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# The partial saphenous nerve injury model of pain impairs reward-related learning but not reward sensitivity or motivation

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**Abstract**

Chronic pain is highly comorbid with affective disorders, including major depressive disorder. A core feature of major depressive disorder is a loss of interest in previously rewarding activities. Major depressive disorder is also associated with negative affective biases where cognitive processes are modulated by the affective state. Previous work from our laboratory has shown that reward-related learning and memory is impaired in rodent models of depression generated through a variety of different manipulations. This study investigated different aspects of reward-related behaviour in a rodent model of chronic pain, the partial saphenous nerve injury (PSNI). Using our reward-learning assay, an impairment in reward learning was observed with no difference in sucrose preference, consistent with a lack of effect on reward sensitivity and similar to the effects seen in depression models. In a successive negative contrast task, chronic pain was not associated with changes in motivation for reward either under normal conditions or when reward was devalued although both sham and PSNI groups exhibited the expected negative contrast effect. In the affective bias test, PSNI rats developed a positive affective bias when treated with gabapentin, an effect not seen in the controls suggesting an association with the antinociceptive effects of the drug inducing a relatively more positive affective state. Together, these data suggest that there are changes in reward-related cognition in this chronic pain model consistent with previous findings in rodent models of depression. The effects seen with gabapentin suggest that pain-associated negative affective state may be remediated by this atypical analgesic.

**Keywords:** Reward, Neuropsychology, Affect, Depression, Rodent



## 1. Introduction

Chronic pain is highly comorbid with affective disorders including major depressive disorder (MDD).[3,33] Despite this, it is not yet understood whether this comorbidity arises through overlapping neurobiology[63] or independent mechanisms.[6] Understanding the mechanisms which contribute to complex conditions such as MDD and/or chronic pain can be challenging in patients. Rodent studies offer a route to better understand these relationships, but methods available are limited.[48,49] Models of behavioural despair were developed to predict antidepressant efficacy but are now often used to test for depression-like phenotypes, despite recent concerns that these tests relate more to stress coping than depression.[9,57] Previous studies have reported depression-like phenotypes in chronic pain models using the forced swim[1,2,5] and tail suspension[21,40,62] tests. However, as well as potential issues relating to what these assays measure, both tests may be confounded by the locomotor or motivational differences in pain models.[38,49] An alternative approach is to measure changes in sensitivity to reward using methods such as sucrose preference tests (SPTs).[34] In chronic pain models, some laboratories report deficits[2,12,16,35,54,60,62] whereas others found no difference.[17,46,47,58] Questions about whether changes in reward sensitivity are a hallmark of MDD have also arisen in a human version, the sweet taste test, which gives mixed results in MDD.[13] No effects in other tests such as intracranial self-stimulation[15] or facial reactions to a delivered sucrose solution[41] have been observed in pain models. Reward processing is complex and involves both reward sensitivity and motivation but is also influenced by prior experiences and reward-related cognition for example, learning and memory, decision-making (for review see Refs. 44,48).

Little studied in the pain field is the role of neuropsychological mechanisms in the development of mood-related symptoms. The potential contribution of neuropsychological mechanisms in MDD was first proposed by Beck in the 1960s, and there has been renewed interest in recent years.[19,20,44,48] Affective state–induced biases in cognition and executive function have been observed in MDD patients and at-risk individuals in domains including attention, learning and memory, decision-making, and emotional interpretation.[7,28,32] Studies in both patients and healthy volunteers have also found that traditional monoaminergic antidepressants can induce acute, positive biases in these domains, effects that may contribute to their clinical efficacy.[20,44] Recent translation of human neuropsychological methods for

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application in preclinical models have also found similar affective biases in rodents.[25,50,53]

In this study, we used the previously characterised partial saphenous nerve injury model (PSNI).[27,59] We tested rats using our reward learning assay (RLA) which we have previously found reveals impairments in putative models of depression.[51,53] We compared effects with assays of reward sensitivity and motivation, including using a successive negative contrast task[11,36] to look at reward motivation during devalue of reward. We also used our affective bias test (ABT)[50] to determine whether acute antinociception would modify affective biases consistent with an acute, treatment-induced change in the affective state (see methods for additional detail).

## 2. Methods

### 2.1. Animals

Studies used 2 cohorts of male Lister-hooded rats (Harlan, United Kingdom) (cohort 1 = reward learning, ABT and SPT cohort 2 = successive negative contrast (SNC) and von Frey, **Figure 1**). In these initial proof of concept studies, only a single sex of animals was used; however, understanding the impact of sex will be an important follow-on study should the hypotheses being tested here be substantiated. We have previously established that affective biases in the ABT are present in both male and female rats,[25] but the lack of a sex-related comparisons is a limitation of the study design. Each cohort of rats were run through the same procedures for the RLA followed by the ABT, and, then, the results for each experiment pooled to provide the final n number. This was necessary as the bowl digging tasks are limited in terms of the numbers of animals which can be run at the same time. Rats for cohort 1 were 280 to 320 g at the start of testing, whereas rats for cohort 2 were 360 to 400 g. All rats were housed in same-treatment pairs, in controlled humidity and temperature conditions and under a 12-hour light-dark cycle (lights off at 0800). Testing was performed during the dark phase of the cycle, under red light, between 09:00 to 18:00. Water was provided ab libitum, and food was restricted to approximately 20 g per day, maintaining rats at ~95% of their free feeding weight matched to their normal growth curve. All procedures were in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and EU2010/63/EU, local institutional guidelines and the International Association for the Study of Pain. All studies were reviewed by the local animal welfare and ethical review body. Procedures are reported in accordance with the ARRIVE guidelines.[29]

### 2.2. Partial saphenous nerve injury surgery

Rats had either sham or PSNI surgery (cohort 1: n = 16/grp), cohort 2: n = 12/grp). We chose this model because it uses a sensory nerve injury and lacks the overt effects on limb function seen with sciatic nerve injury models. Surgeries were as described previously.[26,27,59] In brief, under anaesthesia (5% induction, 2% maintenance isoflurane in $O_2$), a ~1 cm incision was made on the anterior surface of the right hind leg. The saphenous nerve was blunt dissected from surrounding tissues. Proximal to any nerve branches, the nerve sheath was split longitudinally and the nerve gently blunt dissected longitudinally along the midline into 2 sections. A 4-0 nonabsorbable nylon sterile suture (Mersilk, Ethicon) was passed around only the lateral section and tied tightly. The wounds were closed using either the same suture externally (cohort 1) or intradermal absorbable suture (4-0 Vicryl suture, Ethicon) (cohort 2). Intradermal absorbable sutures were used for the second cohort to improve wound healing during recovery. Sham surgery was identical apart from there was no ligation of the saphenous nerve. The timing of surgery relative to behavioural training and testing in the different assays is illustrated in **Figure 1**.

