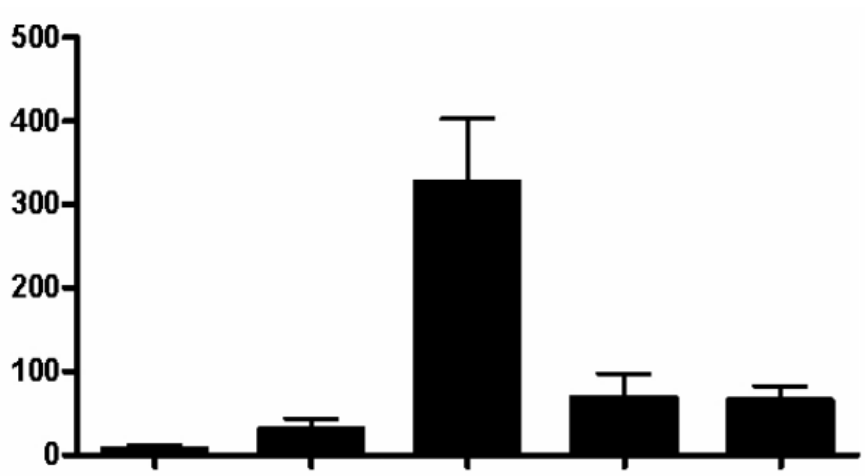
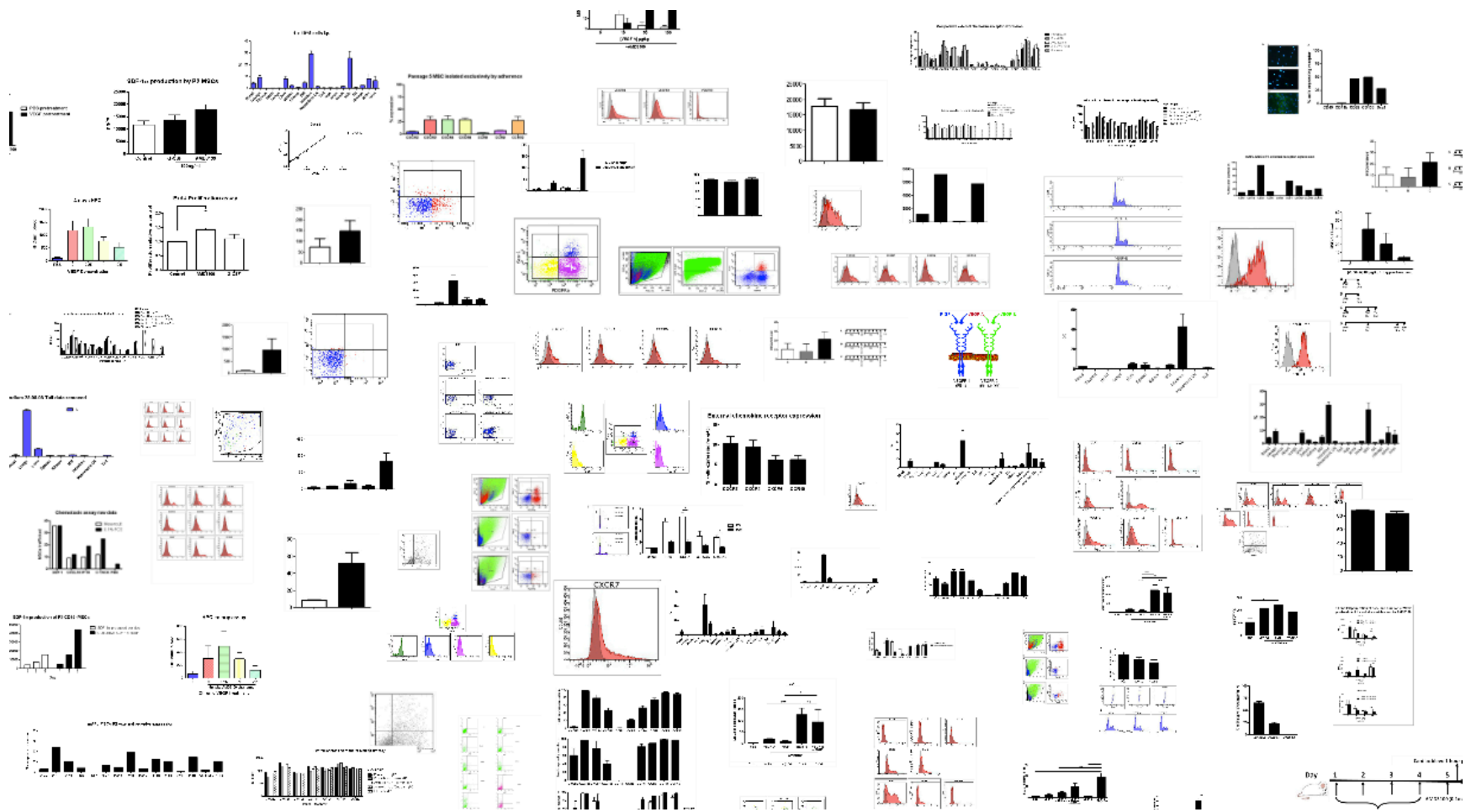


