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Chronic pain impairs cognitive flexibility and engages novel learning strategies in rats

Stephen L. Cowen^c, Caroline E. Phelps^a, Edita Navratilova^a, David L. McKinzie^b, Alec Okun^{a,b}, Omar Husain^b, Scott D. Gleason^b, Jeffrey M. Witkin^b, and Frank Porreca^a

^aDepartment of Pharmacology, University of Arizona, Tucson, AZ, USA

^bNeuroscience Discovery Research, Lilly Research Laboratories, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN, USA

^cDepartment of Psychology, University of Arizona, Tucson, AZ, USA

Abstract

Cognitive flexibility, the ability to adapt behavior to changing outcomes, is critical for survival. The prefrontal cortex is a key site of cognitive control and chronic pain is known to lead to significant morphological changes to this brain region. Nevertheless, the effects of chronic pain on cognitive flexibility and learning remain uncertain. We used an instrumental paradigm to assess adaptive learning in an experimental model of chronic pain induced by tight ligation of the spinal nerves L5/6 (SNL model). Naïve, sham-operated, and SNL rats were trained to perform fixed-ratio, variable-ratio, and contingency-shift behaviors for food reward. Although all groups learned an initial lever-reward contingency, learning was slower in SNL animals in a subsequent choice task that reversed reinforcement contingencies. Temporal analysis of lever-press responses across sessions indicated no apparent deficits in memory consolidation or retrieval. However, analysis of learning within sessions revealed that the lever presses of SNL animals occurred in bursts followed by delays. Unexpectedly, the degree of bursting correlated positively with learning. Under a variable-ratio probabilistic task, SNL rats chose a less profitable behavioral strategy compared to naïve and sham-operated animals. Following extinction of behavior for learned preferences, SNL animals reverted to their initially preferred (i.e., less profitable) behavioral choice. Our data suggest, that in the face of uncertainty, chronic pain drives a preference for familiar associations, consistent with reduced cognitive flexibility. The observed burst-like responding may represent a novel learning strategy in animals with chronic pain.

Keywords

neuropathic pain; executive function; associative learning; decision making

Corresponding author: Stephen L. Cowen, Ph.D., Department of Psychology, University of Arizona, Tucson, AZ 85721, scowen@email.arizona.edu, Tel. 520 626 2615.

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Author Contributions

FP, EN, AO, JMW and DLM designed the study. JMW, AO, DLK, OH, and SDG collected the data. *SLC performed the data analysis.* SLC, CEP, EN, JMW, and FP wrote the paper.

Introduction

Chronic pain induces morphological changes in the prefrontal cortex (PFC) [5; 18], a brain region associated with high-order executive functions such as attention, inhibitory control, and cognitive flexibility [3; 32; 70]. Greater PFC volume and thickness correlate with improved executive function and patients with frontal lesions exhibit impairment [3; 70]. Imaging studies have demonstrated reductions in grey matter in the PFC in patients with chronic pain [5; 18]. However, the possible consequences of pain-related morphological changes on behavior are unknown. One potential consequence is impaired executive function such as reduced cognitive flexibility.

Cognitive flexibility is the ability to adapt behavior to changing outcomes [12] and is associated with increased resilience to negative life events [19], improved academic performance in children [17] as well as better overall quality of life [13]. Patients with chronic pain may be impaired in cognitive flexibility, reflected by reduced performance on tasks such as the trail making test (TMT) [30; 57; 67] and the Wisconsin Card Sorting Task (WCST) [28; 48; 65; 68]. These tasks challenge subjects to adapt to changing rules for trial completion (TMT) or reward (WCST). Impaired WCST performance may be associated with reduced PFC function [6; 44], although not exclusively [20; 49; 50]. The impact of PFC on TMT is less clear [70]. Assessing the impact of pain on behavior arises from the possible relationship of deficits to factors such as medications used for treatment, lifestyle, socioeconomic status and other factors rather than to pain itself. Additionally, the underlying neurobiology of pain-associated morphological changes in the PFC remains unclear, so that establishing causality of pain with cognitive function is difficult. For example, it is currently unknown whether subjects with chronic pain employ alternate learning strategies that allow them to overcome cognitive deficits produced by potentially disrupted frontal function.

