Published in final edited form as:

J Pharm Negat Results. 2016 ; 7(1): 4–11. doi:10.4103/0976-9234.177051.

Rofecoxib-Induced Deleterious Effects Escape Detection by Organismal Performance Assays

Shannon M. Gaukler*, James S. Ruff*, Linda C. Morrison*, and Wayne Potts*

*Department of Biology, University of Utah, 257 S. 1400 E., Room 201, Salt Lake City, Utah 84112

Abstract

Background—Organismal Performance Assays (OPAs) are a unique toxicity quantification method used to assess the safety of potentially toxic compounds, such as pharmaceuticals. OPAs utilize genetically diverse wild mice (*Mus musculus*) housed in semi-natural enclosures wherein exposed individuals compete directly with controls for resources. Previously, OPAs have been successful in detecting adverse effects in mice that were exposed to paroxetine. Here, we further test OPAs utility in pharmaceutical safety assessment by testing OPAs with rofecoxib, a drug with known adverse effects in humans.

Methods—We exposed mice to rofecoxib (~37.5 mg/kg/day) during gestation and into early adulthood. Exposure ceased when individuals were released into enclosures. Five independent populations were established and rofecoxib-exposed individuals ($n = 58$) competed directly with control individuals ($n = 58$) over 28 weeks. Organismal performance was determined by quantifying reproduction, survival and male competitive ability.

Results—In enclosures, rofecoxib-exposed males had equal reproduction, survival and competitive ability. Rofecoxib-exposed females had equal survival compared to controls but experienced 40% higher reproductive output.

Conclusions—The adverse health effects of rofecoxib seen in humans escaped detection by OPAs, just as they had during traditional preclinical assays. These results may be explained by the exposure design (in enclosures, all animals were on the control diet), the relatively short duration of exposure, species differences, or because the health benefits of the drug negated the side effects. Similarly to numerous assays used in preclinical trials, OPAs cannot reveal all maladies, despite their demonstrated sensitivity in detecting cryptic toxicity from numerous exposures.

Keywords

coxib; intra-specific competition; pharmacodynamics; reproductive success; semi-natural enclosures; toxicity assessment

INTRODUCTION

Pharmaceutical development is associated with financial costs of ~\$1.4 billion per compound, and 12–15 years of research per drug are required.^[1] During human clinical trials, 73% of pharmaceuticals that passed preclinical safety assessments fail^[2] and 10% of FDA approved pharmaceuticals are recalled after market release due to unforeseen toxicity.^[3] In addition to human cost in pain, suffering and loss of life, pharmaceuticals that

fail after FDA approval can also cause substantial costs associated with litigation fees and human suffering. One potential solution to increase the detection rate of harmful compounds during preclinical trials is to employ new methodologies. We have developed a unique toxicity assessment tool, known as the Organismal Performance Assay (OPA), which has the potential to be valuable if implemented during preclinical trials.

Preclinical studies were not geared at assessing cardiac safety of rofecoxib. Rather, preclinical studies determine if a pharmaceutical of interest causes mutagenicity, carcinogenicity, teratogenicity and infertility.^[4] Rofecoxib exposure was not found mutagenic in rodent cells, nor was it found to be carcinogenic, teratogenic or cause infertility in rodents.^[4] Cardiac adversity was first suspected after market release during a phase IV clinical trial Vioxx gastrointestinal outcome research (VIGOR) study.^[5] These results were later confirmed by the adenomatous polyp prevention on Vioxx (APPROVe) study, which ultimately lead to the recall of rofecoxib.^[6]

OPAs provide a unique combination of breadth and sensitivity, and provide unambiguous information on the affects of exposures on overall Darwinian fitness. OPAs utilize genetically diverse wild-derived mice (*Mus musculus*) and treatment individuals compete directly with controls for resources, such as food, mates and nesting sites in semi-natural enclosures. The use of wild-derived mice is important as they have cohabitated with humans since the beginning of agriculture and thus demonstrate normal behaviors in man-made structures, behaviors that many laboratory strains no longer possess.^[7] Performance of individuals is measured on an ultimate level in terms of Darwinian fitness (i.e., life long reproduction and survival) and a major component of fitness (e.g., social dominance). Quantifying fitness is key in OPAs as the ultimate function of an organism's physiology is the performance of complex behaviors that facilitate reproduction.