### 2.3. Measurement of mechanical nociceptive behaviour

Mechanical thresholds were measured in the ipsilateral and contralateral hind paws using the up–down von Frey method.[8] Rats were initially habituated to the testing apparatus (290 [length] × 170 [width] × 240 mm [height]) over one session lasting 60 minutes. Rats from cohort 1 failed to show reduced activity in the boxes over the course of this first habituation, and during subsequent attempts (once a week over 11 weeks), this cohort continued to move in response to the presence of the tester, and so, von Frey testing could not be completed. Cohort 1 rats were previously trained in a manually run bowl digging task with a lot of handler interaction, and this may have interfered with the habituation process. Rats from cohort 2 habituated after one session and proceeded to von Frey testing. Testing was performed on weeks −1(day-4), 1(day 4), 6 (day 46), 10 (day 75) postsurgery (**Figure 1**). Rats were placed into the test apparatus and left for 30 to 60 minutes. von Frey hairs were applied perpendicularly to the foot in the area of innervation by the saphenous nerve (lateral edge of the plantar surface). Von Frey (vF) withdrawal responses were determined from a stimulus: response relationship generated using a modified up–down method[8] starting with an 8 g vF force and using 2 g, 4 g, 6 g, 8 g, 10 g, 15 g, and 26 g forces as needed. If a hind paw withdrawal response was seen in response to 8 g, a lower vF stimulus was used, and if no withdrawal response occurred, a higher stimulus force was used until a response was seen. If no withdrawal response was seen in response to 8 g, a higher vF stimulus followed until a response was seen, at which point the force applied was reversed. This reversal process between consistent withdrawal and nonwithdrawal until 5 reversals had been completed. From this, mechanical thresholds were determined using the Dixon's equation as described in Refs. 8,14.

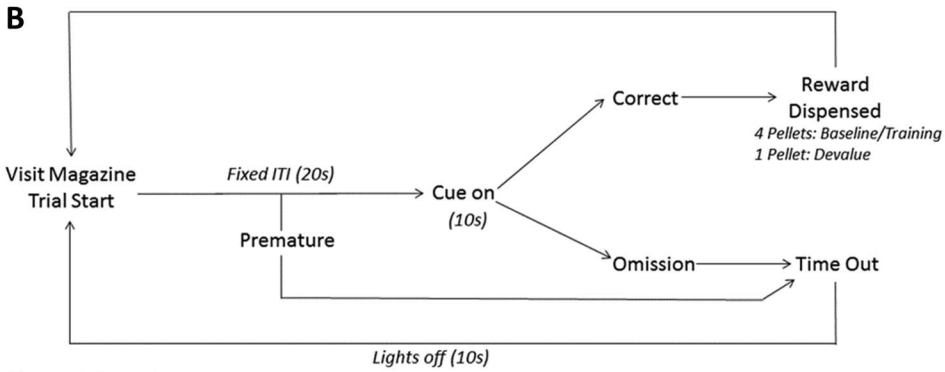
### 2.4. Measurement of affective state–related behaviours

To quantify reward deficits linked to depression-like phenotypes, we used our novel RLA.[44] This assay was developed to quantify in animals' deficits in reward-related cognition based on the hypothesis that the loss of pleasure in previously rewarding activities, which is a hallmark of MDD Diagnostic Statistical Manual version V (DSM-V), is related to these deficits. Unlike behavioural despair models, which are now believed to be limited to a predictive model of antidepressant efficacy,[9,57] our reward-learning assay has been shown to be sensitive to phenotypic changes associated with different risk factors for MDD including early life adversity[51] and treatment with prodepressant drugs.[53]

We also tested animals in the ABT[50] to determine whether acute antinociception would modify affective biases consistent with a treatment-induced change in the affective state. The ABT has undergone extensive validation using pharmacological and psychosocial manipulations of the affective state.[24,25,43,50] We have been able to establish that the task can detect both positive and negative changes in the affective state through quantification of the arising affective bias. The ABT, unlike the RLA, uses a within-subject design and a fixed reward value and so is not a measure of reward learning per se but quantifies acute affective

**A**

| Assay | Associative learning session 1 | Associative learning session 2 | Associative learning session 3 | Associative learning session 4 | Preference test |
|---|---|---|---|---|---|
| RLA | Substrate A, 2 rewards | Substrate B, 1 reward | Substrate A, 2 rewards | Substrate B, 1 rewards | Substrate A vs B, random reinforcement |
| ABT | Substrate A 1 reward gabapentin pre-treatment | Substrate B 1 reward vehicle pre-treatment | Substrate A 1 reward gabapentin pre-treatment | Substrate B 1 reward vehicle pre-treatment | Substrate A vs B, random reinforcement |




**C**

**Cohort 1**

| Study Stage | Training | | Reward Learning Assay | Surgery | | Reward Learning Assay | ABT (data not included) | Gabapentin ABT | SPT |
|---|---|---|---|---|---|---|---|---|---|
| Weeks Post Surgery | -3 | -2 | -1 | 0 | 1 | 2 | 3-8 | 9 | 10 |

**Cohort 2**

| Study Stage | Pre-Surgery SNC | | | Surgery | Post-Surgery SNC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Von Frey | | Von Frey | | Von Frey | | | Von Frey |
| Weeks Post Surgery | -3 | -2 | -1 (day -4) | 0 | 1 (day 4) | 2-5 | 6 (day 46) | 7 | 8-9 | 10 (day 75) |

**Figure 1.** Overview of the methods for the RLA and ABT (panel A), SNC task (panel B), and a timeline of the interventions for cohort 1 and 2 (panel c). The schematic included for the RLA and ABT illustrate the substrate, reward, and treatments for an example rat. Substrates, day of associative learning, and treatment were all counter-balanced with different rats receiving different substrate-reward pairings under each condition and with the order of treatments counter-balanced. ABT, affective bias test; RLA, reward learning assay; SNC, successive negative contrast.

state-induced changes in a specific reward memory. Biases arise when the animal experiences a positive or negative affective state at the time of learning, and this generates either a shift towards or away from the treatment-paired reward-association during a subsequent preference test. In a recent study, we have also been able to demonstrate that the assay is highly sensitive to individual differences in an animal's positive affective experience.[24]