The use of preclinical models allows assessment of the influence of chronic pain on cognitive function under controlled conditions in homogenous subjects. Despite the reduced complexity of the rodent PFC, this region shares many homologous roles in executive control with primates (as reviewed in [32]), and the morphology of the rat PFC has been shown to be similarly altered in chronic pain states [2; 11; 14; 31; 42; 62]. Rodents in chronic pain are unable to learn platform relocation in the Morris Water Maze [36; 46] or a bowl digging attentional set shifting task [37; 38], suggesting impaired cognitive flexibility. However, the influence of other aspects of cognition and alternate learning strategies have not been studied in these tasks.

In the present study, naïve, sham-operated, or nerve-injured rats were tested in an operant protocol of fixed ratio (initial learning), progressive ratio (motivation), reversal (adaptive learning), and variable ratio reversal (cognitive flexibility and decision making). This design allowed measurement of multiple aspects of cognition as well as testing for effects of chronic pain on coping strategies, memory consolidation, and motor impairment. Our data suggest that chronic pain promotes novel adaptive learning strategies that are not fully capable of ameliorating impaired cognitive flexibility.

Methods

Animals

Male, Sprague Dawley rats (Harlan Laboratories Inc., Indianapolis, IN) weighing 250 to 350 g were housed in climate-controlled rooms on a 12-hour light–dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee at Eli Lilly, and these procedures were in accordance with guidelines set by the National Institutes of Health and the International Association for the Study of Pain. Animals were monitored throughout the duration of the study to reduce any unnecessary stress and/or pain. Subjects were divided experimentally into SNL (N = 16), sham (N = 15), and naïve (N = 12) populations. Animals were housed individually for the duration of the experiment (~11 weeks) to control feeding conditions during training periods involving food restriction. During food restriction (~4 weeks), animals were monitored, handled, and tested in the operant chambers by experienced personnel. Data on motivational responses were collected from the same cohort of rats and previously reported [51].

Spinal nerve ligation

The L5/L6 surgical procedure was used to produce experimental neuropathic pain as previously described [33]. The L5 and L6 spinal nerves (left side) were tightly ligated with 4-0 silk suture and the wound was closed. Sham-operated rats were prepared in the same manner as the SNL rats but without ligation of the L5/L6 spinal nerves. Naïve rats had no surgery or other conditions placed upon them. Animals were acclimated in the housing facility for a minimum of 7 days before experimentation. All rats were monitored for any signs of motor impairments and assessed for overall health. SNL induced allodynia was confirmed in all animals using von Frey filaments at post-surgery days 14-120 [33]. Compared with sham controls, SNL rats exhibited significantly decreased tactile withdrawal thresholds throughout the course of the experiment, confirming the presence of mechanical allodynia (tested on Days 14, 21, 28, 35, 42, 56, 90, and 120 post-SNL). Mean withdrawal thresholds ranged between 3.9-5.2 g for SNL rats and 14.1-15.0 g for sham controls; $F(1,208) = 988.3$, $P < 0.0001$ for main effect of Treatment; see **Fig. 3E** in reference [50]). Using a 6 g exclusion threshold for the presence of allodynia, no SNL rats were excluded from the study.

Operant conditioning

Each rat was trained on a series of operant tasks that assessed associative learning. Prior to training and on day 16 post-surgery, food restriction was implemented so as to reduce body weights to 85-90% of their free-feeding weights. Training began on post-surgery day 21 and the training regimen is summarized in Fig. 1 and described below. The Choice task (Task 3) and the Variable Reinforcement task (VR, Task 6) are the main targets of analysis; performance on the Progressive Ratio task (PR, Task 2) has been reported previously [51].