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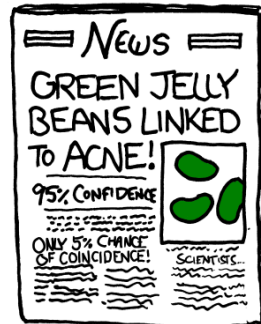
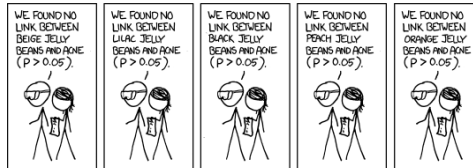
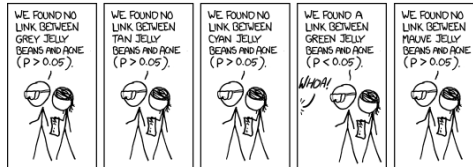
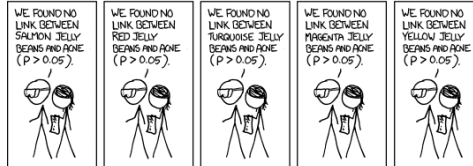
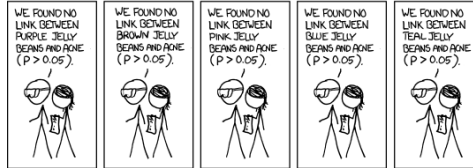
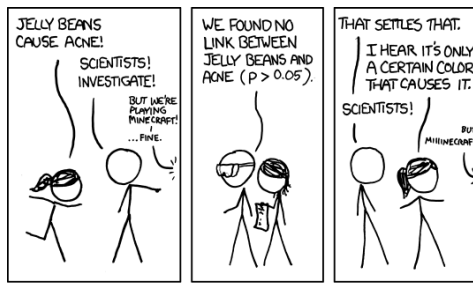
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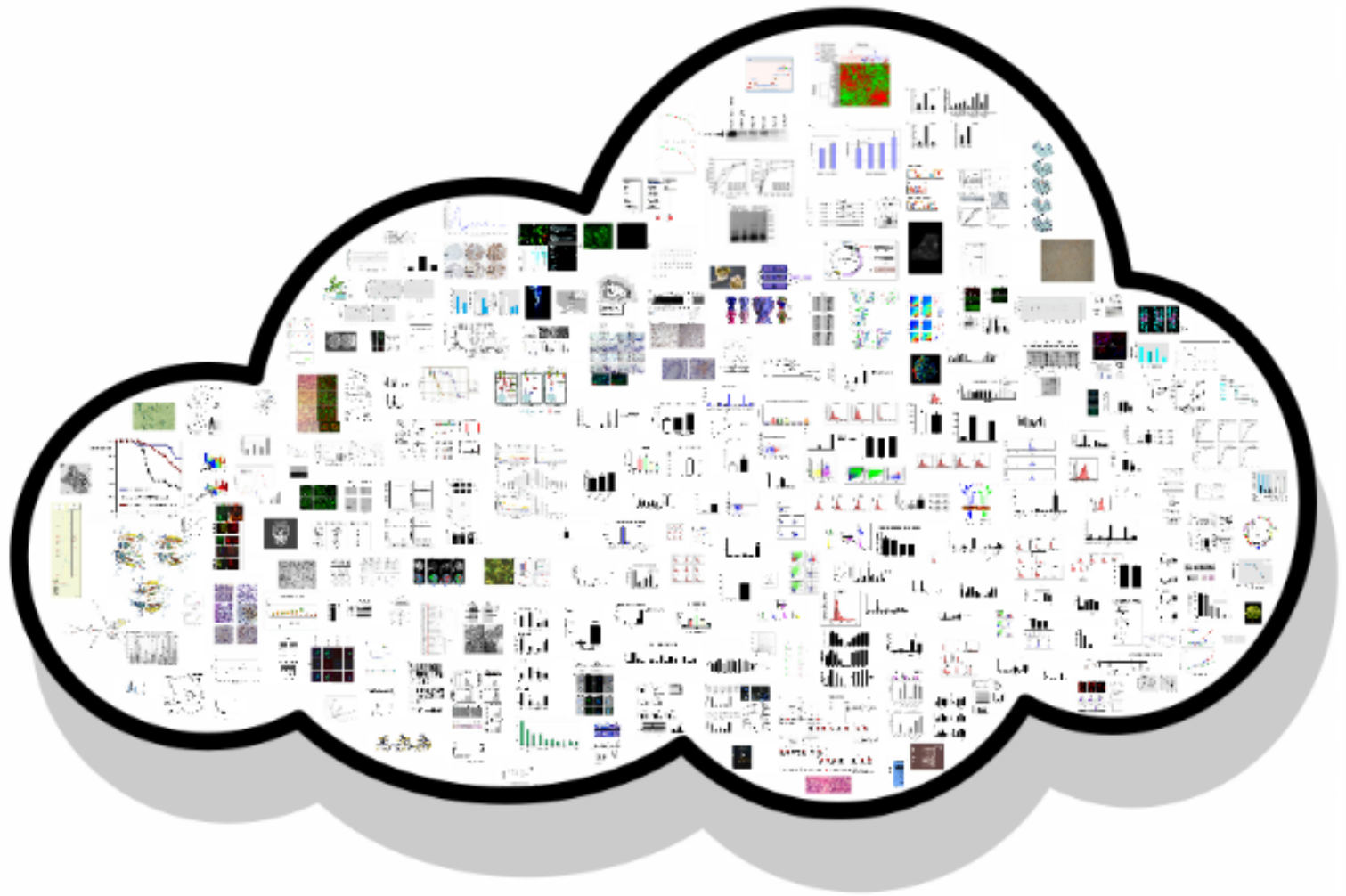
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Nature 455, 757-763 (9 October 2008) | doi:10.1038/nature07327; Received 18 January 2008; Accepted 8 August 2008

Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*

Jane M. Carlton^{1,2}, John H. Adams³, Joana C. Silva^{4,5}, Shelby L. Bidwell¹, Hernan Lorenzi⁶, Elisabet Caler⁷, Jonathan Crabtree^{8,9}, Samuel V. Angilak^{3,8}, Emilio F. Merino¹⁰, Paolo Amedeo¹, Qin Cheng³, Richard M. R. Coulson^{11,12}, Brendan S. Crabb^{13,14}, Hernando A. del Portillo^{13,14}, Kobby Essien^{15,16}, Tamara V. Feldblyum⁵, Carmen Fernandez-Becerra¹³, Paul R. Gilson¹⁴, Amy H. Gueye¹⁷, Xiang Guo⁴, Simon Kang¹⁸, Taco W. A. Koop¹³, Michael Korsinczyk^{2,19}, Esmeralda V.-S. Meyer²⁰, Vish Nene^{4,5}, Ian Pausen^{11,21}, Owen White^{4,5}, Stuart A. Ralph²², Qinghu Ren¹, Tobias J. Sargeant^{11,23}, Steven L. Salzberg⁸, Christian J. Stoeckert¹⁵, Steven A. Sullivan², Marcio M. Yamamoto²⁴, Stephen L. Hoffman²⁵, Jennifer R. Wortman^{5,7}, Malcolm J. Gardner^{1,27}, Mary R. Galinski¹⁹, John W. Barnwell²⁶ & Claire M. Fraser-Liggett^{5,7}