OPAs have been used to quantify the adverse effects of a broad array of treatments. Most recently OPAs have demonstrated their sensitivity by quantifying the effects of the pharmaceutical paroxetine (Paxil®), an anti-depressant belonging to the selective serotonin re-uptake inhibitor drug class currently available on the market.^[8] Additional OPAs studies have detected adverse effects from cousin and sibling-level inbreeding, harboring a selfish gene and consuming added sugar at human relevant levels.^[8–13] In all of these studies, OPAs found substantial deleterious effects that were missed by current methodologies. OPAs are capable of revealing mammalian toxicity with high sensitivity because when wild mice live under social conditions, even small declines in behavior or physiological performance are revealed in OPA endpoint measures. As OPAs challenge all physiological systems simultaneously, reduced physiological function will be detectable by the inability of treatment individuals to perform comparable to controls.

The focus of this study was to use a drug with known adverse effects to determine if OPAs would indicate a decrease in fitness when the mice were exposed at a dose regime near therapeutic levels. We selected rofecoxib (Vioxx®, Merck; Whitehouse Station, NJ), a selective non-steroidal anti-inflammatory drug (NSAID) that was prescribed to patients to relieve arthritic pain. The FDA approved rofecoxib in 1999, but was recalled by Merck in 2004 after the drug was shown to increase the risk of cardiovascular events when patients

took the drug >18 months.^[6] During its time on the market, ~107 million prescriptions of rofecoxib were dispensed,^[14] to 80 million patients^[15] generating a revenue of \$5 billion dollars in sales.^[16] More than 27,000 cardiac events were associated with the use of rofecoxib^[17] and Merck has paid more than \$6 billion in lawsuit settlements and legal fees.^[18]

Here we use rofecoxib to further test OPAs as a broad screening tool for adverse effects by determining if rofecoxib exposure causes fitness declines in mice. If rofecoxib exposure adversely affects any physiological system, we predict that exposed individuals will suffer survival, dominance and reproduction declines relative to controls. If OPAs are successful in detecting rofecoxib-induced adversity, these results will provide additional evidence that OPAs could potentially be a powerful tool if implemented during preclinical studies.

MATERIALS AND METHODS

Animals

Genetically diverse, wild-derived house mice were used in this experiment. Wild mice possess natural behaviors needed to function within semi-natural environments.^[7] The wild mice used in this experiment were from the 12th generation of the colony described in Meagher et al.^[10] Genetic diversity of this colony was assessed in the 11th generation and found to be comparable to wild populations.^[19] Animals were provided food and water *ad libitum* and maintained on a 12:12 hr light:dark cycle. The University of Utah IACUC approved all procedures and protocols.

Drug exposure

Dosing was achieved by incorporating 12.5 g of rofecoxib (AK Scientific Inc. Union City, CA; Molecular formula: C₁₇H₁₄O₄S) into 50 kg of rodent chow (TD.130006; Harlan Teklad, Madison, WI). As wild mice eat approximately 3 g per day and weigh approximately 20 g^[20] individuals will ingest ~0.75 mg of rofecoxib per day or ~37.5 mg/kg/day. Using a metabolic rate conversion factor, this is equivalent to a human dose of ~3.0 mg/kg/day, or a daily dose of ~182.4 mg, assuming the average human weighs 60 kg.^[20] Rofecoxib was prescribed at doses 12.5 – 50 mg/d;^[4] thus animals in this experiment were exposed to a dose ~3.5 fold higher than human therapeutic dose, but is within the range of doses in preclinical studies.^[4]

