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A mouse model for chronic pain-induced increase in ethanol consumption

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Abstract

Chronic pain conditions are often co-morbid with alcohol abuse. “Self-medication” with alcohol introduces a host of problems associated with the abuse of alcohol which over time has the potential of exacerbating the painful condition. Despite the prevalence of chronic pain being associated with alcohol abuse, rodent models which mimic the co-morbid conditions are lacking. In the present study, we model osteoarthritis (OA) in C57BL/6J mice by surgically destabilizing the medial meniscus (DMM). Sham operated mice served as controls. Thirteen weeks after surgery, DMM but not sham operated mice exhibited pronounced incapacitance of the surgically-manipulated hindlimb compared to the non-surgically manipulated hindlimb. At this time, the mice were exposed to the two-bottle ethanol choice, beginning with 2.5% with a gradual increasing to 20%. Compared to sham controls, DMM mice consumed more EtOH and preferred EtOH over water at the 20% EtOH concentration. Histological analysis verified that the DMM mice exhibited significant damage to the articular cartilage and osteophyte growth compared to sham controls and these measures of the severity of OA correlated with the amount of ethanol

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

intake. Thus, the combination of the DMM model of OA with the enhanced two-bottle ethanol choice is a potential preclinical approach in mice by which the basis of the co-morbid association of alcohol abuse and chronic pain conditions can be explored.

1. Introduction

Chronic pain and alcohol abuse are tremendous burdens to individuals and to society. Frequently, individuals resort to "self-medication" with alcohol in an effort to relieve pain. This is readily apparent in clinical studies which have demonstrated that approximately 28% of patients with chronic pain disorders such as arthritis also abuse alcohol [17]. It is reported that younger males suffering from arthritis are much more likely than females to self-medicate with alcohol and that this correlates with pain continuity, frequency, depression, frustration and marital status [17]. While much effort has been exerted trying to investigate the underpinning causes of chronic pain and alcohol abuse separately, relatively few studies have investigated how chronic pain might instigate alcohol abuse.

While acute alcohol has been demonstrated to provide an analgesic effect under some painful conditions [6; 19; 20], this use of alcohol is greatly limited due to acute intoxication, addiction/chronic abuse, and the introduction of maladaptations which can lead to cardiovascular disease, cancer, cirrhosis and other medical problems. Chronic alcohol abuse can also lead to tolerance which can minimize its analgesic action and exacerbate allodynic and nociceptive disorders [for review see 8; 11]. Furthermore, in trauma centers, patients who abuse alcohol are more susceptible to harmful drug interactions [1].

One limiting factor that has contributed to a lack of examination of the basis of the co-morbidity of chronic pain and alcohol abuse is the lack of an appropriate rodent model. The present study was designed to determine if using a combination of a well-established model of chronic pain and alcohol preference/consumption in mice – surgical destabilization of the medial meniscus (DMM) [7; 9; 14; 16] and the two-bottle ethanol choice [2], respectively – is appropriate for studying chronic pain-induced ethanol intake. A positive outcome would allow further investigation of this approach as an appropriate avenue to investigate the basis of the co-morbidity of pain and alcohol intake that results in alcohol abuse clinically.

2. Materials and Methods

2.1 Animals

Ten C57BL/6J male mice (Jackson) were initially housed in groups of up to 4 in plastic-bottomed cages. Animals were housed in a temperature- and humidity-controlled room under a 12:12 h light:dark cycle with lights on from 0700 to 1900 h. Access to food and water was provided *ad libitum*. Mice were group housed with up to 3 littermates until two weeks prior to incapacitance testing at which time they were individually housed. All *in vivo* experiments were conducted with a protocol approved by the Institutional Animal Care and Use Committee at The University of North Carolina at Chapel Hill. Animal use was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2 Destabilization of the medial meniscus and incapacitance testing

The DMM model of OA, initially established by Glasson et al. [9] was performed as previously described [14]. Mice were 12 weeks of age at the time of DMM or sham surgery. OA was induced by transection of the meniscotibial ligament in the right knee. The sham-operated controls underwent the same surgery except that the meniscotibial ligament was not cut. Mice were allowed free activity in their home cages after surgery.