Lever Press Acquisition (Task 1) and Progressive Ratio Testing (Task 2)—

Animals were first trained on a fixed-ratio (FR) task for 4 days (Task 1) followed by training on a PR task (Task 2). Training on these tasks began on post-operative days 21-23 when rats were placed in 2-lever operant responding chambers (12.0" L × 9.5" W × 8.25" H, Med

Associates, VT, USA) and underwent training for 90 min/day on sessions 1-3 (Fig. 1A). During all training sessions, presses on the right lever were followed by a 2 second tone and delivery of 1 sucrose pellet (Dustless Precision Pellets (45 mg; Bioserv, Flemington, NJ)) for each press (FR1 schedule). Presses on the left did not result in food delivery during Tasks 1 and 2. The maximal number of pellets delivered in a single session was 50, but not all rats reached 50 pellets on a given day. On the 4th training day, (post-operative day 24) the rats were again allowed to lever press for food; however, the session length was decreased to 30 minutes. Rats that did not acquire 50 pellets on this day were excluded from the experiment (n = 2 rats). On day 25, the progressive ratio testing started with the reinforcement schedule set such that the number of lever presses required to receive a single pellet increased after each pellet delivery (PR schedule). The initial PR schedule (PR1) was as follows: (1, 1, 1, 2, 2, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80). At the highest ratio, rats had to press the lever 80 times to obtain food. After completion of the PR1 schedule on day 26, food restriction in the home cage was stopped and rats had free access to chow until the end of post-surgery day 41. Rats were tested again on day 27 with the PR1 schedule. On day 28, the reinforcement schedule was made more challenging by increasing the ratios more rapidly. The progressive ratio schedule 2 (PR2) for day 28 was as follows: (1, 2, 4, 8, 16, 32, 64).

Choice Task (Task 3)—Choice testing began on post-surgery day 29, which was the first day following the PR experiment (Fig. 1A). In this FR task, rats received a single sucrose pellet per lever press of the right lever and 4 pellets per press of the left lever. Performance on this task was quantified as the percent of trials in which rats selected the high-reward lever. Rats were tested without food restriction for the first 8 experimental sessions of Task 3. After the 8th session, rats were food restricted and tested for an additional 5 sessions. Experimental sessions lasted 60 min or 50 food pellet deliveries, whichever occurred first, after which the chamber became inactive and subsequent responding was without consequence. Food restriction during days 21-28 was used to speed training. Restriction was not present during the initial phase of Task 3 (days 29-41) to eliminate potential anti-allodynic effects of food restriction on SNL animals, as well as to improve translation between rodent and human studies as human patients with chronic pain are not food restricted.

FR1 and Cue Association Tasks (Tasks 4 and 5)—By design, training on the choice task resulted in all animals developing a preference for the high-reward (left) lever (Fig. 2). To reduce the preference for the higher reward lever following Task 3, rats received 2 days of training where both levers were active and reinforced on an FR1=1 pellet schedule (Task 4). Testing occurred for 30 min or until the rats received 50 total pellets. Rats were food restricted for both experimental sessions. To further reduce bias for a single lever, these two FR1 sessions were followed by a light-following task (Task 5) in which a light appeared over one randomly chosen lever. When the light was on, the lever was active and would deliver 1 pellet when pressed. After the lighted lever was pressed, the light randomly appeared over one of the levers again to signal a new active lever. The session lasted 30 min or ended at 50 food pellet deliveries, whichever came first.

Variable-Ratio Choice Testing (Task 6)—The variable-ratio (VR) task began on post-surgery day 56. In this task, both levers were active and the right lever delivered one pellet per press on 8 of 10 presses, while the left lever delivered 4 pellets of reward on 3 of 10 presses. Consequently, the left lever was designated as the high-reward lever as the average reward per 10 presses was 12 pellets, while the average reward delivered on the right lever was only 8 pellets. Given the schedule just described, the variability in the VR schedule was low. This low-variance VR schedule was chosen to ensure consistent reinforcement probabilities. Experimental sessions lasted 60 min or 50 food pellet deliveries, whichever occurred first.

Statistical methods

All statistical analyses were conducted with Matlab® 2017a. Differences in response rates between groups were assessed using Student's t-tests or analysis of variance (ANOVA) with Tukey-Kramer or Bonferroni-Holm multiple comparisons corrections. Significance was set at $\alpha = 0.05$ for all tests. Learning rates for the choice and probabilistic tasks (percent high-reward) were determined for each animal by computing the slope of the least-squares regression line that fit the session-by-performance data (e.g., Fig. 2A).