Sixty breeding pairs were selected for this experiment and divided into two treatments, rofecoxib-exposed or control. All breeders were individual housed eight days prior to pairing; females in the rofecoxib treatment started exposure at this time while males in the rofecoxib treatment started exposure five days prior to pairing. Breeding pairs were kept together until a maximum of three litters were produced. All offspring were weaned at 28 days of age, standard for our wild derived mice, and housed with same sex siblings. Upon weaning, litter size, sex and weight were recorded. Offspring were kept on their respective treatment until adults and released into the OPA enclosures; this duration of rofecoxib exposure maximized the ability of OPAs to detect health consequences as once released into the semi-natural enclosures, all animals were fed the control treatment. Currently, there is no

way to keep animals on their respective treatments while they are free ranging during OPAs and switching the rofecoxib-exposed animals to the control treatment was a more conservative approach of detecting fitness impacts rather than switching the control individuals to rofecoxib.

OPA enclosures

Enclosures have previously been described in Ruff et al.^[12] Briefly, there are 11 independent enclosures ~30 m². Each enclosure is divided into six territories by hardwire mesh that is easily climbed, but adds a component of spatial complexity. Additionally, each enclosure had four optimal territories and two suboptimal territories. Optimal territories consisted of a large storage bin with multiple dark nesting sites and direct access to food whereas suboptimal territories consisted of light-exposed nesting sites (Figure 1). All territories contained *ad libitum* access to food and water and were kept on a 12:12 hr light:dark cycle.

Five independent OPA populations were established and four were maintained for 28 weeks. One population was terminated at 11 weeks due to 100% control male mortality. Populations consisted of 8–10 males and 12–18 females for a total of 116 animals (42 male, 74 female); these animals are referred to as founders. Half of the mice of each sex were from the rofecoxib-exposed treatment while the remaining half served as controls. The population structure allowed for direct competition between treatment and control individuals. Enclosure space and population size created a population density reported within the range observed in the wild.^[21]

Upon release into enclosures male mice had a mean age of 16.31 (SD ± 5.24) weeks old and females were 15.34 (SD ± 5.31) weeks old. To allow males to establish territories and prevent incidental matings, males from both treatments were released into the enclosures with non-experimental females. One week later, non-experimental females were removed and replaced with experimental females from both treatments. To eliminate any inbreeding, no populations contained opposite sex individuals related at the cousin level or below. Three populations consisted of one set of brothers, four populations consisted of one or two sister pairs, four populations consisted of sister triplets and one population consisted of sister quadruplets. When relatedness occurred, it was balanced between treatments. Populations in the wild typically contain related individuals.

Reproductive success

Founder reproductive success was determined by genetically analyzing offspring born in the enclosures. Offspring were first removed at week eight, then during five-week intervals, referred to as pup sweeps. This time interval, prevented offspring from reaching sexual maturity and from confounding the reproductive success data. During pup sweeps, offspring were removed, sacrificed and a tissue sample was collected for genetic analyses. A total of 1,138 samples were collected with a mean of 227.60 ± 104.50 (M ± S.D.) offspring per population.

A population-level approach was used to determine reproductive success in three of the five populations, this approach is extensively described in Meagher et al.^[10] Briefly, founder individuals from each treatment were selected upon non-overlapping sex specific allelic

variants, females upon the mitochondrial genome and males on the Y-chromosome. To control for confounding effects, such as segregating genes linked with the markers, reciprocal markers were assigned across populations. Mitochondrial genotypes were assessed in 860 samples (three of five populations) and obtained for 100% of offspring. Of the 1,138 offspring, 570 Y-chromosome genotypes were obtained (from all five populations), suggesting that 100% of all males were typed if the sex ratio was 1:1. In one of the populations not typed with the above method, female reproductive success was determined by parentage analysis using multiple microsatellite loci to gain more knowledge for another study and has been extensively described in the supplementary information. The final population was not genotyped as the results of this population would not have influenced the overall trends of female reproductive success results to meet our predictions.

