AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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David A. Ricks
Chairman and CEO
Eli Lilly and Company
Via email: ricks_david@lilly.com

Melissa Stapleton Barnes
Senior Vice President, Enterprise Risk Management, and Chief Ethics and Compliance Officer
Eli Lilly and Company
Via email: barnes_melissa_s@lilly.com

Dear Chairman Ricks and Senior Vice President Barnes,

On behalf of People for the Ethical Treatment of Animals (PETA) and our more than 6.5 million members and supporters, I am writing to ask that Eli Lilly and Company (Eli Lilly) discontinue use of the Forced Swim Test (FST) in its behavioral experiments involving animals.

Since 1993, Eli Lilly has published at least 19 manuscripts and submitted at least 11 patent applications that describe the use of the FST in experiments involving mice and rats. I have listed these references below. In publications, Eli Lilly-affiliated authors have described the FST as a model or test of behavioral despair[i] and a test capable of demonstrating antidepressant activity, effects, qualities of compounds being tested.[ii] However, the applicability of an animal's behavior during the FST to their mood, or to human depression, or to the utility of a compound for treating human depression has been refuted. A thorough discussion of this matter is presented in the document, "The Invalidity of the Forced Swim Test" (attached).

In brief, animals, typically mice or rats, are made to swim in a cylinder of water. They swim frantically, trying to find an escape, until they stop struggling and subsequently float. The claim is that when mice spend more time floating, they are deemed to be more "depressed." This claim is made in spite of the evidence that floating is actually a learned and adaptive behavior, one that saves energy and is beneficial for survival.[iii] Individual animals who are quicker to float also save energy and are less likely to sink, meaning that animals who more rapidly pick up on this reality, and spend less time struggling, are simply learning this adaptive behavior more readily.

Some claim that the forced swim test is a screening tool for antidepressant activity, since, sometimes, mice who are given human antidepressant drugs will swim more and float less. However, the immobility response also occurs after treatment with drugs that do not have antidepressant effects at all, such as antihistamines, caffeine, and other miscellaneous drugs.[iv] Time spent swimming vs. floating is also influenced by the genetic strain of an animal and experimental variances, such as water depth or temperature.[v]

Affiliates:
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Six[vi] compounds identified in Eli Lilly's published animal experiments, beginning in 1993, have been tested in humans. For only two[vii] of these compounds did the authors' *interpretation* of an animal's behavior during the FST predict a *potential* efficacy or inefficacy of the compound's antidepressant-like action in humans; however, *none* of the compounds identified is currently approved as a treatment for human depression. For over half of the compounds identified, the authors' interpretation of what an animals' behavior during the FST means for humans, or the efficacy of the compound in human depression, was *not* corroborated in human trials. **This data suggest that, for the past 25 years of your studies, a *certain interpretation* of an animal's behavior during FST will predict the *potential* efficacy of a compound for use in human depression only 33 percent of the time, which is less than chance (50 percent), and so far has a zero percent chance of predicting the successful use of a compound for human depression during this time**.

In published papers, Eli Lilly-affiliated authors have described attempts to improve the reliability and translatability of the FST. In 2003, Bai and colleagues found that two strains of mice, C57Bl/6 and NIH-Swiss, were different in their behavior during the FST and concluded that the "neural circuitry mediating this behavior in these tests is not identical" between even these strains of the same species.[viii] The authors cite 1988 and 1992 studies that demonstrate that the FST can correctly identify compounds that are already known to be antidepressants with around 90 percent accuracy. However, the ability to identify classic antidepressants does not take into account the FST's likeliness for both Type I and Type II errors when testing novel compounds. How many promising compounds has Eli Lilly kept on the shelf because they do not give the desired results in the FST? Despite continued and widespread use of the FST in basic research and preclinical trials, novel antidepressants to treat patients who do not respond to the definitive drug classes are yet to be approved.

In 2017, Eli Lilly-affiliated Yuen and colleagues published experiments that attempted to make the FST more quantitatively translatable to humans in regards to dosing of compounds. They found that "human doses can be over-or under-predicted by many fold when using the traditional approach" of dose estimation.[ix] The authors noted other limitations of the FST, such as the fact that rats are "generally less sensitive than mice in the FST when given the same doses of a drug."[x] Over the years, experimenters have tweaked the FST to fit what they wanted it to show. They have added elements such as a pre-test, increased the types of behaviors measured during the test, and changed other arbitrary aspects of the experimental setup until the results fit their hypotheses or the animal they wanted to use.[xi] Would it not be more scientifically, and ethically, valid to instead look for a validation method with construct validity instead of subjecting animals to unnecessary torment?