Within-session learning was quantified in two ways. The first approach measured learning as the difference between the percent in which the animals selected the high-reward lever during the last $\frac{1}{3}$ of trials minus the percent of high-reward lever presses during the first $\frac{1}{3}$ of trials (Percent High Reward Last Third – Percent High Reward First Third). The second approach involved fitting a least-squares regression line to the pattern of left-right responses per session coded as 0s and 1s (e.g., 0 0 0 1 0 1...). The slope of this fit was also used as a measure of within-session learning. Learning between sessions (an indication of memory consolidation) was calculated as the percent of high-reward lever presses in the first $\frac{1}{3}$ of trials on day $D+1$ minus the percent of high reward choices on day D .

The temporal structure of lever-press responding was assessed using an established measure of the 'burstiness' of the arrival times of events [63]. This measure quantifies burst responding as the average dissimilarity between adjacent inter-trial intervals. It is calculated as follows: a value near 0 indicates a perfectly regular inter-press interval, values near 1 indicate a Poisson-distributed series of intervals, and values above 1 indicate that there were bursts. We chose this measure as it is more robust relative to other measures of bursting to slow changes in response rates [63]. This was a concern as slow changes within days (e.g., due to satiety or fatigue) and between days (e.g., potentially due to learning) were present.

Results

Learning rates

A goal of the first phase of training was to develop a preference for the right lever. An initial preference for the right lever was established in all groups by only reinforcing right presses on FR and PR tasks (Tasks 1-2, Fig. 1A). No differences between groups were observed, as has been previously reported [51]. This indicates that SNL animals are not deficient in initial learning and motivation.

In Task 3, the reinforcement density of left lever increased, making it more profitable (right lever: 1 pellet/press, left lever: 4 pellets/press). Assessment of lever preference on day 1 of Task 3 indicated that animals expressed a strong preference for the previously reinforced (right) lever (mean preference for high-reward lever: naïve = 90% of trials, sham = 93% of trials, SNL = 96% of trials, one-way ANOVA, $F(2,40) = 0.73$, $p = 0.49$). All animals learned to prefer the previously non-reinforced, but now high reinforcement density, left lever over subsequent sessions.

Learning rates were quantified as the slope of the least-squares line fitting the session-by-performance data in Fig. 2A. Analysis was restricted to sessions 1-8 as these sessions did not involve food restriction (food restriction began on session 9). SNL rats were slower to adapt to the new contingency than were sham and naïve rats (Fig. 2B, one-way ANOVA $F(2,40) = 5.79$, $p = 0.006$, $\omega^2 = 0.18$).

We hypothesized that some high-reward (i.e., left lever) selections from SNL animals resulted from increased response variability. To investigate this, learning curves were re-computed by only coding high-reward selections that occurred on 3 consecutive trials, an approach that eliminated infrequent and un-sustained high-reward choices. As with the original % high-reward press measure, this measure also revealed clear differences between SNL and control groups (Fig. 2C, one-way ANOVA $F(2,40) = 8.82$, $p = 0.0007$, $\omega^2 = 0.27$). Furthermore, the effect size was considerably higher using this measure ($\omega^2 = 0.27$ vs. $\omega^2 = 0.18$), an observation that further suggests that SNL animals harbored a strong preference for the previously preferred (i.e., right) lever. These effects did not appear to result from deficits in motor activity or motivation as SNL animals responded at similar rates (Fig. 3A, One-way ANOVA $F(2,40) = 0.30$, $p = 0.74$) and completed as many trials (Fig. 3B, ANOVA, $F(2,40) = 0.67$, $p = 0.52$) as animals in the naïve and sham groups.

It was conceivable that some between-group differences were due to an anti-allodynic effect of food restriction. Contrary to this prediction, mechanical paw withdrawal responses measured in SNL rats before and during food restriction were 4.56 ± 0.64 g and 3.86 ± 0.78 g (SEM), respectively (as previously reported [51]), indicating that no anti-allodynic response was present.