### *2.5. Training for the reward-learning assay and affective bias test*

All training and testing was performed in a 40 $cm^2$ clear Perspex arena in which 2 glazed pottery bowls (10 cm in diameter) were placed against 1 wall. Training and testing were performed as described previously in Refs. 25,50,53. In brief, rats were first trained to dig to obtain a food pellet reward (40-mg precision pellets, TestDiet, Sandown Scientific, United Kingdom) with the amount of digging substrate (sawdust only during training) increased to a final depth of ~2 cm. Once trained, rats could be tested in either a RLA or an ABT using the same basic protocol. A major advantage of the bowl digging set-up is that the same motor effort is required for either a correct or incorrect choice and recording measures of associative learning (trials to criteria), motivation, and motor function (latency to dig) during the discrimination learning sessions provide additional control data for any nonspecific effects. The standard testing protocol was performed over 5 days and used a within-subject design. During each discrimination learning session, the rat was presented with 2 digging substrates in discrete trials: one rewarded (CS+, A or B) and one nonrewarded (CS-, C). A crushed-up food pellet was added to the unrewarded substrate to reduce olfactory cues. Rats were allowed to explore both bowls and make a choice of which to dig in. As soon as the rat chose a bowl to dig in, that is, nose or paw(s) went below the surface of the substrate, the other was removed. For each trial, bowls were positioned in a pseudo-random order, left or right to prevent spatial learning. The session was complete when either the rat achieved criterion (6 consecutive correct trials) or completed 20 trials without achieving criterion. No digging within 20 seconds was counted as an omission and trials to criterion, as well as the latency to dig

in each trial, were recorded to determine any nonspecific effects of treatment. On the fifth day, the 2 previously rewarded substrates (CS+, A and B) were presented together for the first time, and preference was determined over 30 trials. Positions of the bowls were pseudo-random, and trials were randomly reinforced with a single food pellet, such that the probability of reward was 1 in 3 for each substrate. For all studies the substrates, order of presentation and treatments (where appropriate) were fully counter-balanced to remove any experimental bias. Substrates used for these experiments were as follows: aspen chip bedding, cypress bedding, woodchips, hygiene cloth, exfoliating gloves, small beads, wool, and mouse bedding.

#### 2.5.1. Reward-learning assay

To test if rats would develop a reward-induced positive bias, individual substrates (CS+, A or B, randomised to high or low reward) were paired with either one (low) or 2 (high) food pellets during the discrimination learning sessions (days 1–4, **Fig. 1**). Rats were then presented with both the high and the low value reward-paired substrates together on the final day, and their choices recorded over 30 trials. During the preference test, all trials were rewarded with a single pellet reward randomly presented in either substrate A or B. A reward-induced positive bias was observed when rats made more choices for the substrate previously associated with the higher value reward.

#### 2.5.2. Affective bias test

In this version of the assay, the absolute value of the reward is the same for all the discrimination learning sessions, a single reward pellet. However, one of the substrate-reward associations is learnt after a control manipulation whereas the other was learnt after treatment with an analgesic on a separate day (**Fig. 1**). (Rats were initially tested using morphine, venlafaxine, amitriptyline, or vehicle [saline]. However, the venlafaxine positive control failed to elicit a positive bias in the sham group possibly due to stress associated with intraperitoneal dosing which we have found can induce a negative bias in the assay[52] [data not shown]). For the gabapentin experiment and to facilitate oral dosing, before the experiment, rats were trained to drink strawberry milkshake from a syringe for 1 week. This then enabled us to avoid the stress associated with oral gavage or intraperitoneal administration by administering the treatment in this palatable form. The gabapentin was dissolved in milkshake at a 1 mL/kg dose volume, and rats would immediately drink all the milkshake from the syringe, when it was presented through the cage bars, 1 hour before the learning session. In this study, both sham and PSNI rats were treated with either gabapentin (50 mg/kg, oral in strawberry milkshake, 1 mL/kg dose volume, 400 mg capsules, Arrow Generics) or vehicle (strawberry milkshake, 1 mL/kg dose volume), counter-balanced over the 4 pairing sessions (drug treatment on day 1 and 3 or 2 and 4) with the same treatment paired with either substrate A or B. Rats did not receive any treatments before the choice test. During the choice test, rats were presented with substrates A and B together for the first time and their choices recorded over 30 trials. Based on our previous validation of this assay, a positive bias during the choice test was interpreted as rats being in a more positive affective state during learning of the substrate-reward association relative to that during control conditions.[50] The dose used has been shown previously to reduce mechanical and cold allodynia in PSNI[59] and when given orally in the L5/L6 spinal nerve ligation model at this dose and time-point.[22]

### 2.6. The sucrose preference test

The SPT was adapted from the original protocol[61] and has been previously described in Ref. 53. Rats were first habituated to the 2 drinking solutions, water and 1% sucrose, before testing. Starting 72 hours before the test, 1% sucrose replaced normal drinking water in the home cage for 48 hours, with the aim to prevent neophagia during the test. This was then replaced by normal drinking water for 24 hours. Before testing, rats were water restricted for 4 hours before being housed individually in new cages and given the choice of 2 bottles: one containing normal drinking water and the other 1% sucrose. The side of the sucrose/water was counterbalanced across cages, and the total test time was 1 hour with bottles weighed pretesting and posttesting.

### 2.7. Successive negative contrast

Training and testing were performed as described previously.[36,42] In brief, all training and testing used 5-hole operant boxes (Med Associates, Sandown Scientific, United Kingdom) controlled by K-Limbic software. Only the middle aperture was used for responding, with reward pellets dispensed into a magazine on the opposite wall of the chamber. Rats were trained to initiate a trial by a nose-poke in the magazine. After initial continuous reinforcement training where the magazine light is illuminated with each reward delivery, the food reward and magazine light become associated. In subsequent stages, the light in the magazine alone is illuminated, and rats learn they need to nose poke to initiate a trial. After a fixed intertrial interval (ITI), the stimulus light would come on in the central aperture, and the rats had to nose-poke this aperture within a limited hold (LH) period. Correct responses were rewarded with 4 food pellets (40 mg precision pellets, TestDiet, Sandown Scientific, United Kingdom) delivered to the magazine. Throughout training, both stimulus and LH duration were decreased, whereas the ITI was increased, from a starting duration of 2 seconds. The final stage had a stimulus duration of 10 seconds, LH of 10 seconds, and ITI of 20 seconds. Each session consisted of 50 trials. Once rats had met criteria for 2 consecutive trials, at this final stage, they were considered trained. To establish baseline, presurgery performance, rats were then tested for 10 sessions, including 3 devalue, on weeks postsurgery-3 (day-17) and 2 (day-13 and day-10). Devalue sessions were always run as single session with a least 2 days between the test. For each devalue session, a correct response was rewarded with only a single pellet rather than the training and baseline level of 4 pellets. We have previously shown that this reduction in reward outcome results in a negative contrast effect consistent with the criteria previously described by Crespi.[11] Based on previous studies, reducing the expected value of the reward increases correct and collection latency and omissions and reduces premature responses.[11,36] As our primary interest was to compare the response of rats in chronic pain to controls during baseline and devalue sessions, we did not include control groups which only ever received the lower value reward.