There is a clear need to develop new therapeutics to treat human depression. Only small numbers of patients respond to available treatments, which themselves have severe shortcomings.[xii] However, the use of animal experiments in an effort to generate these treatments has been criticized as a major contributor to failure rates in this area.[xiii] Animal models of human depression lack many important aspects of model validity. Hendrie and Pickles argue that multiple failures on the part of animal experimenters are to blame for lack of progress in this field, namely falling trap to "logical flaws" and "false assumptions."[xiv]

The FST is so traumatic to animals that it is often used as a stressor in itself,[xv] in an effort to create a sense of helplessness. To quote Dutch animal behaviorists Franz Josef van der Staay, Saskia S. Arndt, and Rebecca E. Nordquist, "If evidence accumulates that the intended goal/purpose cannot be reached, then one should consider abandoning further development of the model."[xvi] This group also pointed out

that in all cases, "benefits must outweigh the ethical costs of the animals. These costs include pain and suffering, distress and death."[xvii]

In summary, the FST does not reliably predict successful novel treatments for human depression—nullifying any scientific justification for carrying out the test; and it causes acute suffering and distress to the animals who are used—presenting a compelling ethical argument against using the test. We therefore ask that Eli Lilly immediately discontinue its use of the FST in behavioral experiments involving animals.

May we meet to discuss this important matter?

Sincerely,

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Use of the Forced Swim Test by Eli Lilly and Company

1. Benvenga MJ, Leander JD. Antidepressant-like effect of LY228729 as measured in the rodent forced swim paradigm. *European Journal of Pharmacology*. 1993;239:249-252.
2. O'Neill, Michael, inventor; Eli Lilly and Company Limited, assignee. Use of moxonidine for the treatment of depression. WO1999020278A1. April 29, 1999.
3. Perry, Kenneth Wayne, inventor; Eli Lilly and Company, assignee. Potentiation of pharmaceuticals. US006000043A. May 23, 2000.
4. Bai F, Li X, Clay M, Lindstrom T, Skolnick P. Intra- and interstrain differences in models of "behavioral despair." *Pharmacology, Biochemistry and Behavior*. 2001;70:187-192.
5. Li X, Tizzano JP, Griffey K, Clay M, Lindstrom T, Skolnick P. Antidepressant-like actions of an AMPA receptor potentiator (LY392098). *Neuropharmacology*. 2001;40:1028-1033.
6. O'Neill MF, Osborne DJ, Woodhouse SM, Conway MW. Selective imidazoline I2 ligands do not show antidepressant-like activity in the forced swim test in mice. *Journal of Psychopharmacology*. 2001;15(1):18-22.
7. Role of 5-HT(1A) and 5-HT(1B) receptors in the mediation of behavior in the forced swim test in mice. *Neuropsychopharmacology*. 2001;24:391-398.
8. Skolnick, Phil, inventor; Eli Lilly and Company, assignee. Combination therapy for treatment of depression comprising an antidepressant and an ampa receptor potentiator. WO 01/89530A1. May 11, 2001.
9. Knobelsdorf, James Allen, inventor; Eli Lilly and Company, assignee. (bis)sulfonamide derivatives. PCT/US01/10829. May 23, 2001.