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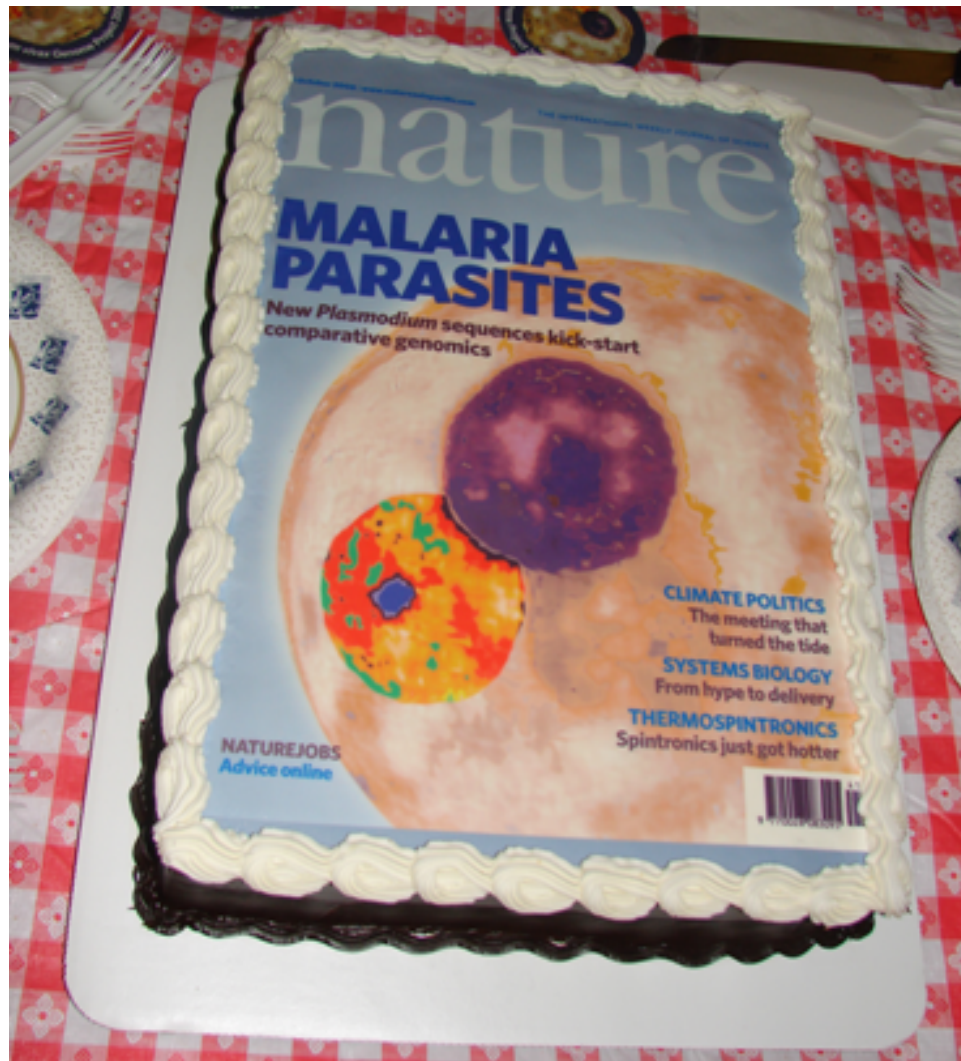
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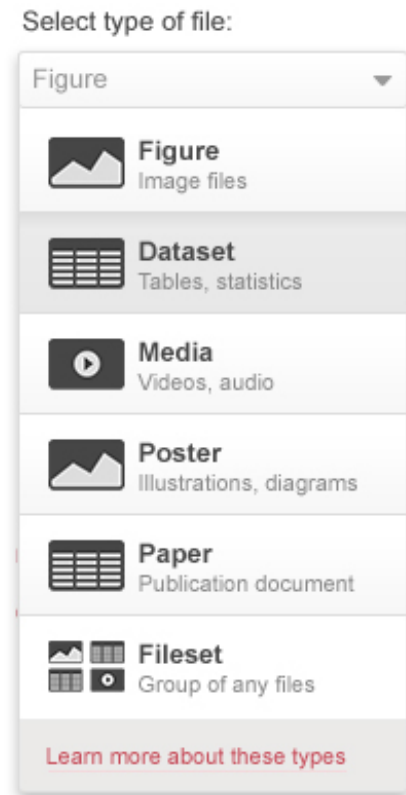
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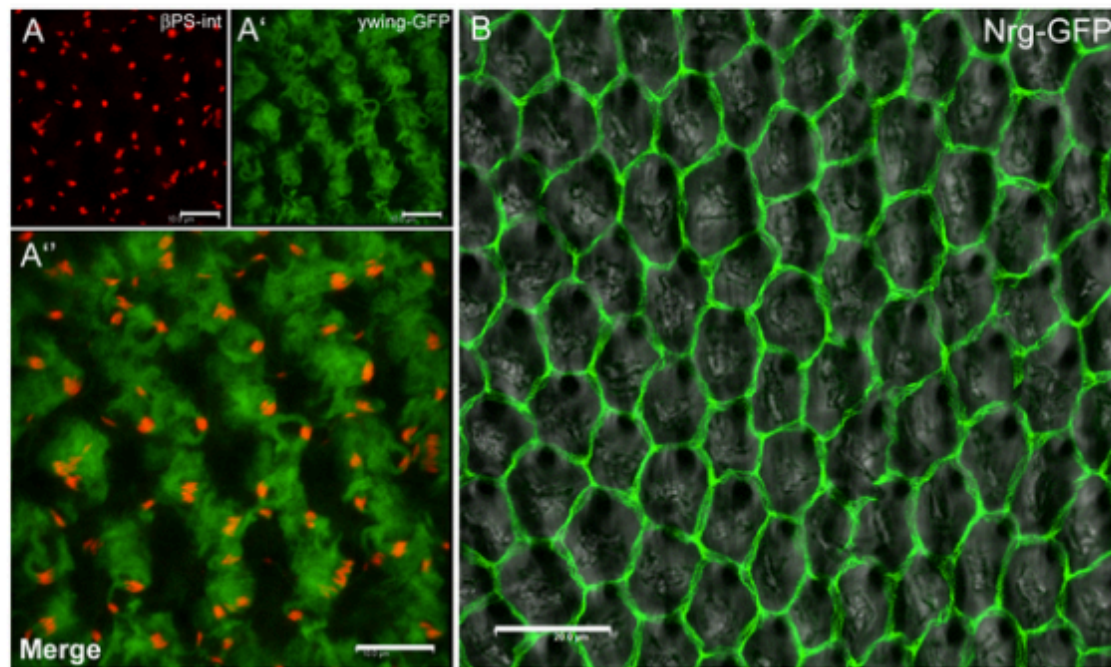
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The organization of *Drosophila* wing epithelial cells after wing inflation



