Neuropathic rats exhibit profound thermal hyperalgesia on a cortex dependent pain behavioral measure

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INTRODUCTION

• Pain perception in animals can only be determined by evaluating behavioral cues in response to some intervention. Although various methods have been used in assessing simple reflexive pain behaviors, considerably less effort has been dedicated to measure supraspinal nociceptive behaviors.

•The main objectives of this study were

1) to develop a fully automated thermal pain behavior assessment tool, named AlgoTrack, 2) to evaluate pain behavior in response to thermal innocuous and noxious stimuli, 3) in awake unrestrained rodents in a task that requires learning and cortical circuitry, and 4) to produce a more objective and quantitative measure of pain behavior in animals. The method enables characterizing the complete stimulus-response curve for all temperatures of interest in a given rat or mouse. Outcomes on AlgoTrack were compared to other standard pain assessment tools.

METHODS

• Animals & surgery

Three groups of adult Sprague-Dawley rats were used: SNI (n=10), CCI (n=8) and sham (n = 8). Adult mice (n=6) were also used.

• Pain behavioral testing

All operated animals (CCI, SNI and sham rats) were studied behaviorally from 2 weeks prior to surgery up to 4 weeks post surgery. Mechanical and cold sensitivities as well as thermal responses (using AlgoTrack, Hot Plate, and Plantar tests) were examined.

•AlgoTrack

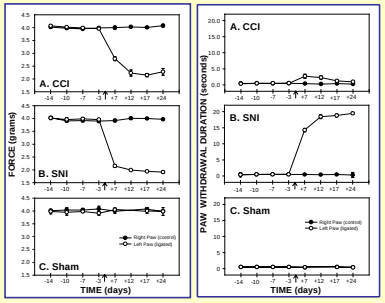
This device consists of a small rectangular box with two hemi-chambers with separate heating plates whose temperatures are computer-controlled. When the plate, where the animal is standing, is quickly heated up to the desired temperature, the animal avoids the noxious stimulus typically by escaping to the opposite non-heated side; the escape latency and temperature are automatically recorded.

1 AlgoTrack Prototype

RESULTS

2 Mechanical and cold hypersensitivities

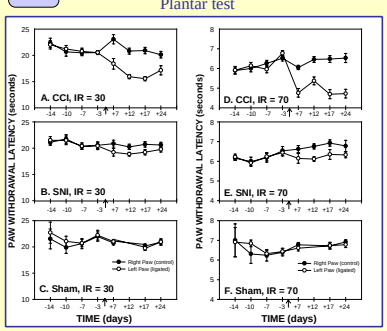
Mechanical (Von Frey filaments) | Cold (Acetone Drop Test)



Mechanical and cold sensitivity time-courses for CCI (A), SNI (B) and sham (C) rats:
Mechanical paw-withdrawal thresholds of the ligated paw were significantly attenuated after ligation (indicated by an arrow) in the CCI and SNI models as compared to the right paw (control), and to sham.
Paw withdrawal duration (seconds) to acetone applied to the ligated paw was significantly increased after ligation in SNI and CCI animals as compared to the right paw (control) and to sham.. Cold sensitivity change was smaller in CCI animals than in SNI rats.

3 Heat hyperalgesia

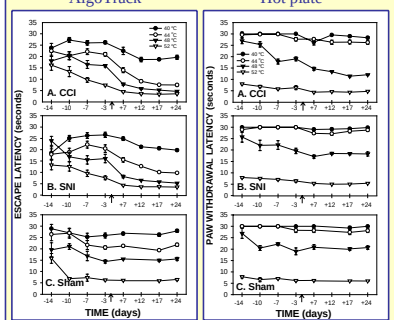
Plantar test



Time-course for the Plantar test in CCI, SNI and sham rats, performed at 2 Infra Red (IR) intensities, 30 (A, B, and C) and 70 (D, E, and F).
Paw withdrawal latencies of the ligated paw decreased in CCI and SNI rats 7 days post ligation as compared to the control (right) paw and to sham animals. Thermal hyperalgesia, as assessed on Plantar test, was much smaller in SNI than CCI rats.