After baseline testing, rats were split into 2 counter-balanced groups and underwent sham or PSNI surgery as described above. After 1-week recovery, rats were returned to behavioural testing using the final training stage. Rats were tested over 4 sessions to re-establish a stable baseline. They were then tested

for 4 sessions under baseline conditions to determine if there were any effects of nerve injury before progressing to testing rats during both baseline and devalue sessions. Rats were tested in 6 devalue sessions on days 7 to 45 postsurgery.

### 2.8. Statistical analysis

#### 2.8.1. Experiment design and methods to reduce bias

The sample size used was based on a power calculation using GPower3.1.9.2 and previous data from the laboratory using the bowl digging tasks and operant SNC.[50] The experimenter was blind to drug treatment, but not the surgery group, for all the behavioural testing except the von Frey studies in which rats were pseudorandomly placed into the test boxes by a different researcher to the one undertaking the test. All drug dosing and factors such as digging substrate and order of the pairing sessions for the RLA and ABT were counter-balanced to reduce experimental bias.

All graphs were created in GraphPad Prism version 5.03, and this was used for *t* test analysis in the tasks specified below. SPSS Statistics 23 IBM was used to conduct repeated measure analysis of variance (ANOVA) and post-hoc analysis when appropriate. A Huynh–Feldt correction was used to correct for violation of sphericity. Where significant main effects or interactions were observed ($P < 0.05$), effects were further analysed using post-hoc tests with appropriate correction for multiple comparisons, for example, Bonferroni for within-subject comparisons and Sidak for between subject.

Mechanical thresholds were determined using Dixon's equation: mechanical threshold $= (10[xf + k\delta])/10000$.[8,14] In which xf is the last force used, k is a constant specific to the response pattern (defined in Refs. 8,14), and δ is the average difference between each force used. The results for the normality test (Shapiro–Wilk test) for the specific groups and time-points found most of the data were normally distributed (only 3 of the 16 values were significant), and Levene's test satisfied the assumptions for RM ANOVA. Therefore, differences between sham and PSNI and presurgery baselines were analysed using a mixed model ANOVA with treatment GROUP (between-subject factor) and DAY (within-subject factor). Data from the ipsilateral and contralateral paws were analysed using separate ANOVAs.

Data from the RLA and ABT study are presented as percentage choice bias. This was calculated as {[(number of choices of gabapentin or 2 pellet-paired substrate)/(number of choices of vehicle or 1 pellet-paired substrate)] × 100}−50. This gave a percentage preference in which a positive value denotes a bias towards the gabapentin/2-pellet paired substrate, and a negative value indicates a preference towards the vehicle/1-pellet paired substrate. Statistical analysis was a one-tailed, unpaired *t* test for comparison between sham and PSNI rats as well as individual 1 sample *t*-tests against a theoretical mean of 0% choice bias.

Percentage preference for the sucrose solution in the SPT was determined as [weight of sucrose solution consumed/(weight of sucrose solution consumed + weight of water solution consumed)] × 100. Total fluid consumed is represented in grams. Comparison between sham and PSNI rats was conducted by a 2-tailed *t* test in GraphPad Prism.

For the SNC task, variables recorded were as follows: number correct, omissions (not responding within the LH), and premature (response before the light stimulus) alongside both correct and collection latencies (time to respond to stimulus and time to collect reward respectively). In contrast with the 5-choice serial reaction time task, there were no incorrect responses recorded in this task as only the central aperture was in use. Data were analysed using a mixed model ANOVA with treatment GROUP (between-subject factor), TIME (within-subject factor), and SESSION (within-subject factor).

## 3. Results

### 3.1. Partial saphenous nerve injury–induced mechanical allodynia

Consistent with previous studies, rats receiving nerve injury developed allodynia with reduced mechanical threshold in the ipsilateral (**Fig. 2A**) but not contralateral paw (**Fig. 2B**). There were no significant main effects on contralateral thresholds, but GROUP, DAY, AND GROUP × DAY were all significant on the ipsilateral paw (GROUP $F(1,21) = 13.285$ $P = 0.002$, DAY ($F(2.814, 59.091) = 13.194$ $P < 0.001$), and GROUP*DAY interaction ($F(2.814, 59.091) = 3.910$ $P = 0.02$ **Fig. 2A**). Postsurgery ipsilateral measurements, made on days 46 and 75, showed reduced threshold for the PSNI rats compared with sham (day 46 $P = 0.001$ day 75 $P = 0.006$), and all PSNI ipsilateral postsurgery measurements were lower than presurgery values (day 4 $P = 0.004$ day 46 $P < 0.0001$, and day 75 $P < 0.0001$).

### 3.2. Impaired reward learning after partial saphenous nerve injury

Before surgery, both treatment groups developed a reward-induced positive bias in the RLA. When rats were retested 14 days postsurgery, using new substrate-reward associations, compared with the null hypothesis of 0%, the sham group developed a reward-induced positive bias (1 sample $t_{15}=2.936$, $P= 0.01$) but the PSNI animals did not (**Fig. 3A**). A pairwise comparison between sham and PSNI also found that the reward-induced positive bias was attenuated in PSNI animals (*t* test, $t_{30} = 3.144$, $P = 0.002$). There were no differences between groups during the discrimination learning sessions in either trials to criteria or latencies postsurgery (**Figs. 3B, C**, respectively). These control measures suggest rats were not impaired in their ability to learn the associations during the initial pairing sessions and had no overt motor impairments. The effects observed were therefore specific to impairments during retrieval in the choice test and suggest an impaired ability to use the prior information about reward value to guide decision-making.

### 3.3. Gabapentin induces a positive affective bias in partial saphenous nerve injury rats alone

Treatment with gabapentin (50 mg/kg, oral) induced a positive bias in the PSNI treated group compared with controls (1-tailed $t_{28} = 2.011$, $P = 0.027$, **Fig. 4A**) and a tendency to positive bias when compared against the theoretical mean of 0% bias (1 sample *t* test, $t_{13} = 1.836$, $P = 0.089$). Sham rats did not show any bias and neither group showed any effect of treatment on trials to criterion (total number of trials taken to achieve criteria of 6 consecutive correct trials) or the latency to dig (**Figs. 4B,C**), suggesting there was no effect of drug on learning and memory or general motor function.