10. Skolnick, Phil, inventor; Eli Lilly and Company, assignee. Combination therapy for treatment of depression. US 2003/0092770A1. May 15, 2003.
11. Li X, Witkin JM, Need AB, Skolnick P. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell and Molecular Neurobiology*. 2003;23(3):419-430.
12. Li X, Need AB, Baez M, Witkin JM. Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *The Journal of Pharmacology and Experimental Therapeutics*. 2006;319:254-259.
13. Gehlert DR, Rasmussen K, Shaw J, Li X, et al. Preclinical evaluation of melanin-concentrating hormone receptor 1 antagonism for the treatment of obesity and depression. *The Journal of Pharmacology and Experimental Therapeutics*. 2009;329:429-438.
14. Buezo, Nuria Diaz, inventor; Eli Lilly and Company, assignee. Kappa selective opioid receptor antagonist. US 7,709,522 B2. May 4, 2010.
15. Benito Collado Ana Belen, inventor; Eli Lilly and Company, assignee. Spiropiperidine compounds as orl-1 receptor antagonists. WO 201 1/060035 A1. November 10, 2010.
16. Smith, Stephon Cornell, inventor; Eli Lilly and Co Ltd, assignee. 4-substituted-3-phenylsulfanylmethyl-bicyclo[3.1.0]hexane antagonists of mglur 2/3 useful in the treatment of depressive disorders and sleep disorders. CA2818116. November 18, 2010.
17. Monn, James Allen, inventor; Eli Lilly and Company, assignee. Mglu2 agonists. US 2011/0152334 A1. June 23, 2011.
18. Fell MJ, Witkin JM, Falcone JF, Katner JS, et al. N-(4-((2-(trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: in vivo profiling suggests a link between behavioral and central nervous system neurochemical changes. *The Journal of Pharmacology and Experimental Therapeutics*. 2011;336:165-177.
19. Dressman, Bruce Anthony, inventor; Eli Lilly and Company, assignee. 4-substituted-3-benzyloxy-bicyclo[3.1.0]hexane compounds as GluR 2/3 antagonists. WO 2012/068041. November 15, 2011.
20. Man, Teresa, inventor; Eli Lilly and Company, assignee. Bicyclo(3.1.0)hexane-2,6-dicarboxylic acid derivatives as mglu2 receptor agonist. WO 2012/173850. June 7, 2012.
21. Gleason SD, Li X, Smith IA, Ephlin JD, et al. mGlu2/3 agonist-induced hyperthermia: an in vivo assay for detection of mGlu2/3 receptor antagonism and its relation to antidepressant-like efficacy in mice. *CNS & Neurological Disorders – Drug Targets*. 2013;12(5):554-566.
22. Witkin JM, Overshiner C, Li X, Catlow JT, et al. M1 and m2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly acting antidepressant scopolamine. *The Journal of Pharmacology and Experimental Therapeutics*. 2014;351:448-456.
23. Chappell MD, Li R, Smith SC, Dressman BA, et al. Discovery of (1S,2R,3S,4S,5R,6R)-2-Amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid Hydrochloride (LY3020371·HCl): A Potent, Metabotropic Glutamate 2/3 Receptor Antagonist with Antidepressant-Like Activity. *Journal of Medicinal Chemistry*. 2016;59:10974-10993.
24. Dressman BA, Tromiczak EG, Chappell MD, Tripp AE, et al. Novel bicyclo[3.1.0]hexane analogs as antagonists of metabotropic glutamate 2/3 receptors for the treatment of depression. *Bioorganic & Medicinal Chemistry Letters*. 2016;26:5663-5668.
25. Witkin JM, Rorick-Kehn LM, Benvenga MJ, Adams BL, et al. Preclinical findings predicting efficacy and side-effect profile of LY2940094, an antagonist of nociceptin receptors. *Pharmacology Research & Perspectives*. 2016;4(6):e00275.
26. Martin AE, Schober DA, Nikolayev A, Tolstikov VV, et al. Further Evaluation of Mechanisms Associated with the Antidepressantlike Signature of Scopolamine in Mice. *CNS & Neurological Disorders – Drug Targets*. 2017;16(4):492-500.

27. Hufgard JR, William MT, Skelton MR, Grubisha O, et al. Phosphodiesterase-1b (Pde1b) knockout mice are resistant to forced swim and tail suspension induced immobility and show upregulation of Pde10a. *Psychopharmacology*. 2017;234:1803-1813.
28. Yuen E, Swanson S, Witkin JM. Prediction of human efficacious antidepressant doses using the mouse forced swim test. *Pharmacology, Biochemistry and Behavior*. 2017;161:22-29.
29. Bruns RF, Mitchell SN, Wafford KA, Harper AJ, et al. Preclinical profile of a dopamine D1 potentiator suggests therapeutic utility in neurological and psychiatric disorders. *Neuropharmacology*. 2018;128:351-365.
30. Witkin JM, Shenvi RA, Li X, Gleason SD, et al. Pharmacological characterization of the neurotrophic sesquiterpene jiadifenolide reveals a non-convulsant signature and potential for progression in neurodegenerative disease studies. *Biochemical Pharmacology*. 2018;155:61-70.

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[i] Benvenga MJ, Leander JD. Antidepressant-like effect of LY228729 as measured in the rodent forced swim paradigm. *European Journal of Pharmacology*. 1993;239:249-252.; O'Neill MF, Osborne DJ, Woodhouse SM, Conway MW. Selective imidazoline I2 ligands do not show antidepressant-like activity in the forced swim test in mice. *Journal of Psychopharmacology*. 2001;15(1):18-22.; Li X, Tizzano JP, Griffey K, Clay M, Lindstrom T, Skolnick P. Antidepressant-like actions of an AMPA receptor potentiator (LY392098). *Neuropharmacology*. 2001;40:1028-1033.; Skolnick, Phil, inventor; Eli Lilly and Company, assignee. Combination therapy for treatment of depression. US 2003/0092770A1. May 15, 2003.; Li X, Witkin JM, Need AB, Skolnick P. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell and Molecular Neurobiology*. 2003;23(3):419-430.; Li X, Need AB, Baez M, Witkin JM. Metabotropic glutamate 5 receptor antagonism is associated with antidepressant-like effects in mice. *The Journal of Pharmacology and Experimental Therapeutics*. 2006;319:254-259.; Benito Collado Ana Belen, inventor; Eli Lilly and Company, assignee. Spiropiperidine compounds as orl-1 receptor antagonists. WO 201 1/060035 A1. November 10, 2010.