Burst-like responses in SNL animals

It was conceivable that the temporal pattern of lever-press responding differed between SNL and naïve or sham-operated animals. We hypothesized that poor performance in SNL animals could be due to intermittent disengagement from the task, possibly due to distraction or fatigue and the corresponding need for rest. Specifically, we predicted that SNL animals would respond with short bouts of lever presses followed by long delays. Response sequences consisting of bouts of activity followed by refractory periods are described as ‘bursts’, and various metrics have been used to quantify bursting [61]. We predicted that the degree of bursting would correlate negatively with learning and be highest in SNL rats (i.e., more burst responses in individual rats would correspond to slower learning). Bursting was quantified using the Local Variance metric [63]. Local Variance measures the extent to which patterns of inter-trial intervals violate a Poisson random process. This measure was originally developed to measure burst activity in neurons

(Methods and Fig. 4A). According to this measure, lever presses for SNL rats, but not sham or naïve, occurred in bursts on sessions 1-8 (Fig. 4BC one-way ANOVA, $p_{d1-4} = 0.04$, $p_{d5-8} = 0.006$, $p_{d10-13} = 0.36$, Bonferroni-Holm). Contrary to our hypothesis, Local Variance was positively correlated with performance on each session in Task 3 (Fig. 4D. one-sample t-test, $p_{naïve} = 0.10$, $p_{sham} = 0.48$, $p_{SNL} = 0.007$, Bonferroni-Holm), suggesting that bursting increased with improved learning in SNL rats.

Within-session learning

Impaired associative learning could result from a reduced ability to learn from feedback within a training session. To investigate this possibility, within-session learning was quantified as the slope of a linear fit of trial number to each trial's response (high-reward response coded as 1, low-reward response coded as 0). Thus, this slope indicates the rate the preference for the high-reward arm changes per trial. Results from this analysis are presented in Fig. 5A. No difference in slopes were observed between groups at any time point (Fig. 5B, One-way ANOVA, all uncorrected p values > 0.5). All groups exhibited positive (> 0 slope) within-session learning rates during sessions 1-4 (Student's t-test, $p < 0.003$, Bonferroni-Holm), but not on days 5-8 ($p > 0.1$) or 10-13 ($p > 0.9$). This indicates that in the early trials following a contingency shift, within-session performance improvement occurred comparably across all groups. This finding suggests that SNL rats may not be impaired in their capacity to learn from immediate feedback.

Between-session learning: memory consolidation and memory retrieval

Associative learning deficits could arise from impaired ability to consolidate memories during post-training rest or an impaired capacity to retrieve memories on subsequent training days. Under this hypothesis, memory performance was quantified as the difference in performance on adjacent training sessions. This was calculated as the difference in the percent of high-reward responding during the last 17 trials on day X to the first 17 trials on day X+1. Analysis of this value, which was termed 'consolidation', revealed no group differences at any time point (Fig. 5CD, One-way ANOVA, all uncorrected p values > 0.5). The consolidation measure was < 0 for each group for days 1-4 (t-test, $p < 0.04$, Bonferroni-Holm), but was not significantly different from zero on days 5-8 ($p > 0.2$) and 10-13 ($p > 0.7$).

Variable-ratio performance

After food restriction (sessions 9-13 of Task 3), all groups expressed an equivalent and strong preference for the high-reward (left) lever (Fig. 2BC). The preference for the left lever was then reduced through training on a two-lever FR (Task 4) and cued response task (Task 5) that did not favor either lever for reward contingency or magnitude (Methods). Rats were then conditioned on a variable ratio (VR) schedule (Task 6) that favored the left lever (12 pellets per 10 presses) over the right lever (8 pellets per 10 presses) allowing assessment of the preference for the initially preferred (familiar) right lever (trained in Tasks 1 and 2). SNL animals did not learn the new contingency as they retained their preference for the initially preferred right lever (Fig. 6. t-test, $p_{SNL} = 0.52$, $p_{naïve} = 0.006$, $p_{sham} = 0.01$, Bonferroni-Holm correction) despite the continued loss of food reward. There was a main effect of group (One-way ANOVA $F(2,40) = 6.67$, $p = 0.003$, $\omega^2 = 0.21$) although post-hoc

between-group comparisons (Tukey-Kramer) only yielded a significant difference between SNL and naïve rats.