### 3.4. No changes in reward sensitivity in the sucrose preference test after partial saphenous nerve injury

Both groups developed a sucrose preference with sham (1 sample $t_{15} = 8.864$ $P < 0.0001$ n = 16) and PSNI (1 sample $t_{15} = 3.948$ $P = 0.0013$ n = 16) treated rats showing a preference for 1% sucrose vs water when compared with a hypothetical mean of 0% bias (**Fig. 5A**). There was no difference between groups in percentage preference (**Fig. 5A**) or total fluid consumption (**Fig. 5B**).

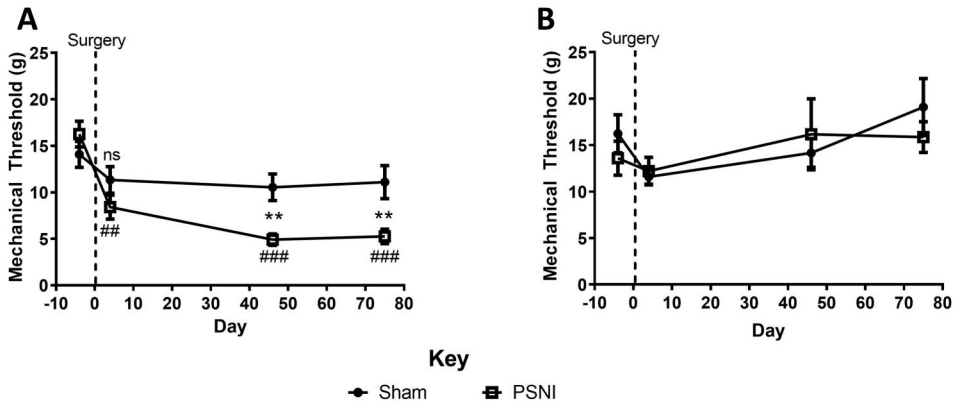



**Figure 2.** PSNI-induced mechanical allodynia in the ipsilateral but not contralateral paw. (A) Ipsilateral, (B) contralateral data shown as mean ± SEM, n = 11 sham n = 12 PSNI. Data were analysed using a repeated measures ANOVA. $^{**}P < 0.01$ between-group difference ## $P < 0.01$, ### $P < 0.001$ within-group difference, ns nonsignificant within-group difference. 1 sham was excluded from analysis for being consistently 2 standard deviations away from the mean. ANOVA, analysis of variance; PSNI, partial saphenous nerve injury.

### *3.5. No evidence of changes in motivation for reward or the successive negative contrast effect after partial saphenous nerve injury*

Consistent with previous studies, the rats trained in the SNC task expressed a devalue effect when the reward outcomes were unexpectedly reduced from 4 to 1 pellet.[36,42] All rats completed the maximum number of trials in all baseline and devalue sessions. During each devalue session, there was an increase in both time to respond to the cue (correct latency, SESSION ($F(1,19) = 60.907$ $P < 0.0001$, **Figure 6A**) and time to collect the reward after delivery (collection latencies, SESSION $F(1,19) = 14.115$ $P = 0.001$, **Fig. 6B**). Rats also reduced their premature responses (SESSION $F(1,19) = 30.766$ $P < 0.0001$, **Fig. 6C**) and increased the number of trials they omitted (SESSION $F(1,19) = 22.258$ $P < 0.0001$, **Fig. 6D**) in the devalue sessions relative to baseline, although post-hoc data did not show a consistent difference unlike between sessions latency data. Postsurgery, both sham and PSNI performed the task at a similar level to their presurgery baseline,

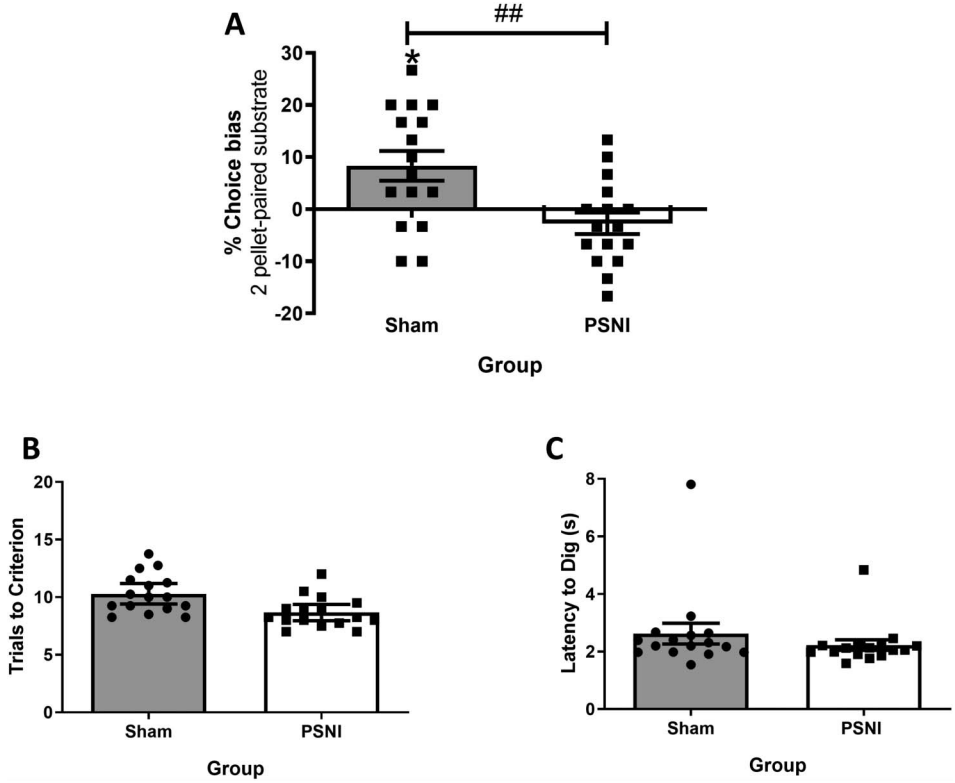



**Figure 3.** Impairments in reward learning in PSNI rats, postsurgery. (A) Postsurgery sham rats preferred the 2-pellet paired substrate (1 sample against null hypothesis of 0% $t_{15} = 2.936$, $P = 0.0102$ n = 16), but PSNI showed no bias. There was a significant difference between groups (1-tailed $t$ test $t_{30} = 3.144$, $P = 0.002$ n = 16 per group). There was no effect of PSNI, when compared with sham, on trials to criterion (B) or latency to dig (C) during discrimination learning sessions. $^{*}P < 0.05$ compared with a null hypothesis of 0% bias, ##$P < 0.01$ PSNI compared with sham rats. n = 16 for both sham and PSNI. Data are expressed as mean ± SEM. PSNI, partial saphenous nerve injury.