Four days prior to incapacitance testing, each mouse was habituated to the environment, handling and restraint necessary for performance of the test. On the test day, thirteen weeks after surgery, mice were assessed for incapacitance of the operated right hindleg as previously described [3] (Figure 1A). Weight bearing was measured by the downward pressure applied to plates of the Incapacitance Meter Tester (IITC Life Science, Woodland Hills, CA., USA). The mean weight (g) of the left and right side was measured over a 5 second interval. Each measurement was repeated 10 times and the average difference in the load-bearing side with the non-surgical (contralateral) paw versus the surgical (ipsilateral) was calculated.

2.3 Two-bottle ethanol choice

Immediately following the determination of incapacitance of DMM mice versus sham controls, we exposed both groups of mice to the two-bottle ethanol choice [2]. Mice had uninterrupted access to both water and ethanol throughout the 24 day trial. Initially, mice were exposed to a concentration of 2.5% ethanol for four days (days 1–4); the concentration of ethanol gradually increased to 5.0% (4 days; days 5–8), 10% (8 days; days 9–16) and 20% (8 days; days 17–24). Each day, the bottles were weighed and placement of the two bottles were switched to control for place preference. Every week, the body weights of the mice were measured. At the end of the trial, mice were rapidly decapitated and their hindlimbs dissected for histological analysis.

2.4 Histological analysis

Hindlimbs from the operated side were dissected and processed for histological analysis as previously described [14]. In brief, hematoxylin and eosin and safranin-O/fast green mid-coronal sections of each stifle joint were obtained and one representative HE-stained section from each joint was graded by an evaluator (VU) blinded to group assignment. The evaluation included the Articular Cartilage Structure (ACS) score (0–12), synovial hyperplasia severity score in the medial compartment (0–3; 0 = 1 cell layer in synovium, 1 = 2–3 cell layers, 2 = 4–5 cell layers, and 3 = 5 or more cell layers), and osteophyte score for the medial tibial compartment (0–3) [13]. An adjacent slide (~ 70 um apart) was stained with safranin O/fast green and the Safranin-O (Saf-O) staining score (0–12) [15] for the medial tibial compartment was obtained.

2.5 Data analysis

GraphPad Prism® (version 6.07) was used to statistically analyze all data. Incapacitance test data were analyzed using a Student's unpaired t-test for differences between sham and DMM subjects. Alcohol consumption (g EtOH consumed per kg body weight) and preference (mL EtOH consumed per total mL liquid consumed) with the two bottle choice

were analyzed using two-way repeated measures analysis of variance (ANOVA). Repeated measures ANOVA were performed independently for the different concentrations of ethanol (2.5, 5.0, 10 and 20%). Alcohol consumption and preference data from day 24 at 20% EtOH concentration was not included in the final data set because of interruption of the night-time drinking cycle due to blood collection and breath analysis (data not shown). Bonferroni's multiple comparisons test was used when appropriate. A $P < 0.05$ was considered significant. Correlation between the incapacitance versus OA histological analyses and grams of EtOH consumed / body weight versus OA histological analyses were determined using Pearson's correlation coefficient. The grams of EtOH consumed in these analyses were the summed total over the entire 20% EtOH exposure. A $P < 0.05$ was considered significant.

3. Results

As a voluntary consummatory study, it was important to ensure that the body weight of the mice did not confound the volumetric intake of EtOH. Therefore, each week of the two-bottle choice test the body weight of each mouse was recorded (grams) and compared between the sham and DMM groups. We found no statistical differences between the two groups at any of the time points [week 1: Sham = 32±1, DMM = 33±5 (P=0.4816, t=0.7380, df=8); week 2: Sham = 32±1, DMM = 33±5 (P=0.8042, t=0.2563, df=8); week 3: Sham = 33±2, DMM = 35±5 (P=0.3837, t=0.9215, df=8).