Discussion

The present study was conducted in matched groups of rats under controlled conditions to identify learning deficits associated with chronic neuropathic pain. We showed that SNL animals retain the ability to learn various reinforcement contingencies; however, in a choice task (Task 3), they were significantly slower than controls at adapting to new and more profitable behaviors. Analysis of the temporal pattern of lever presses revealed that SNL animals employed a unique strategy relative to controls whereby they responded in bursts of presses. This pattern of response may indicate a coping strategy as burst-responding correlated positively with better performance. In a variable-ratio task (Task 6), SNL animals were unable to learn a new association and perseverated on the previously learned high-reward lever at a higher rate than naïve rats. No deficits in initial learning (Tasks 1, 2), within-session learning (Task 3), memory consolidation (Task 3), or motor ability were seen. These data indicate that chronic pain engendered a selective impairment in cognitive flexibility, with SNL rats responding in bursts of lever presses followed by longer delays, a pattern that may represent a coping strategy.

When first trained to associate a lever with reward, naïve, sham, and SNL animals learn at similar rates (Tasks 1 and 2, and as previously reported in [51]), suggesting no deficit in learning the initial association and a specificity for impairment when a new association was introduced. The lack of an initial learning deficit was surprising as impaired learning and memory have been reported in a number of animal models of pain (i.e. neuropathic, inflammatory) in tasks such as the Morris Water Maze [27; 36; 37; 69] (although see [46]), novel object recognition [34; 41; 43; 46; 56; 64; 69], and Y-Maze [23; 45; 69] (although see [22]). This lack of impairment in SNL animals during the acquisition phase of the task (Tasks 1 and 2) may result from the high salience of food reward overcoming deficits in attention produced by chronic pain [16; 35]. Alternatively, Tasks 1 and 2 may represent simpler associative learning behaviors, producing a ceiling effect whereby all groups learn at high rates.

When reinforcement contingencies were altered, SNL animals were slower than sham and naïve controls at acquiring new and optimal behavioral strategies. One factor contributing to this effect may be a reduced capacity of SNL animals to sustain an optimal response strategy. This is suggested by the observation that SNL animals intermittently chose the optimal response, but reverted to the suboptimal and previously profitable lever (Fig. 2C). We suggest that these results are analogous to those found in pain patients tested in the WCST. When the discriminative stimulus for card matching is switched in the WCST of cognitive flexibility [25], pain patients persist longer on the previous rule than healthy controls [28; 65; 68] (although this did not reach significance in [48]). The preference of the SNL animals for the previously profitable lever is consistent with perseverative behavior of pain patients and suggests translational validity of this task for cognitive flexibility. The data also suggest a causal relationship between chronic pain and cognitive impairment. We note

that the mechanisms whereby chronic pain impacts cognitive processing will need further scrutiny to assess possible contributions of pain related states such as anxiety.

Interestingly, when all animals were food restricted (Sessions 9-13 of Task 3) the learning rate for SNL animals became indistinguishable from naïve and sham rats. This could be due to food restriction enhancing motivation in SNL animals; however, our data show no evidence for this as response latencies and the number of completed trials did not differ between groups. Previous reports using rats have also found no motivation deficits for reward under pain conditions (including these SNL animals) [26; 51], although motivational deficits have been seen in a mouse model [60]. Another possibility is that food restriction induced analgesia, which has been observed in an acute and chronic inflammatory pain state [24], although food restriction in our study was very mild by comparison. Further evidence that analgesia due to food restriction was not a major factor is that other studies using similar food-restriction protocols have reported effects of pain on attention [26; 53], learning and memory [10; 39], and decision making [54]. In addition, mechanical paw withdrawal responses measured in SNL rats before and during food restriction were 4.56 ± 0.64 g and 3.86 ± 0.78 g, respectively (as previously reported [51]), demonstrating no anti-allodynic effects of our food restriction protocol. Furthermore, SNL-induced tactile allodynia did not change from day 14 to day 120 post SNL [51]. A limitation of this study design is that all animals were food restricted on the ninth session, so we are unable to determine if food restriction was the cause of this recovery, or if this was the threshold number of training sessions required for SNL rats to fully acquire the new rule.