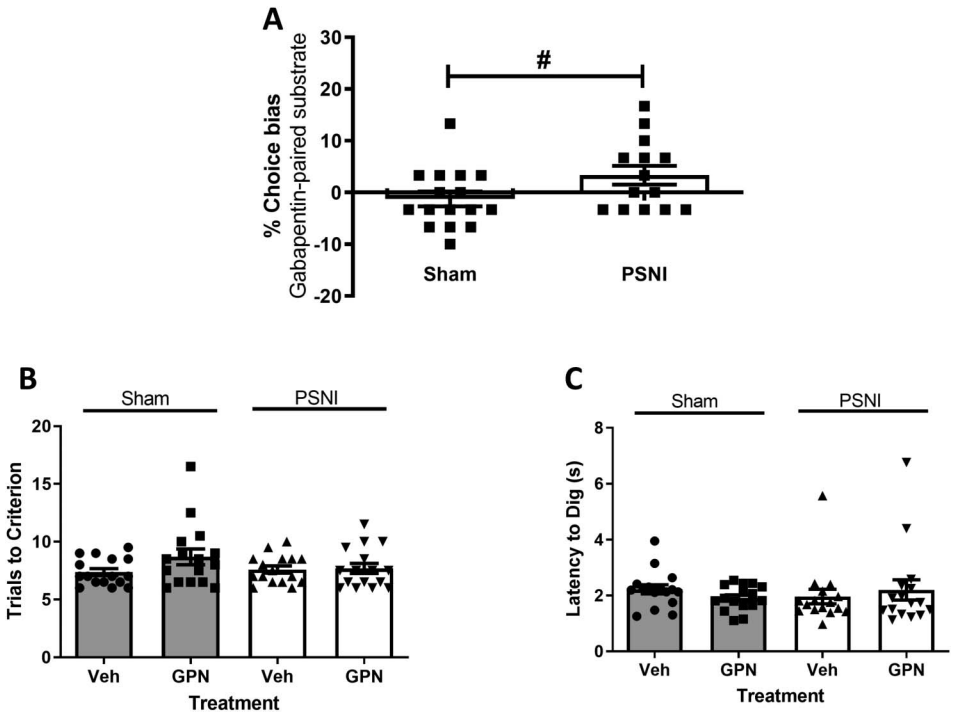



**Figure 4.** Gabapentin induce a positive bias in PSNI rats relative to shams. (A) PSNI rats showed a positive bias towards the substrate learnt after gabapentin treatment compared with controls (1-tailed *t* test $t_{28} = 2.011$ $P = 0.027$) and a tendency to a positive bias compared against the null hypothesis of 0% bias (1 sample *t* test, $t_{13} = 1.836$, $P = 0.089$). There was no effect of gabapentin treatment in either group in terms of associative learning (trials to criterion and number of trials taken to achieve 6 consecutive correct trials) (B) or latency to dig (C) in discrimination learning sessions. n = 16 for both sham and PSNI. Data are expressed as mean ± SEM. PSNI, partial saphenous nerve injury.

with no effect of GROUP or GROUP × SESSION during either baseline or devalue sessions. There were no changes in correct or collection latencies over the period of testing, including after surgery, because there was no significant effect of TIME or interactions with TIME. However, in both sham and PSNI groups, there was a decrease in premature responding over the period of testing (TIME F(3.987,75.758) = 3.950, $P = 0.011$), which was greater for the baseline sessions (SESSION × TIME F(5,95) = 2.810 $P = 0.019$). Omissions generally reduced over testing (TIME F(4.659,88.512) = 2.523, $P = 0.038$) and also reduced more for devalue sessions (SESSION × TIME F(5,95) = 2.539 $P = 0.033$). Overall, there was also no evidence that chronic pain changes motivation to respond for reward in this operant task or the SNC effect.

## 4. Discussion

Consistent with previous studies, we observed that rats receiving a PSNI developed allodynia consistent with a chronic pain phenotype.[27,59] The allodynia developed over time and was present during the behavioural studies. Results from the different measures of reward processing revealed dissociable effects with rats from the nerve injury group exhibiting an attenuated reward-induced positive bias. These effects were not related to changes in reward sensitivity in the SPT or changes in reward motivation. These findings concur with recent data obtained from putative models of depression suggesting that rats experiencing chronic pain exhibit similar impairments in reward processing to rats exposed to known depression risk factors of early life stress,[51] chronic treatment with a prodepressant drug (retinoic acid) or a known, prodepressant immune-based treatment, interferon alpha.[53] In the ABT, rats experiencing chronic pain developed a positive affective bias after treatment with the atypical analgesic, gabapentin, relative to the sham controls. This suggests that gabapentin-induced antinociception induces a relatively more positive state possibly arising from remediation of a negative affective state resulting from chronic pain, but gabapentin itself does not modify affective state. These data provide evidence for a deficit in reward processing which is consistent with a depression-like phenotype arising from chronic pain. Chronic pain animals remain sensitive to reward-related affective biases as seen in the depression models[51] suggesting different underlying mechanisms to those detected in the Pavlovian RLA. The following discussion considers these different measures of reward processing in the context of both chronic pain and depression-related research and possible implications for understanding their comorbidity.

In this study, we used the PSNI model, in which the injury is to a purely sensory nerve, to reduce potential motor effects. There was no evidence of PSNI-induced locomotor deficits in either the bowl digging or SNC tasks, because no differences in response latencies were observed between groups, despite the presence of allodynia. We did observe a small decrease in threshold in the sham group shortly after surgery, but the difference between groups was clear from day 46 and for the remainder of the period of testing consistent with previous findings for this model.[27,59] Alongside, using a model which lacks ventroflexion of the toes, we also used behavioural tasks where any differences in motor function were controlled for within the experiment design. In addition to controlling for motor effects, we did not see any evidence of cognitive impairments in either bowl digging tasks. During the learning phase of these tasks, no differences were