Male competitive ability

Prior to OPA release, all individuals received a PIT (passive integrated transponder) tag (TX1400ST, BioMark, Boise, ID) and a unique ear punch for identification purposes. Two sets of PIT antenna and readers (FS2001F-ISO, BioMark, Boise, ID) were used in this experiment and were rotated twice per week among concurrent populations. PIT tag antennas were placed above each feeding station within the six territories of an enclosure. All PIT tag data were downloaded to a computer containing data logging software (Minimon, Culver City, CA). A male was considered territorial occupant (dominant) in a territory if > 80% of the total reads belonged to him at a particular location. PIT tag data were collected on female mice, however was not analyzed due to the lack of information on female dominance behavior.

Survivorship

Survivorship was assessed by daily non-invasive health checks and extensive enclosure checks during pup sweeps. Extensive checks were not performed at a higher frequency as to not disrupt territoriality formation, which increases infanticidal behavior. Research personnel entered enclosures only to freshen water, fill feeders, rotate PIT tag readers, remove deceased individuals and to conduct pup sweeps. Deceased founders were identified by PIT tag IDs. The date of death was estimated upon the condition of the corpse. Individuals that had died long before research personnel discovered it were given a death date half way between the date it was found and the last date it was read by PIT tag readers.

Statistical analyses

Linear mixed models (LMMs) were used to analyze wean weight and body weight in OPAs as these data are continuous and normally distributed. Generalized linear mixed models (GLMMs) were used to analyze litter size, reproductive success and male competitive ability as these data are discrete counts and conform to either a Poisson distribution (litter size and reproductive success) or binomial distribution (male competitive ability). To use a GLMM with a Poisson distribution, data are logarithmically transformed thus standard errors (SE) are asymmetric as values reported in the results section have been back-transformed. Both LMMs and GLMMs were conducted in R 3.0.2 using the lme4 library.^[22, 23] P-values were calculated for LMMs with the Swatterthwaite approximation under the lmerTest function.^[24] Cox proportional hazard models (PH) were used for survivorship; therefore, χ^2

were calculated to inform p-values in conjunction with degrees of freedom (JMP 9.0.3, SAS institute Inc., Cary NC). A complete description of statistical analyses can be found in the supplementary information.

RESULTS

Breeding cage

No difference in wean weight was detected due to rofecoxib exposure (LMM; female offspring, $t = -0.80$, $P = 0.42$; male offspring, $t = -1.28$, $P = 0.20$). At the model intercept (litter one), rofecoxib-exposed female offspring weighed on average of 10.60 g (SEM ± 0.66), while controls weighed an average of 11.13 g (± 0.33). No effect of time (LMM; $t = -0.45$, $P = 0.65$) or time by treatment (LMM; $t = 0.76$, $P = 0.45$) was detected. Rofecoxib-exposed male offspring weighed on average 11.05 g (± 0.81) and controls weighed on average 12.09 g (± 0.37). There was a significant increase in male weight over time (LMM; $t = 2.85$, $P < 0.01$), but this trend was consistent across treatments (LMM; $t = 0.04$, $P = 0.97$). For a complete readout of mixed model results for breeding cage data, see supplementary Table 1.

A trend was detected where rofecoxib-breeders produced smaller litters (GLMM; $z = -1.70$, $P = 0.09$; Figure 2). In first litters (model intercept), rofecoxib-exposed breeders produced an average of 3.09 pups (SEM $+0.69$, -0.57), while control breeders produced 4.36 pups ($+0.48$, -0.43). No effect of time (GLMM; $z = 0.30$, $P = 0.77$) or time by treatment (GLMM; $z = 1.08$, $P = 0.28$) was detected; suggesting the trend of reduced litter size was consistent over time.