Findings from previous experiments support the observation that animal models of chronic pain have reduced executive function. For example, rodent models of chronic neuropathic pain exhibit reduced performance in a bowl-digging test of attentional set shifting [37; 38] and platform relocation in the Morris Water Maze [36; 46]. The choice task (Task 3) used in the present study had the advantage of allowing further investigation of the nature of the deficit, such as learning across training sessions and the temporal dynamics of responding. The observation of burst responding in SNL animals indicates that these animals responded reliably for short intervals followed by periods of inactivity. This could result from animals becoming intermittently distracted or disengaged. This interpretation is supported by previously reported attentional impairments in animal models of pain [7; 26; 41; 43; 53; 64] and chronic pain patients [15; 47; 52]. It was therefore surprising that burst responding in SNL animals correlated positively with learning, suggesting that burst responding could be an adaptive mechanism for overcoming pain-induced cognitive impairment. To the best of our knowledge, this is the first time that an alternative behavioral strategy to a cognitive task has been described in a pain model as a potential compensatory mechanism.

The variable ratio task studied here is similar to the rodent gambling task, a task in which animal models of pain prefer levers with infrequent high rewards that yield a lower overall return relative to levers with frequent but low reward amounts [29; 54]. Therefore, it was surprising that SNL rats in Task 6 avoided the high-reward density lever given that pain should lead to a preference for infrequent and high rewards. This could be related to the type of pain and chronicity of the pain because the previous gambling-task studies used an inflammatory pain model [29; 54] whilst this was neuropathic. From the perspective of

cognitive flexibility, the present data and data from the rodent gambling task are consistent SNL rats being impaired in shifting preference in the face of environmental pressure. Although rodent and human gambling tasks are often interpreted as tests of probabilistic decision making, many tasks demand that subjects overcome the initial preference for the high-reward and low overall return option. Thus, subjects with chronic pain may not be impaired in probabilistic behaviors per se, but in their capacity to adapt to and overcome well-learned associations.

In summary, these data indicate that animals with chronic neuropathic pain exhibit long-lasting impairment in their capacity to shift away from previously learned associations. These effects were not due to reduced motivation or physical capacity to respond, and therefore represent a selective impact of chronic pain on cognitive flexibility. Furthermore, analysis of the temporal pattern of presses indicated that SNL animals responded in bursts. This unique response pattern may reflect an adaptive response for coping with chronic neuropathic pain. Whether similar results would occur with other types of pain remain to be explored. The reduced capacity of SNL rats to adapt to changing contingencies suggests that chronic pain drives a preference for habitual responses. Habitual responding occurs during stress, whereby behavioral control is shifted away from cortical structures to the dorsal-striatum [8; 58]. Shifts from goal-directed to habitual behaviors has not previously been explored in chronic pain. Habitual responding may be a contributing factor to depression, post-traumatic stress disorder, and addiction [59]. A shift to habitual responses in chronic pain could contribute to these comorbidities. Indeed, chronic pain is strongly associated with stress [66], and stress and pain are associated with disrupted dopamine function [1; 9; 55] and reduced prefrontal volume [4; 5]), changes that could negatively impact executive function. Interestingly, chronic pain and stress may have opposing effects on synaptic reorganization within frontal circuits [40; 42; 61]. Taken together, our results suggest that chronic pain encourages a preference for familiar associations and reduces cognitive flexibility. Cognitive flexibility plays a key role in goal-directed behaviors, problem solving, and adapting to changing environments. Thus, reduced cognitive flexibility could have a far-reaching negative impact on the lives of patients with chronic pain.

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Summary

Rats with chronic neuropathic pain were impaired relative to controls in learning optimal behavioral strategies in the face of changing environmental demands. This suggests that chronic pain disrupts executive functions such as adaptive learning and cognitive flexibility. Specific changes in behavior patterns demonstrated the emergence of a novel learning strategy under conditions of chronic pain. Dysregulation of cognitive flexibility adds an important new dimension to the understanding of the pathophysiological impact of chronic pain.

Figure 1.
Timeline and behavior. A) Training timeline. Task 1: Right lever reinforced 1 pellet/press, FR schedule. The left lever was inactive. Task 2 and 3: PR schedule. One pellet delivered after ratio completion. Task 3: Contingency Shift. The left lever now delivered 4 pellets/press and the right lever delivered 1/press. Task 4: Both levers delivered 1 pellet/press. Task 5: Reward lever cued with a light. Task 6: VR task where the right lever delivered one pellet on 8/10 presses and the left (optimal) lever delivered 4 pellets on 3/10 presses. B) Performance on session 6 of Task 3. Subjects (rows) sorted by percent high-reward choices.

Figure 2.