3.1 DMM mice exhibit an increase in alcohol preference compared to sham surgery mice

Mice subjected to the DMM surgery exhibited pronounced incapacitance of their surgically-impaired hindlimb compared to sham controls 13 weeks post-surgery (P=0.0016, t=4.664, df=8) as confirmed by a Student's t-test (Figure 1B). The incapacitance of the mice correlated with certain measurements of the degree of OA severity in DMM and sham control mice, as measured with Safranin-O and hematoxylin and eosin staining (Figure 1C). Pearson's product-moment correlation demonstrated no significant correlation between the change in weight (g) between the contralateral and ipsilateral paw and the scores for articular cartilage structure (r=0.6255, P=0.0531) (Figure 1D), but did reach statistical significance with Safranin-O staining (r=0.8522, P=0.0017) (Figure 1E), osteophyte score (r=0.8595, P=0.0014) (Figure 1F) and the score for synovial hyperplasia (r=0.7773, P=0.0081) (Figure 1G).

Immediately following confirmation of incapacitance, mice were subjected to a two-bottle ethanol choice of gradually increasing concentrations of EtOH (2.5, 5.0, 10 and 20%) (Figure 2). Two-way repeated measures ANOVA was performed on each concentration of EtOH. A significant interaction between DMM treatment and time was measured at 10 ($F_{7,56} = 3.812$, $P = 0.0019$) and 20% EtOH ($F_{6,48} = 4.827$, $P = 0.0006$) for alcohol consumption (Figure 2A). A significant effect of DMM was measured at the 20% EtOH concentration ($F_{1,8} = 15.95$, $P = 0.0050$) and alcohol preference ($F_{1,8} = 14.66$, $P = 0.0006$) (Figure 2B). A significant increase in consumption of EtOH by DMM mice versus sham controls was confirmed on days 16 (P<0.001), 18 (P<0.0001), 20 (P<0.01), 21 (P<0.01) and 22 (P<0.01) and preference for EtOH on days 18 (P<0.0001), 20 (P<0.05) and 22 (P<0.05)

with Bonferroni's multiple comparison's test. Importantly, no differences were measured in body weight or water consumption between the sham and DMM groups at any time point (data not shown).

3.2 Severity of osteoarthritis (OA) correlates with degree of alcohol consumption

Certain measurements of the OA severity in DMM and sham control mice, as measured with Safranin-O and hematoxylin and eosin staining, correlated with the amount of alcohol consumed. Pearson's product-moment correlation confirmed a significant correlation between the grams of 20% EtOH consumed per kg body weight and the scores for articular cartilage structure ($r=0.9608$, $P<0.0001$) (Figure 3A), Safranin-O staining ($r=0.8208$, $P=0.0036$) (Figure 3B) and osteophyte score ($r=0.8608$, $P=0.0048$) (Figure 3C). The score for synovial hyperplasia did not correlate significantly with ethanol consumption ($r=0.1742$, $P=0.6304$) (Figure 3D). No significant correlation was found between incapacitance of the mice and the amount of ethanol consumed ($r=0.5144$, $P=0.1282$) (Figure 3E).

4. Discussion

In this study, we show that the DMM mouse model of OA induced a robust increase in EtOH intake and preference compared to sham controls. Mice which exhibit pronounced incapacitance of the surgically-impaired hindlimb proceeded to consume more ethanol per body weight at the highest concentration of EtOH (20%). Furthermore, significant correlations were demonstrated between EtOH intake and post-mortem analyses of articular cartilage damage and osteophyte growth –histological measurements which both determine the severity of the OA. This finding suggests the possibility that the degree of ethanol intake in mice can be predicted by the severity of OA.

A recent study by González-Sepúlveda et al. [10] in a mouse model of neuropathic pain demonstrated that partial sciatic nerve ligation also resulted in an increase in alcohol consumption and long-term anxiety- and depressive-like behaviors. However, consumption of alcohol did not attenuate nociceptive behaviors in this protocol. In a separate study by Smith et al. [18], no increase in EtOH preference with the two-bottle choice and a mouse model of chronic, inflammatory pain was observed. In this latter study, administration of Complete Freunds Adjuvant to the hindpaw did not induce an increase in ethanol intake. Taken together, current data from this and other studies suggest that mouse models of DMM-induced OA and partial sciatic nerve ligation-induced neuropathic pain, but not chronic inflammatory pain, result in an increase in alcohol intake in mice. Thus, these mouse models could possibly allow suitable studying of the co-morbidity of chronic pain and alcohol abuse.

