Does Genetic Susceptibility to Persistent Post-op Pain (Thermal Hyperalgesia) Correlate with Other Phenotypes in the Mouse Phenome Database (MPD)?

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Background

Thermal pain withdrawal after nerve injury is well accepted as a measure of experimental and clinical pain. We hypothesized that genetic susceptibility to a maladaptive response following nerve injury is due to common pathways that affect pain processing, perception, and neuropsychiatric responses (i.e., anxiety). In this study, we measured thermal hyperalgesia after nerve injury in 16 inbred mouse strains, by calculating the Persistent Pain Index (PPI). We calculated heritability and compared our PPI neurobehavior phenotype with datasets from other laboratories that measured pain and anxiety phenotypes in multiple inbred strains in the Jackson Laboratories Mouse Phenome Database (MPD).

Methods

Chronic construction injury (CCI) of sciatic nerve was used as a model of persistent post-operative pain. Briefly, the left sciatic nerve was exposed and 3 loose 6.0 silk ligatures were loosely placed around the dissected nerve. Baseline nociception and post-CCI thermal hyperalgesia were tested in each animal using a mobile infrared heat lamp device, positioned underneath the targeted hind paw. Baseline measurements were obtained two days prior to CCI surgery. Behavioral tests were performed at Baseline, Days 1, 7, 14 and 21 after CCI. The PPI was calculated as the area under the curve based on cumulative thermal measurements (Hargreaves) over 21 days. Strains of inbred mice with greater PPI are demonstrating less thermal hyperalgesia, as measured by minimal change in their withdrawal latency over time. Using the MPD, we compared our thermal PPI data with thermal hypersensitivity phenotype data from Lariviere et al.,[1] and anxiety phenotype data from O'Leary et al,[2] using Pearson coefficients.

Calculating the Persistent Pain Index

This method incorporates the Baseline adjusting for this highly variable measure across inbred strains. The PPI also calculates the area under the curve for the thermal pain responseo ver 21 days. Strains of inbred mice with minimal change in PPI are demonstrating less neuropathic pain behavior, as measured by thermal hyperalgesia. [Baseline mean thermal hypersensitivity is positively correlated with mean PPI. The Pearson correlation coefficient is 0.795 (P<0.0002). The relationship between Baseline and PPI persists when the Baseline to Day 1 interval is removed from the PPI calculation (data not shown). Nonetheless, this relationship is not entirely predictive of the thermal response after injury because those strains that have the highest and lowest PPI are both approximately mid-way in the Baseline rank order. The equation for the thermal PPI calculation was as follows:

PPI =AUC_{Total} = Auc1+Auc2+Auc3+Auc4= Withdrawal Latency (WL)

Auc1= (1-0) x (WL at Day 1 + WL at Day 0)/2

Auc2= (7-1) x (WL at Day 7 + WL at Day 1)/2

Auc3=(14-7) x (WL at Day 14 + WL at Day 7)/2

Auc4=(21-14) x (WL at Day 21 + WL at Day 14)/2

Inbred mice with a greater reduction in withdrawal latency after nerve injury over 21 days (i.e., demonstrating greater pain behavior) will produce a lower PPI. Importantly, this approach is less sensitive to daily fluctuations in response minimizing the number of animals needed for these experiments. For this important reason, we chose not to use difference from Baseline for summing the area where variability in the Baseline would have a much greater impact on the overall response

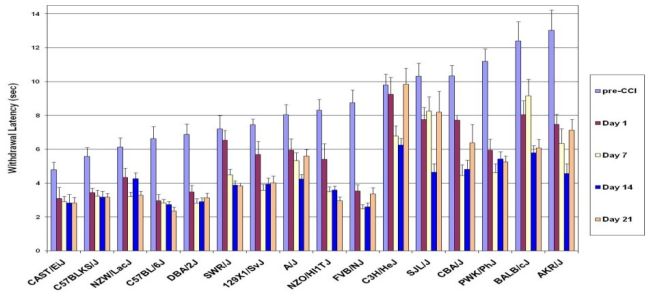

Figure 1. Strain Distribution Pattern (SDP) of Thermal Hypersensitivity After CCI Peripheral Nerve Injury. Ten-week-old male mice (n = 8-20) underwent CCI and withdrawal latency from thermal stimulation was measured on Days 1, 7, 14, and 21 after CCI. The strains were ranked left to right from the lowest to highest mean pre-CCI withdrawal latency Baseline values.