OPA measurements

Rofecoxib-exposure did not affect female body weight at the model intercept (week zero of LMM; $t = 0.21$, $P = 0.83$; Figure 3A). Rofecoxib-exposed females weighed on average 19.68 g (SEM ± 1.02) and controls weighed on average 19.47 g (± 0.97). Females gained weight over time, likely due to pregnancy (LMM; $t = 12.02$, $P < 0.0001$), and this effect was consistent between treatments (LMM; $t = -0.81$, $P = 0.42$). Rofecoxib-exposed males showed a marginally significant trend of reduced weight at the model intercept (week zero) when compared with controls (LMM; $t = -1.78$, $P = 0.08$; Figure 3B). Rofecoxib-exposed males weighed on average 20.13 g (± 0.73) and controls weighed on average 21.43 g (± 0.51). Males from both rofecoxib and control treatments gained weight over time (LMM; $t = 4.54$, $P < 0.0001$) and a treatment by time effect was detected (LMM; $t = 1.983$, $P < 0.05$), suggesting that rofecoxib-exposed males gained more weight over time compared to controls. For a complete readout of mixed model outputs for enclosure weight data, see supplementary Table 2.

Rofecoxib-exposure increased female reproductive success by 40% relative to controls. At model intercept (week eight) rofecoxib-exposed females had significantly more offspring than controls (GLMM; $z = 3.89$, $P < 0.0001$; Figure 4A) with a mean of 28.76 offspring per population (SEM $+3.44$, -3.07). Control females had a mean of 18.53 offspring per population ($+4.24$, -3.44). No effect of time (GLMM; $z = 0.39$, $P = 0.70$) or time by

treatment interaction (GLMM; $z = 1.20$, $P = 0.23$) was detected; suggesting rofecoxib-exposed females had more offspring throughout the duration of the study. For a complete readout of mixed model results for reproduction in enclosures and competitive ability, see supplementary Table 3.

Rofecoxib exposure did not impact male reproductive success (GLMM; $z = 0.19$, $P = 0.85$; Figure 4B). At model intercept (week eight) rofecoxib males sired on averaged 10.31 male offspring (SEM +1.87, -1.41) per population. Control males had an average of 10.03 male offspring (+1.96, -1.64) per population. No effect of time was detected (GLMM; $z = 1.38$, $P = 0.17$). A trend was detected in which rofecoxib-exposed males produced fewer male offspring over time (GLMM, $z = -0.05$, $P = 0.09$).

Male competitive ability was not impacted by treatment. At week three (model intercept) control males occupied 30% of territories, rofecoxib-exposed males occupied 30% leaving 40% of territories unoccupied (GLMM; $z = 0.08$, $P = 0.94$; Figure 5). The percent of undefended territories is not unusual because 2/6 (or 33%) of the territories are suboptimal and often difficult to defend. There was a marginally significant increase in the number of territories being occupied over time (GLMM; $z = 1.72$, $P = 0.09$); but no time by treatment interaction occurred (GLMM; $z = -0.27$, $P = 0.79$), suggesting that males from both treatments occupied more territories over time.

Control females ($n = 74$) had increased mortality, however, the data were not analyzed due to overall low mortality; only two control females died while 100% of rofecoxib-exposed females survived (Figure 6A). No significant differences were detected in male mortality between treatments (PH; $\chi^2 = 0.04$, $P = 0.83$; Figure 6B). Mortality rate did not differ in replicate populations (PH; $\chi^2 = 4.85$, $P = 0.30$), nor was there a difference in the effect of treatment among populations (PH; $\chi^2 = 2.92$, $P = 0.57$).

DISCUSSION

In cages, rofecoxib exposure did not affect wean weight of offspring, but a trend was detected where rofecoxib-exposed litters were smaller than control litters. In enclosures, rofecoxib-exposed males had equal reproduction, survival and competitive ability. In enclosures, rofecoxib-exposed females also had equal survival compared to controls, but experienced 40% higher reproductive output.