[ii] Bai F, Li X, Clay M, Lindstrom T, Skolnick P. Intra- and interstrain differences in models of "behavioral despair." *Pharmacology, Biochemistry and Behavior*. 2001;70:187-192.; Li X, Witkin JM, Need AB, Skolnick P. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell and Molecular Neurobiology*. 2003;23(3):419-430.; Witkin JM, Overshiner C, Li X, Catlow JT, et al. M1 and m2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly acting antidepressant scopolamine. *The Journal of Pharmacology and Experimental Therapeutics*. 2014;351:448-456.; Chappell MD, Li R, Smith SC, Dressman BA, et al. Discovery of (1S,2R,3S,4S,5R,6R)-2-Amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid Hydrochloride (LY3020371·HCl): A Potent, Metabotropic Glutamate 2/3 Receptor Antagonist with Antidepressant-Like Activity. *Journal of Medicinal Chemistry*. 2016;59:10974-10993.; Dressman BA, Tromiczak EG, Chappell MD, Tripp AE, et al. Novel bicyclo[3.1.0]hexane analogs as antagonists of metabotropic glutamate 2/3 receptors for the treatment of depression. *Bioorganic & Medicinal Chemistry Letters*. 2016;26:5663-5668.; Bruns RF, Mitchell SN, Wafford KA, Harper AJ, et al. Preclinical profile of a dopamine D1 potentiator suggests therapeutic utility in neurological and psychiatric disorders. *Neuropharmacology*. 2018;128:351-365.; Witkin JM, Shenvi RA, Li X, Gleason SD, et al. Pharmacological characterization of the neurotrophic sesquiterpene jiadifenolide reveals a non-convulsant signature and potential for progression in neurodegenerative disease studies. *Biochemical Pharmacology*. 2018;155:61-70.

[iii] Molendijk ML, de Kloet ER Immobility in the forced swim test is adaptive and does not reflect depression. Psychoneuroendocrinology. 2015;62:389-391.

[iv] Arai I, Tsuyuki Y, Shiomoto H, Satoh M, Otomo S. Decreased body temperature dependent appearance of behavioral despair in the forced swimming test in mice. *Pharmacological Research*. 2000;42:171-176.; Schechter MD, Chance WT. Non-specificity of "behavioral despair" as an animal model of depression. *European Journal of Pharmacology*. 1979;60:139-142.

[v] De Pablo JM, Parra A, Segovia S, Guillamon A. Learned immobility explains the behavior of rats in the forced swimming test. *Physiology and Behavior*. 1989;46:229-237.; Jeffrys D, Funder J. The effect of water temperature on immobility in the forced swimming test in rats. *European Journal of Pharmacology*. 1994;253:91-94.; Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology* 2001;155:315-322.

[vi] moxonidine, CERC-501, LY354740, biperiden, scopolamine, LY2940094

[vii] scopolamine, LY2940094

[viii] Bai F, Li X, Clay M, Lindstrom T, Skolnick P. Intra- and interstrain differences in models of "behavioral despair." *Pharmacology, Biochemistry and Behavior*. 2001;70:187-192.
[ix] Yuen E, Swanson S, Witkin JM. Prediction of human efficacious antidepressant doses using the mouse forced swim test. *Pharmacology, Biochemistry and Behavior*. 2017;161:22-29.
[x] Yuen 2017
[xi] Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends in Pharmacological Sciences*. 2002;23(5):238-245.; Petit-Demouliere B, Chenu F, Bourin M. Forced swimming in mice: a review of antidepressant activity. *Psychopharmacology*. 2005;177:245-255.
[xii] Hendrie C, Pickles A. The failure of the antidepressant drug discovery process is systemic. *Journal of Psychopharmacology*. 2013;27(5):407-416.
[xiii] Garner JP. The significance of meaning: Why do over 90% of behavioral neuroscience results fail to translate to humans, and what can we do to fix it? *ILAR Journal*. 2014;55(3):438-456.; Hendrie 2013
[xiv] Hendrie 2013
[xv] de Kloet ER, Molendijk ML. Coping with the forced swim stressor: Towards understanding an adaptive mechanism. *Neural Plast*. 2016;2016:6503162.
[xvi] van der Staay FJ, Arndt SS, Nordquist RE. Evaluation of animal models of neurobehavioral disorders. *Behavioral and Brain Functions*. 2009;5:11.
[xvii] van der Staay 2009