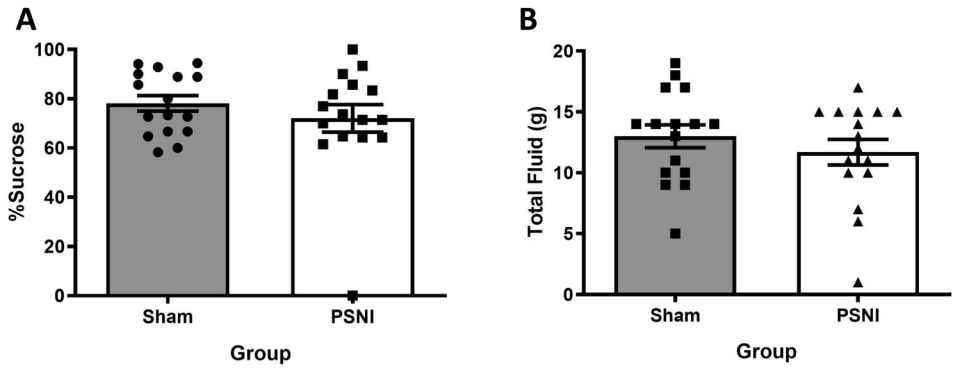



**Figure 5.** No deficits in reward sensitivity in PSNI rats in the SPT. (A) There was no difference between sham and PSNI in percentage sucrose consumed over 1 hour (1-tailed *t* test $t_{30} = 0.9501$ $P = 0.1748$ n = 16 in each group). Both sham and PSNI groups had a significant preference from the null hypothesis of 50%: sham (one sample $t_{15} = 8.864$ $P < 0.0001$ n = 16), PSNI (1 sample $t_{15} = 3.948$ $P = 0.0013$ n = 16). (B) PSNI did not induce any significant changes in fluid consumption. n = 16 both sham and PSNI. Data are expressed as mean ± SEM. PSNI, partial saphenous nerve injury.

observed between groups. Rats had similar trials to criterion during learning in the RLA and ABT. This is consistent with the lack of learning deficit seen in other chronic pain models and reward-motivated learning tasks[10,41] although it should be noted that cognitive deficits in spontaneous tasks such as novel objective recognition have been observed.[30,31,39,55,62]

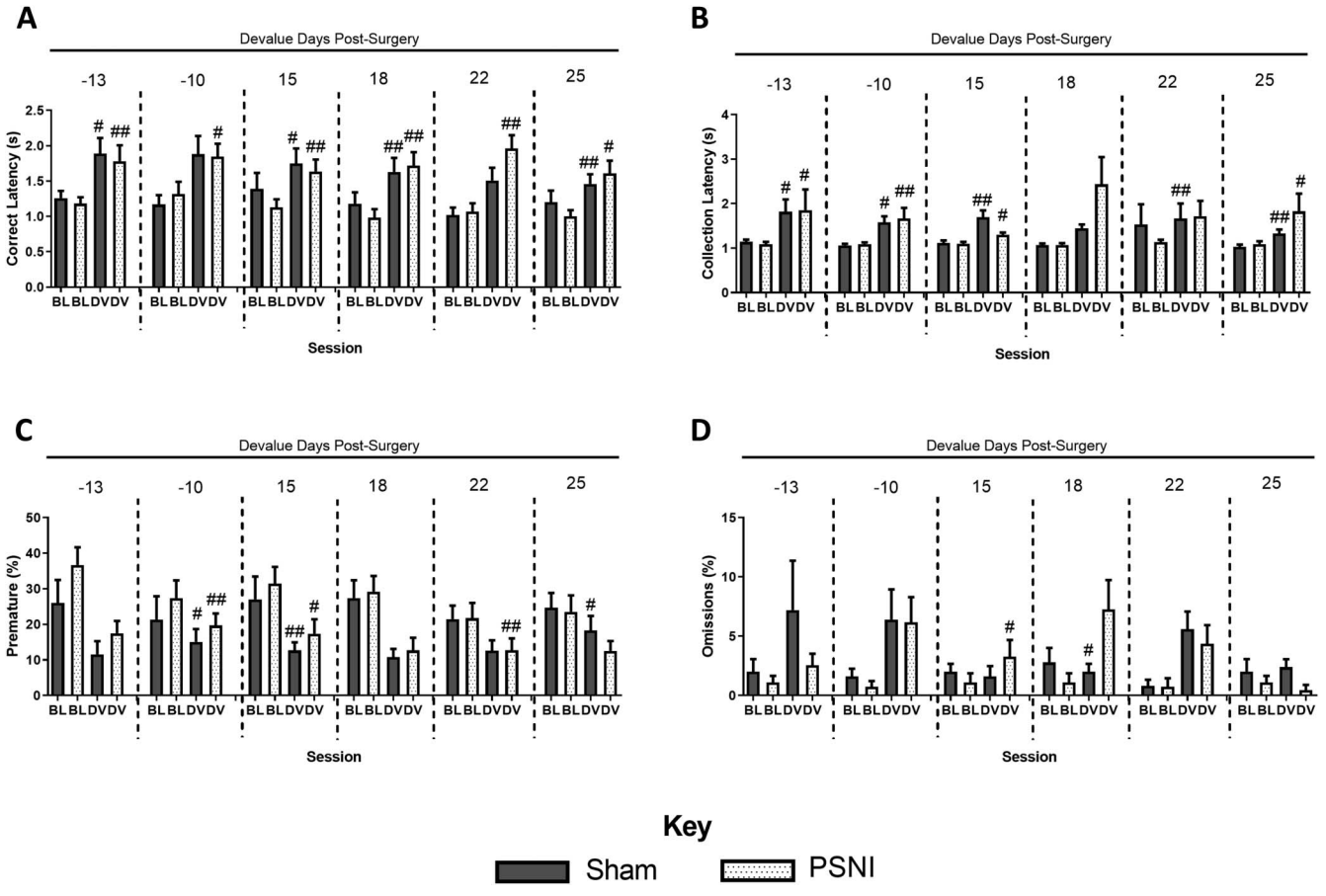



**Figure 6.** Neither motivation to respond for reward nor the SNC effect was affected by PSNI. There were no differences between groups during baseline testing for any of the measures recorded. When the number of food pellets for a correct response (nose-poke in the central aperture when lights were on) was reduced from 4 to 1 pellet, both sham and PSNI rats showed a similar devalue effect. Similar increases in correct latency (A) (time to nose-poke after light on in the aperture) and collection latency (B) (time to collect reward after it was dispensed) were seen in both sham and PSNI groups. There was a significant main effect and decrease in premature responding (responses made during the ITI) (C) and an increase in omissions (failing to respond during the limited hold) (D), but again, there were no differences between sham and PSNI rats. Data shown as mean ± SEM, sham n = 10, PSNI n = 12. Data were analysed by repeated measures ANOVA. #$P < 0.05$ ##$P < 0.01$ ###$P < 0.001$ significant difference between preceding baseline and devalue session, taking sham and PSNI data together. 1 sham was excluded for consistently being 2 standard deviations away from the mean in pain testing, another for operant box error. BL: baseline session in which a correct response was rewarded with 4 pellets. DV: devalue session in which the number of pellets for a correct response was reduced to 1 pellet. ANOVA, analysis of variance; ITI, intertrial interval; PSNI, partial saphenous nerve injury; SNC, successive negative contrast.

