant Task of Behavioral Flexibility

In the present study, MIA treatment did not impair operant performance and operant measures of behavioral 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,⁶⁷ and impaired spatial reversal learning in the watermaze.⁵ These measures do appear to have translational relevance as chronic pain patients with fibromyalgia and primary Sjögren syndrome with small fiber neuropathy show impaired behavioral flexibility on the Wisconsin Card Sorting Test.^{68,69} 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.⁷⁰ The absence of impairments in operant performance and behavioral 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 behavior was not associated with cognitive impairment in Lister hooded 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.⁴ There is, however, evidence that chronic pain caused by knee OA causes forebrain changes,¹³⁻¹⁵ 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.^{11,12} 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 behavioral assays that correspond more directly to the ones used in the present study, such as a virtual DMP test^{22,71} and Cambridge Neuropsychological Test Automated Battery (CANTAB) assays of executive functions, including behavioral flexibility.⁷² There are several

factors that may interact with chronic pain to impair cognitive function in patients, and which impact the translational relevance of the experimental models. First, aging is a key contributor to cognitive decline.⁷³ This is relevant as OA is an age-associated disorder,⁷⁴ and the average age of participants in the 2 recent studies showing a negative impact of chronic OA pain on cognition in human participants was >60 years at the start of data collection.^{11,12} Therefore, aging may interact with chronic OA pain to impair cognitive function. In support of this possibility, impairments in behavioral flexibility associated with chronic pain have been reported to be age-dependent in both rodent⁵ and human studies.⁷⁵ Moreover, recent neuroimaging evidence suggests that aging may increase the vulnerability of a brain circuit including the PFC and hippocampus to chronic lower back pain,⁷⁶ 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.¹² Third, prescribed OA pain medication may contribute to cognitive impairments.² In particular, a negative impact of long-term opioid treatment on cognitive task performance has been reported in patients with chronic pain.^{66,77} 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 modeled experimentally in animals.

Author Contributions

SG, GJH, SGW, VC, and TB: Study concept and design. **SG:** Data acquisition. **SG, GJH, VC, and TB:** Data analysis. **SG, VC, and TB:** Manuscript – original. **GJH and SGW:** Manuscript – review and editing.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jpain.2023.04.002](https://doi.org/10.1016/j.jpain.2023.04.002).

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