In contrast to the study by González-Sepúlveda et al. [10], our present investigation did not assess whether EtOH ingestion reduced nociceptive behaviors. In a DMM study, anti-nociception would have been confirmed with reduced incapacitance of the DMM-impaired hindlimb. However, because this was a voluntary consummatory experiment, measuring changes in incapacitance after EtOH consumption would not have been suitable. The amount of EtOH consumed would need to be controlled in order for such a measurement of incapacitance across subjects to be valid which is not feasible with voluntary consumption.

Furthermore, while most EtOH consumption took place during the dark cycle, there were stark individual differences in the exact time of consumption. Therefore, the time of consumption would need to be controlled for to examine the analgesic effects of alcohol. Finally, the major finding of this study is that chronic osteoarthritic pain leads to enhanced EtOH consumption and this is not diminished by the lack of incapacitance measurements after EtOH consumption. However, this could make for a future direction whereby alcohol is involuntarily consumed via gavage or an intra-intestinal catheter. Then, measurements on the effects of alcohol on incapacitance could be made in a controlled manner and at the same time determine if an increase in alcohol consumption underpins an increase in the severity of OA or vice versa. In the study by González-Sepúlveda et al., they showed no significant reduction in nociceptive behaviors with alcohol – a finding which is in contrast to both rodent [5] and human studies [6; 19; 20]. However, in addition to introducing several maladaptations including cancer, liver disease, etc., chronic alcohol intake has been demonstrated to exacerbate painful conditions over time [1; 4]. Therefore, a wide breadth of scientific literature concludes that alcohol should not be used as an analgesic.

One possible confounding aspect of our data is the low EtOH intake for the sham group of C57BL/6 mice. Studies with this strain of mice have shown consumption as high as 15 g EtOH per kg body weight [21]. However, a consideration must be made to the age of the mice at the time of exposure to the two-bottle choice. In mice, adult naïve mice consume less EtOH than adolescents [12]. At the time of the two-bottle choice in our paradigm, mice are 23–25 weeks of age and lasts until 28 weeks of age. Therefore, we presume that the decrease in EtOH consumption in the sham controls compared to other studies is due to the age of the animals.

In summary, we demonstrate that following induction of OA using DMM surgery, a well characterized model of OA in mice, there is a large increase in alcohol preference compared to sham surgery mice. In addition to having positive face validity to the human co-morbid conditions of osteoarthritis and chronic alcohol abuse, it also can serve as a model for chronic pain-induced alcohol consumption in general. With this model, investigations can be made with novel analgesics that aim to eliminate or reduce alcohol abuse in chronic pain patients. Given the enormous burden to individuals and society, further studies which utilize this model to decipher the mechanisms of chronic pain-induced alcohol consumption are warranted.

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References

1. Askay SW, Bombardier CH, Patterson DR. Effect of acute and chronic alcohol abuse on pain management in a trauma center. Expert Rev Neurother. 2009; 9(2):271–277. [PubMed: 19210200]
2. Bachmanov AA, Tordoff MG, Beauchamp GK. Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin Exp Res. 1996; 20(2):201–206. [PubMed: 8730208]

3. Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, Schrier DJ, Kilgore KS. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage. 2003; 11(11): 821–830. [PubMed: 14609535]
4. Brown RA, Cutter HS. Alcohol, customary drinking behavior, and pain. J Abnorm Psychol. 1977; 86(2):179–188. [PubMed: 858835]
5. Campbell VC, Taylor RE, Tizabi Y. Antinociceptive effects of alcohol and nicotine: involvement of the opioid system. Brain Res. 2006; 1097(1):71–77. [PubMed: 16730342]
6. Cutter HS, O'Farrell TJ. Experience with alcohol and the endogenous opioid system in ethanol analgesia. Addict Behav. 1987; 12(4):331–343. [PubMed: 2825469]
7. Driscoll C, Chanalaris A, Knights C, Ismail H, Sacitharan PK, Gentry C, Bevan S, Vincent TL. Nociceptive Sensitizers Are Regulated in Damaged Joint Tissues, Including Articular Cartilage, When Osteoarthritic Mice Display Pain Behavior. Arthritis Rheumatol. 2016; 68(4):857–867. [PubMed: 26605536]
8. Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. Neurosci Biobehav Rev. 2012; 36(10):2179–2192. [PubMed: 22975446]
9. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage. 2007; 15(9):1061–1069. [PubMed: 17470400]
10. Gonzalez-Sepulveda M, Pozo OJ, Marcos J, Valverde O. Chronic pain causes a persistent anxiety state leading to increased ethanol intake in CD1 mice. J Psychopharmacol. 2016; 30(2):188–203. [PubMed: 26681793]
11. Jochum T, Boettger MK, Burkhardt C, Juckel G, Bar KJ. Increased pain sensitivity in alcohol withdrawal syndrome. Eur J Pain. 2010; 14(7):713–718. [PubMed: 20018536]
12. Kakihana R, McClearn GE. Development of Alcohol Preference in Balb/C Mice. Nature. 1963; 199:511–512. [PubMed: 14058626]
13. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, Shah M, Thompson EW. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum. 2009; 60(12):3723–3733. [PubMed: 19950295]
14. Loeser RF, Olex AL, McNulty MA, Carlson CS, Callahan MF, Ferguson CM, Chou J, Leng X, Fetrow JS. Microarray analysis reveals age-related differences in gene expression during the development of osteoarthritis in mice. Arthritis Rheum. 2012; 64(3):705–717. [PubMed: 21972019]
15. McNulty MA, Loeser RF, Davey C, Callahan MF, Ferguson CM, Carlson CS. A Comprehensive Histological Assessment of Osteoarthritis Lesions in Mice. Cartilage. 2011; 2(4):354–363. [PubMed: 26069594]
16. Miotla Zarebska J, Chanalaris A, Driscoll C, Burleigh A, Miller RE, Malfait AM, Stott B, Vincent TL. CCL2 and CCR2 regulate pain-related behaviour and early gene expression in post-traumatic murine osteoarthritis but contribute little to chondropathy. Osteoarthritis Cartilage. 2016
17. Riley JL 3rd, King C. Self-report of alcohol use for pain in a multi-ethnic community sample. J Pain. 2009; 10(9):944–952. [PubMed: 19712901]
18. Smith ML, Li J, Ryabinin AE. Increased alcohol consumption in urocortin 3 knockout mice is unaffected by chronic inflammatory pain. Alcohol Alcohol. 2015; 50(2):132–139. [PubMed: 25451237]
19. Wolff HG, Hardy JD, Goodell H. Measurement of the Effect on the Pain Threshold of Acetylsalicylic Acid, Acetanilid, Acetophenetidin, Aminopyrine, Ethyl Alcohol, Trichlorethylene, a Barbiturate, Quinine, Ergotamine Tartrate and Caffeine: An Analysis of Their Relation to the Pain Experience. J Clin Invest. 1941; 20(1):63–80. [PubMed: 16694809]
20. Woodrow KM, Eltherington LG. Feeling no pain: alcohol as an analgesic. Pain. 1988; 32(2):159–163. [PubMed: 3362554]
21. Yoneyama N, Crabbe JC, Ford MM, Murillo A, Finn DA. Voluntary ethanol consumption in 22 inbred mouse strains. Alcohol. 2008; 42(3):149–160. [PubMed: 18358676]