Deficits in reward processing in MDD in patients use methods which are not readily translated to animal studies.[44] Questionnaire measures of anhedonia in patients are complicated by their subjective nature, and it is not always obvious whether the deficits relate to the ability to experience pleasure, the ability to anticipate reward or reward-related cognition[48] Recently, it has been suggested that reward deficits in MDD may relate more to reward-related memory rather than inherent reward sensitivity.[48,56] There is no consensus on the presence of deficits in reward sensitivity in chronic pain models. Some studies report reduced reward sensitivity in the SPT,[2,12,16,35,54,60,62] and others did not.[17,46,58] Other methods to assess reward sensitivity such as intracranial self-stimulation[15] and changes in facial expressions in response to reward[41] also found no deficits in chronic pain models. Here, similar to some putative models of MDD,[51,53] PSNI displayed no changes in reward sensitivity in the SPT but failed to develop a reward-induced positive bias in a novel bowl-digging task. We only tested a 1 hour, 1% SPT based on the original model designed to test for stress-induced reward deficits[61] and to use the same methods as previously reported for our depression model;[51] however, other protocols have been used and may give different results. A separate cohort of rats also showed no changes in motivation for reward in the SNC task under normal conditions or after reward devalue. Taken together, these data suggest that the PNSI pain model exhibits specific impairments in reward learning similar to effects seen in rodent models of depression.[53] These deficits are independent of any change in reward sensitivity and do not seem to be related to motivation as measured in the SNC task.

The ABT has been extensively investigated using antidepressant and prodepressant manipulations.[25,43,50] The finding that PSNI rats exhibit a significantly more positive bias towards gabapentin suggests the rewarding aspects of antinociception are driving a positive bias. It would be interesting to test if antidepressant drugs, also used to treat chronic pain, induce similar positive biases, although we know from previous studies that similar treatments induce positive biases in control rats.[50] The findings with gabapentin are different because it did not have any apparent effects in sham rats suggesting a specific interaction with the pain state. Although both the ABT and RLA investigate reward-related behaviours, the tasks are designed to look at different aspects of reward-related cognition. In the RLA, animals learn to associate cue-reward associations under the same core affective state, and biases are induced by the recall of the reward value predicted by the cue. In the ABT, the reward value is fixed with the arising biases correlated with the short-term affective state change induced alongside the treatment-paired cue. Previous studies in depression models suggest that animals with impaired reward learning are still able to develop affective state–induced biases, and although positive biases remain unaffected, negative biases are potentiated.[51] Although insights into the neuropsychological mechanisms which underlie these different behaviours are limited, the evidence to date suggests the reward-learning and reward-related affective biases are not mediated by the same neuropsychological mechanisms. Previous studies using conditioned place preference, to assess the rewarding effect of pain relief with gabapentin, found similar results in which chronic neuropathic pain rats, but not sham, preferred the gabapentin-paired chamber.[4] The dose used in the current study has previously been shown to reduce allodynia in the PSNI pain model.[59] Although this is only a single study in the ABT and has some limitations to the findings, it may be that this task offers a novel approach to assessing new analgesics with a focus on the affective component of pain especially in the context of neuropsychological deficits.

The SNC task investigated how rats respond to an unexpected change in reward outcome. The contrast effect results in animals showing a lower level of motivation to respond than the level of control animals that have only ever experienced the lower value reward.[11,36] In this study, similar responses to the devalue sessions were observed in both the PSNI group and sham controls. All rats exhibited similar increases in correct and collection latencies as well as main effects of the session on omissions and premature responses when the reward decreased from 4 to 1 reward pellet(s). There was also no difference observed between groups during the baseline sessions. Some studies have suggested that negative affective states may potentiate the devalue effect;[18] however, the results, here, do not suggest the PSNI-induced pain modifies this behaviour. To the best of our knowledge, this is the first study to investigate motivation for reward in an SNC task in a chronic pain state, although other studies have looked at motivational behaviour in progressive ratio tasks.[22,41,45] Current findings are inconclusive with one study reporting a reduction in breakpoint, indicating reduced motivation for reward,[45] whereas others found no effects.[22,41] In our study, motivation for immediate reward is not impaired in chronic pain and further supports our proposal that deficits in reward learning may be more relevant to reward-related impairments which can present in chronic pain patients.

Chronic pain is commonly associated with symptoms of MDD.[3] The results presented here show that the relatively mild PSNI model exhibits similar impairments in reward learning to those seen, using the same assay, in putative models of depression.[51] These impairments in reward-induced positive bias are specific and dissociable from other aspects of reward processing with rats showing no impairment in either reward sensitivity or motivation. Alleviating the pain in this model with gabapentin, resulted in a more positive affective bias relative to the vehicle-treated state, although with only a trend level effect observed. These findings concur with other pharmacological and phenotypic studies into the neuropsychological processes which may contribute to MDD.[51,53] Our laboratory has shown that acute prodepressant treatments induce negative affective biases in the ABT, and, when experienced chronically, also induce impairments in reward-related cognition.[51,53] This suggests that chronic pain may affect mood through similar neuropsychological mechanisms, that is, pain generates a negative affective state which, when experienced chronically, leads to deficits in reward learning. New methods which can be used to assess the potential efficacy of novel analgesics and that reduce the reliance on changes in sensory processing are needed.[37] By quantifying changes in affective state either acutely using the ABT or chronically through measurements of reward-related cognition, it may be possible to develop new analgesics which are also efficacious on the emotional component of chronic pain. One limitation of the current studies was the use of only a single sex. Although we have shown that similar affective biases are observed in male and female rats,[25] it will be important that studies with a specific focus on understanding if there are sex differences in these effects are undertaken.

## Conflict of interest statement

The authors have no conflicts of interest to declare in relation to the work presented in this article. E.S.J. Robinson has current or previously obtained research grant funding through PhD studentships, collaborative grants, and contract research from Boehringer Ingelheim, Compass Pathways, Eli Lilly, MSD,

Pfizer, and SmallPharma but in areas unrelated to the work presented here. L.F. Donaldson has previously received funding through PhD support, collaborative grants or licencing agreements from GSK, AstraZeneca, and Philogene in areas unrelated to the work presented here. She is a founder equity holder in and consultant to a University of Nottingham spin-out company Exonate Ltd (splicing kinase inhibitor development for ophthalmology). Exonate Ltd has a research collaboration agreement with Janssen Pharmaceuticals unrelated to the work presented here. She is also a founder equity holder and director of EmendaTherapeutics Ltd (CDC-like kinase inhibitor development for oncology and analgesia). She has received no financial support from either Exonate or EmendaTherapeutics Ltd. B.M. Lumb has previously obtained research grant funding through PhD studentships and collaborative grants from Eli Lilly and GSK but in areas unrelated to the work presented here. The remaining authors have no conflict of interest to declare.

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