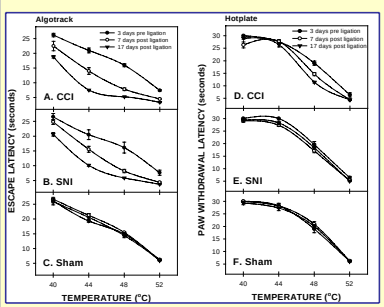
4 Heat hyperalgesia

AlgoTrack | Hot plate



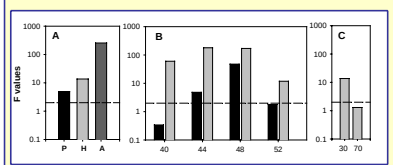
AlgoTrack and Hotplate test time-courses for CCI (A), SNI (B) and sham (C) rats.
AlgoTrack test was performed for all animals at 4 temperatures (40, 44, 48 and 52ºC). Escape latencies were significantly reduced in CCI and SNI animals at all temperatures following ligation as compared to sham, and as compared to pre-ligation. The decrease in escape latencies was maximal at day 12 post-ligation and was maintained throughout the period of testing.
The Hotplate test for CCI (A), SNI (B) and sham (C) rats, performed at 4 temperatures (40, 44, 48 and 52ºC). Paw withdrawal latency decreases were most evident in CCI rats when tested at 48ºC.

5 AlgoTrack v.s. Hotplate test



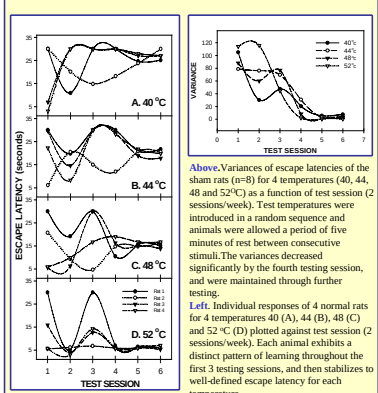
Comparison of the temperature-escape/paw withdrawal responses between AlgoTrack and Hotplate
CCI (A, D), SNI (B, E), and sham (C, F) animals were tested at 3 days prior and at 7 and 17 days post-ligation. On the hotplate test, paw withdrawal latency decreases post-ligation are small and observed mainly in CCI rats. On the AlgoTrack test, both CCI (A) and SNI (B) rats exhibit attenuation in their escape times in response to all temperatures.

6 Comparing between Plantar, Hotplate and AlgoTrack for thermal hyperalgesia for SNI animals



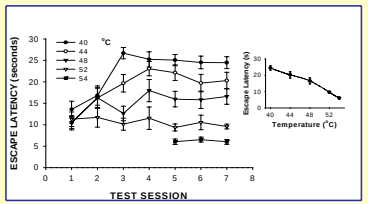
Comparison of thermal pain behavior outcomes in SNI rats between Plantar, Hotplate, and AlgoTrack tests.
A) F-values for contrasting thermal pain behavior pre-ligation (3 days prior) to post-ligation (days 7, 17 and 24) on the Plantar test (P; for both 30 and 70 IR intensities); on the Hotplate test (H; for all test temperatures: 40, 44, 48, 52 ºC); and on the AlgoTrack test (A; for the same test temperatures as on Hotplate).
B) F-values for contrasting between SNI rats and sham rats on AlgoTrack test (gray bars) and Hotplate test (black bars) in post-ligation days (7, 17 and 24), for each applied temperature indicated.
C) F-values for contrasting between SNI rats and sham rats on Plantar test in post-ligation days, for the IR intensities indicated. The y-axis is in log scale and covers 4 decades. The dashed line is for F = 2.0, which delineates threshold for significance.

7 Performance on AlgoTrack is learned behavior



Above. Variances of escape latencies of the sham rats (n=8) for 4 temperatures (40, 44, 48 and 52ºC) as a function of test session (2 sessions/week). Test temperatures were introduced in a random sequence and animals were allowed a period of five minutes of rest between consecutive stimuli. The variances decreased significantly by the fourth testing session, and were maintained through further testing.
Left. Individual responses of 4 normal rats for 4 temperatures 40 (A), 44 (B), 48 (C) and 52 ºC (D) plotted against test session (2 sessions/week). Each animal exhibits a distinct pattern of learning throughout the first 3 testing sessions, and then stabilizes to well-defined escape latency for each temperature.

8 Performance of mice on AlgoTrack test



AlgoTrack time-course for normal mice.
Algotrack was performed at 4 temperatures initially (40, 44, 48 and 52 ºC), and supplemented with an additional test temperature (54 ºC) in the last 3 sessions (2 sessions/week). Escape times stabilize after four test sessions and are maintained. The insert shows thermal stimulus-response in these mice in the last test session.

CONCLUSIONS

• We introduced a new method designed to emphasize central (cortical-cognitive) over peripheral (spinal-reflexive) aspects of the animals' response to painful thermal stimuli, and compared it with other well-established behavioral tests of pain.

•AlgoTrack requires an initial period of training. Rats and mice seem to need at least 4 testing sessions over a 2-week period to learn that escaping to the other plate eliminates the stimulus.

• The new method uncovered significant changes of heat sensitivities in a rat model of neuropathic pain.

• AlgoTrack objectively quantifies thermal pain *perception*, thus is a promising new tool to help uncover central aspects of pain (and its treatment) in animal models under a variety of conditions.

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