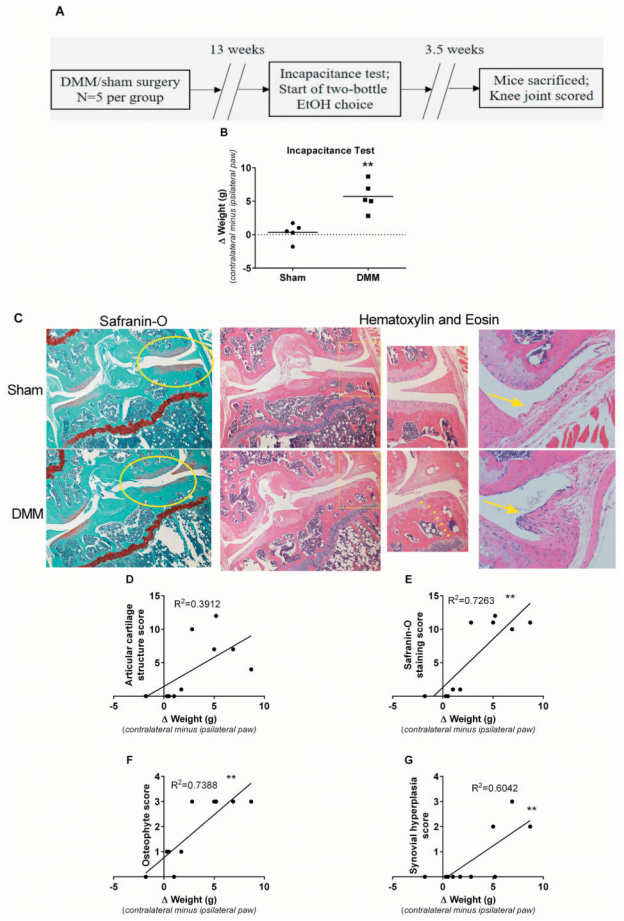

Figure 1.

Incapacitance testing of mice after surgical destabilization of the medial meniscus (DMM) or sham control surgery. (A) Timeline of *in vivo* experiments: 13 weeks after DMM or sham surgery, incapacitance of the hindlimb was measured and the two-bottle ethanol choice over 3.5 weeks (24 days) commenced. (B) Measurement of incapacitance of the surgically-impaired DMM hindlimbs compared to sham controls. N=5. **P<0.01. (C) Histological evaluation of cartilage damage (left; Safranin O/fast green staining), osteophyte growth (middle; HE staining) and synovial hyperplasia (right; HE staining) on the hindlimb of DMM and sham-operated mice. Correlation analyses of incapacitance of surgically-manipulated hindlimb with (D) articular cartilage structure score, (E) Safranin-O staining score, (F) osteophyte score but not (G) synovial hyperplasia. N=5 (correlation graphs show both Sham and DMM data points). **P<0.01.