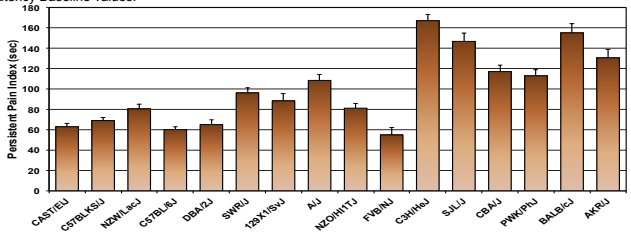

Figure 2. Thermal Persistent Pain Index (PPI) in 16 Inbred Strains of Mice. Withdrawal latency response (WL)(sec) from thermal stimulation (Hargreaves) was measured at Baseline and after CCI on Days 1, 7, 14, and 21. The PPI was calculated as the area under the curve from the Baseline response through Day 21 as four separate intervals using the trapezoid approach.

Table 1. Heritability Estimates for Thermal PPI Responses

Response Measure	Inter-Strain Variance	Intra-Strain Variance	Heritability	Effective Factors
PPI	1134	379	0.75	2.73

Table 1. Heritability is estimated as the ratio of among-strain variance to total variance (sum of inter-strain and intra-strain variances) for 16 inbred strains.

Pearson Correlation

Variable	With Variable	N	Correlation	P-value
Thermal PPI	Thermal Sciatic Nerve Ligation	7	0.29461	0.5437
Thermal PPI	Thermal Carrageenan	7	0.81766	0.0215*

Table 2. Mouse Phenome Database (MPD) comparison of thermal PPI from chronic constriction Injury (CCI) of the sciatic nerve with thermal hypersensitivity nociception data by Lariviere et al.[1]

Pearson Correlation

Variable	With Variable	N	Correlation	P-Value
Thermal PPI	EPM_openarms_av	10	-0.64554	0.0423*
Thermal PPI	EPM_closedarms	10	-0.49916	0.147
Thermal PPI	EPM_grooming	10	-0.58396	0.077
Thermal PPI	EPM_#closedarms	10	-0.45257	0.1967
Thermal PPI	EPM_fecalboli	10	0.39141	0.274
Thermal PPI	EPM_headdips	10	0.8248	0.0019*
Thermal PPI	EPM_stretchattends	10	0.23793	0.521
Thermal PPI	EPM_urinepuddles	10	0.13809	0.7131
Thermal PPI	EPM_closedarms_rat	10	-0.50075	0.1454

EPM=Elevated Plus Maze

Table 3. Mouse Phenome Database (MPD) comparison of thermal PPI from chronic constriction Injury (CCI) of the sciatic nerve with elevated plus maze anxiety data from O'Leary et al.[2].

Research Findings

1. Calculations of the thermal Persistent Pain Index (PPI) were based on thermal hypesenstivity measurements in 16 inbred strains. We found interstrain vaiability of the thermal PPI phenotype with a heritability of 75%. Calculation of effective factors was on the order of 2.73, which estimates the number of independently segregating genes with equivalent phenotypic effects that can account for the observed strain differences.
2. Comparison with two datasets in the Mouse Phenome Database (MPD) showed that there was statistically significant Pearson coefficients for the following: 1) head dipping anxiety behavior (r=0.8248, P=0.0019), 2) thermal hyperalgesia after carrageenan hindpaw inflammation (r=0.8177, P=0.0215).

Discussion

1.Thermal PPI after chronic constriction injury (CCI) is a heritable trait. When compared to another database that studied mice after thermal hypersensitivity, the thermal PPI correlated with thermal carrageen pain as opposed to neuropathic pain. This is consistent with the contention that the CCI model is characterized by inflammatory processes involved with suture ligation around the sciatic nerve.

2. The most interesting finding is the correlation with our thermal PPI data with head dipping behavior. Dalvi et al[3] reports that in mice treated with diazepam, fluamazenil only reversed head dipping behavior, suggesting that anxiolysis is mediated by both flumazenil sensitive $GABA_A$ receptors and flumazenil insensitive $GABA_A$ receptors. Future studies are underway in characterizing the genes involved in the thermal PPI phenotype and how they affect GABA receptor functioning.

References

1. Pain 2002;97:75-86
2. Behav Genet 2013;43:34-50
3. Pharmacol Biochem Behav 1999;62:727-734