Contingency shift performance. A) Task 3 performance as percent high-reward choices/session. B) Mean and $\pm$ SEM for high-reward choices/session for all groups. INSET: Learning curve slope for sessions 1-8. The learning rate in SNL was lower than naïve and sham groups (One-way ANOVA $F(2,40) = 5.79$, $p = 0.006$, $\omega^2 = 0.18$, Inset: * = significant comparisons following Tukey-Kramer correction). C) Learning adjusted for response variability. Percent of high-reward choices on 3 consecutive trials. INSET: Learning curve slope. SNL exhibited a reduced slope when compared to naïve and sham and the effect size was larger than in B (One-way ANOVA $F(2,40) = 8.82$, $p = 0.0007$, $\omega^2 = 0.27$).

Figure 3.

Response latencies and number of completed trials (Task 3). A) The time between each lever press is indicated on the y axis. Response rates were high during the initial sessions and decreased as training progressed. The increase was likely due to the low rate of reinforcement during the initial trials (due to ‘errors’) and the extensive training on the PR task. In addition, animals received more rewards per trial as training progressed which also slowed response times. Rates increased again following food restriction. There was no difference between groups in the rates (slopes) at which the inter-trial intervals increased from sessions 1-8 (ANOVA, $F(2,40) = 0.30$, $p = 0.74$). Mean and $\pm$ SEM. B) Number of completed trials by session. No difference between groups was found in the number of completed trials per session (ANOVA, $F(2,40) = 0.67$, $p = 0.52$). The decrease in completed trials from sessions 3-8 was likely due to satiation as animals regularly received 4 pellets per trial and were not food restricted.

Figure 4.

Temporal response patterns. A) Schematic illustration of how the Local Variance (LV) measure scales with bursting (top), Poisson (middle), or regular (bottom) leverpress responding when the average response rate is held constant. B) Local variance quantifies the temporal clustering or “bursting” of lever-presses based on inter-press intervals. Mean and $\pm$ SEM. C) Local variance on sessions 1-4, 5-8, and 10-13 (One-way ANOVA, $p_{d1-4} = 0.04$, $p_{d5-8} = 0.006$, $p_{d10-13} = 0.36$, Bonferroni-Holm). Significant between-group differences indicated as *'s, $\alpha = 0.05$. D) Local variance was positively correlated with learning high-reward arm preference in SNL rats (one-sample t-test, $p_{naïve} = 0.10$, $p_{sham} = 0.48$, $p_{SNL} = 0.007$, Bonferroni-Holm).

Figure 5.

Learning within sessions and between adjacent sessions. A) Within-session learning was quantified as slope of the regression line that fit the high (coded as 1) and low (coded as 0) lever presses on each trial. A positive slope indicates within-session learning. Mean and $\pm$ SEM. B) Analysis indicated no group differences in within-session learning at any time point (One-way ANOVA, all uncorrected p values > 0.5). All groups did exhibit positive (> 0 slope) within-session learning rates during sessions 1-4 (Student's t -test, $p < 0.003$, Bonferroni-Holm for multiple comparisons), but not on days 5-8 ($p > 0.1$) or 10-13 ($p > 0.9$). C) Between-session learning was assessed by comparing estimated performance at the end of session D to performance at the start of session D+1 as determined by regression analysis of high and low reward lever presses. D) Analysis indicated no group differences in between-session learning at any time point (One-way ANOVA, all uncorrected p values > 0.5). The measure of consolidation for all groups was below zero for each group for days 1-4

(Student's t-test, $p < 0.04$, Bonferroni-Holm for multiple comparisons), but was not significantly different from zero on days 5-8 ($p > 0.2$) and 10-13 ($p > 0.7$).

Figure 6.

VR performance. A) Percent of high-reward lever choices. Mean and $\pm$ SEM. B) Mean

learning curve slopes. Slopes were significantly > 0 for naïve and sham but not SNL

(Student's t-test, p SNL = 0.52, p naïve = 0.006, p sham = 0.01, Bonferroni-Holm).

Between-group comparisons only revealed a difference between naïve and SNL (horizontal

black line). One-way ANOVA $F(2,40) = 6.67$, $p = 0.003$, $\omega^2 = 0.21$, Tukey-Kramer

correction).