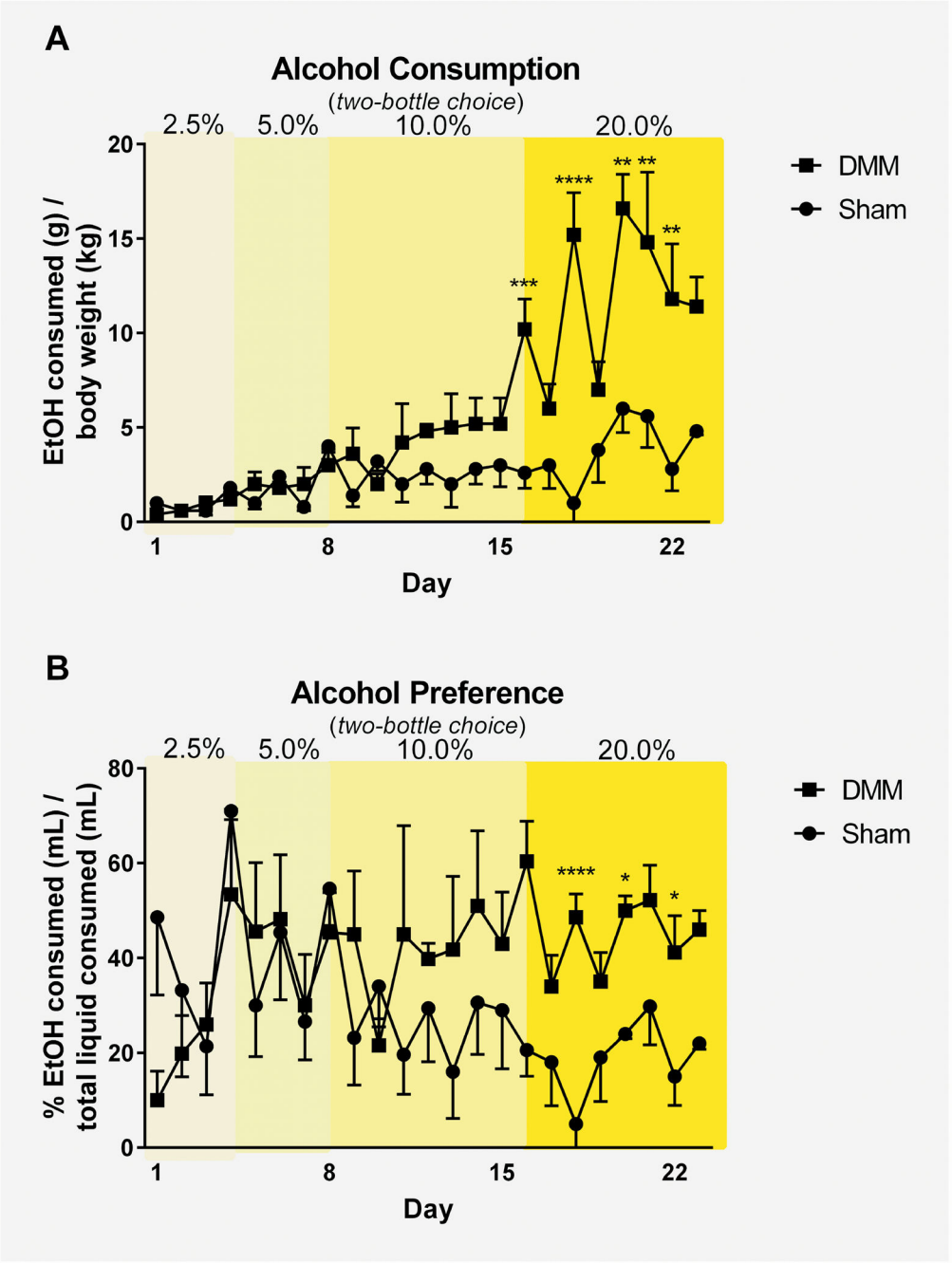

Figure 2.

Measurements of the (A) consumption of ethanol and (B) preference of ethanol versus water in DMM and sham-operated mice over the course of the two-bottle choice. Mice were given the choice between water and 2.5 (days 1–4), 5.0 (days 5–8), 10 (days 9–16), and 20% (days 17–23) EtOH. DMM mice consumed greater quantities of ethanol per kg of body weight at days 16, 18, 20, 21 and 22 and showed a greater preference for ethanol on days 18, 20 and 22 compared to sham controls. N=5. **** P<0.0001, ***P<0.001. **P<0.01, *P<0.05.

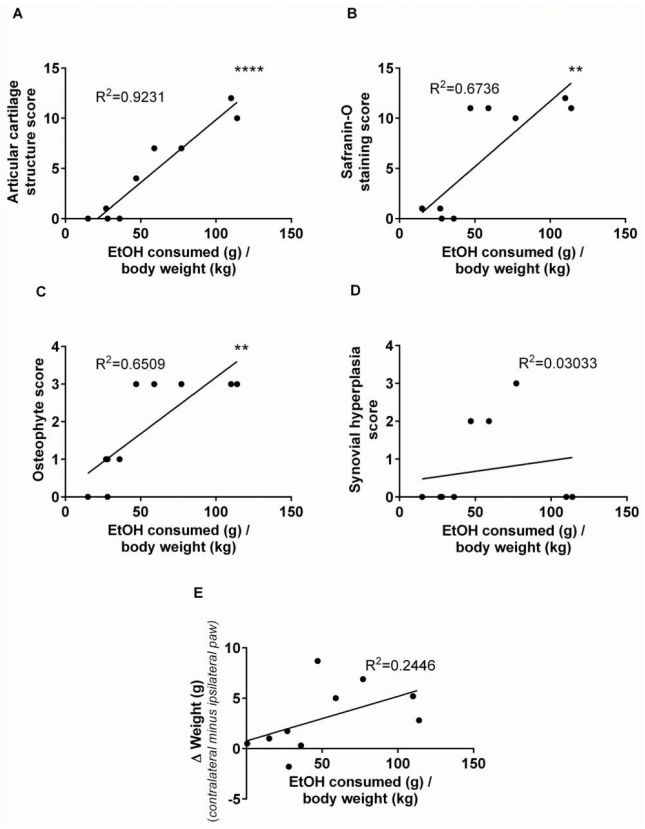

Figure 3.

The amount of 20% EtOH consumed correlated significantly with (A) articular cartilage structure score, (B) Safranin-O staining score, (C) osteophyte score but not (D) synovial hyperplasia. The amount of 20% EtOH consumed did not correlate significantly with (D) incapacitance of the surgically-manipulated hindlimb. N=5 (correlation graphs show both Sham and DMM data points). **** P<0.0001, **P<0.01.