One possible explanation for our observed results is that the drug did not cause lasting deleterious effects and after exposure ceased, allowing exposed individuals to rebound to average fitness. A second explanation is perhaps animals in this experiment were not exposed to rofecoxib long enough to induce fitness effects. Animals were exposed to rofecoxib during gestation and to ~15–16 weeks of age whereas cardiac adversity was not detected in humans until after taking the prescription for >18 months. Another possible explanation is that although cardiac events occurred in 27,000 people, an estimated 80 million people took the drug,^[15] suggesting < 0.4% of people who took the drug had a cardiac event. Due to such a small overall percentage of cardiac adversity in humans, this effect would not have been statistically detectable in five populations of mice with a total of

116 individuals. Lastly, perhaps rofecoxib-exposure does not cause the same adverse effects in mice as it did in humans and thus differences between species may be why adverse effects were not detected by OPAs. Rofecoxib-exposed litters tended to be smaller when born in cages. This result is in line with previous research on NSAIDs that have shown these drugs can cause transient infertility,^[25] as prostaglandin synthesis is important for normal ovulation.^[26] Disruption of prostaglandin synthesis was also suggested to be the cause of increased embryo loss in rofecoxib-exposed rats during preclinical studies.^[4]

Although rofecoxib-exposed litters tended to be smaller in cages, rofecoxib-exposed females had 40% more offspring when compared to controls when they were in enclosures. These contrasting results are interesting because the main variable that changed was the type of environment these animals were in and because increased reproduction was detected in a treatment where the immune system is targeted. Tradeoffs exist between pregnancy and the immune system and because rofecoxib suppresses the immune system, perhaps less energy was allocated towards immune function and more energy was allocated to reproduction.^[27] The fact that we detected a positive impact on female reproduction within a semi-natural environment does indicate that normal physiological systems were being disrupted. Thus, such results could be treated as a red flag during pharmaceutical development, triggering further investigation into what physiological system was displaying abnormal behavior.

CONCLUSION

The adverse health effects of rofecoxib seen in humans escaped detection by OPAs; however, rofecoxib-induced adverse effects were also missed by current preclinical methodologies. These results may be explained by the exposure design (animals competing in enclosures were removed from rofecoxib prior to release into semi-natural enclosures), the relatively short duration of exposure, species differences, or because the health benefits of the drug negated (or outweighed in the case of female reproduction) the side effects. Similar to all other assays used in preclinical trials, OPAs cannot reveal all maladies, despite their demonstrated sensitivity in detecting cryptic toxicity from numerous exposures. OPAs ability to detect adverse health outcomes in a variety of other treatments, suggests their implementation during pharmaceutical development would be beneficial. In addition to the proximate-level animal studies, OPAs would be useful because they quantify fitness on an ultimate level that provides clear interpretation of overall health. This is achieved because the nature of the OPA is that it challenges most physiological systems simultaneously and consequently toxicity that reduces performance of any physiological system (e.g., cardiorespiratory, metabolic or neurological) is likely to be detected in the endpoint measures of this assay (such as survival and reproductive success). Further development of the OPA as a tool for drug safety evaluation is needed, such as evaluating additional pharmaceuticals that have known adverse effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Miller H. How is the FDA really doing? Genet Eng Biotechnol News. 2012; 32:6–8.
2. Lipsky MS, Sharp LK. From idea to market: the drug approval process. J Am Board Fam Pract. 2001; 14:362–7. [PubMed: 11572541]
3. Schuster D, Laggner C, Langer T. Why Drugs Fail - A Study on Side Effects in New Chemical Entities. Curr Pharm Des. 2005; 11:3545–3559. [PubMed: 16248807]
4. Merck. VIOXX® (rofecoxib Tablets and Oral Suspension). [updated 2002, cited 2014 April]. Available from: http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4090B1_14_O-FDA-Tab-I-1.htm
5. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, et al. Comparion of Upper Gastrointestinal Toxicity of rofecoxib and Naproxen in Patients with Rheumatoid Arthritis. N Engl J Med. 2000; 343:1520–1528. [PubMed: 11087881]
6. Keane, W. Merck Announces Voluntary Worldwide Withdrawal of VIOXX. [updated 2004, cited 2014 April]. Available from: <http://www.pbm.va.gov/vacenterformedicationsafety/vioxx/DearHealthcareProfessional.pdf>
7. Nelson AC, Cauceglia JW, Merkley SD, Youngson NA, Oler AJ, Nelson RJ, et al. Reintroducing domesticated wild mice to sociality induces adaptive transgenerational effects on MUP expression. Proc Natl Acad Sci. 2013; 110:19848–53. [PubMed: 24248373]
8. Gaukler SM, Ruff JS, Galland T, Kandaris KA, Underwood TK, Liu NM, et al. Low-dose paroxetine exposure causes lifetime declines in male mouse body weight, reproduction and competitive ability as measured by the novel organismal performance assay. Neurotoxicol Teratol. 2015; 47:46–53. [PubMed: 25446017]
9. Carroll LS, Meagher S, Morrison L, Penn DJ, Potts WK. Fitness effects of a selfish gene (the Mus t complex) are revealed in an ecological context. Evolution. 2004; 58:1318–1328. [PubMed: 15266980]
10. Meagher S, Penn DJ, Potts WK. Male-male competition magnifies inbreeding depression in wild house mice. Proc Natl Acad Sci. 2000; 97:3324–9. [PubMed: 10716731]
11. Ilmonen P, Penn DJ, Damjanovich K, Clarke J, Lamborn D, Morrison L, et al. Experimental infection magnifies inbreeding depression in house mice. J Evol Biol. 2008; 21:834–41. [PubMed: 18312317]
12. Ruff JS, Suchy AK, Hugentobler SA, Sosa MM, Schwartz BL, Morrison LC, et al. Human-relevant levels of added sugar consumption increase female mortality and lower male fitness in mice. Nat Commun. 2013; 4 doi: 10.1038/ncomms3245.
13. Ruff JS, Hugentobler SA, Suchy AK, Sosa MM, Tanner RR, Hite M, et al. Compared to sucrose, previous consumption of fructose and glucose monosaccharides reduces survival and fitness of female mice. J Nutr. 2015; 145
14. Krumholz HM, Ross JS, Presler AH, Egilman DS. What have we learnt from Vioxx? Br Med J. 2007; 334:120–3. [PubMed: 17235089]
15. Jüni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet. 2004; 364:2021–9. [PubMed: 15582059]
16. Drazen JM. COX-2 Inhibitors - A Lesson in Unexpected Problems. N Engl J Med. 2005; 352:1131–1132. [PubMed: 15713947]
17. Horton R. Vioxx, the implosion of Merck, and aftershocks at the FDA. Lancet. 2004; 364:1995–6. [PubMed: 15582041]
18. Loftus, P. Merck Books \$950 Million Vioxx Charge. [updated 2010, cited 2014 April]. Available from: <http://online.wsj.com/article/SB10001424052702304155604575581923223447954.html>
19. Cunningham CB, Ruff JS, Chase K, Potts WK, Carrier DR. Competitive ability in male house mice (Mus musculus): genetic influences. Behav Genet. 2013; 43:151–60. [PubMed: 23291957]
20. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. Fed Am Soc Exp Biol J. 2008; 22:659–61.
21. Sage, R. The Mouse in Biomedical Research. Academic Press; New York: 1981. p. 40-90.

22. Bates D, Maechler M, Bolker B, Walker S, Christensen R, Singmann H. lme4: linear mixed-effects models using Eigen and S4. R Packag version. 2014(1):1–5.
23. R Development Core Team. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria. 2013
24. Kuznetsova A, Brockhoff R, Christensen R. lmerTest: Tests for random and fixed effects for linear mixed effects models (lmer objects of lme4 package). R Packag version. 2013:2.0–3.
25. Østensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. *Arthritis Res Ther*. 2006; 8:209. [PubMed: 16712713]
26. Sugimoto Y. Failure of Parturition in Mice Lacking the Prostaglandin F Receptor. *Science*. 1997; 277:681–683. [PubMed: 9235889]
27. Sheldon BC, Verhulst S. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol*. 1996; 5347:317–321.

Figure 1.

Figure 3.

Figure 4.

Figure 5.

Figure 6.