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Description

Upon emergence of *Drosophila* from the pupal case its wing exhibits a shriveled morphology. The wing tissue at this point is enclosed within a semi-constrained cuticle. As a final step in wing morphogenesis the fly pumps hemolymph into the compacted structure ([Lehmacher et al. 2009](#)) so that the inflation results in the formation of a wing blade.

It is possible to fix the epithelial cells of a freshly-inflated wing blade after creating small nicks along the wing margin. This permits immunolabeling procedures followed by the visualization of cellular details using confocal microscopy. **Panel-A** above shows the result of this "nick-approach" after which the fixed wing (4% Paraformaldehyde) was incubated with an anti-BetaPS-integrin MAb (6G11, DSHB, Univ. of Iowa, USA) first and then with AlexaFluor594 secondary antibody. This wing also expressed GFP ([Kiger et al. 2007](#)). **Panel A'-A''**: The betaPS-integrin adhesion sites

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students knew more about the author and they could read the book if they were interested. Suggestions to further improve the use of literature excerpts were also obtained. Common among these were to also use Nepali language excerpts (16 respondents), use more excerpts (8), use excerpts with simpler language (6), use more excerpts from the medical field (5), provide photocopies of the excerpts beforehand to the students (4), and provide more time for the activity (4 respondents).

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5	1.00	2.00	1.00	1.00	2.00
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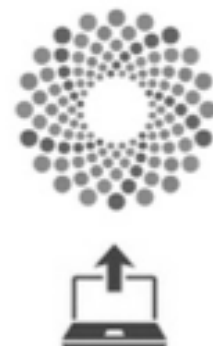
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Accumulation of an Antidepressant in Vesiculogenic Membranes of Yeast Cells Triggers Autophagy

Jingqiu Chen¹, Daniel Korostyshevsky², Sean Lee, Ethan O. Perlsstein¹

¹ Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, United States of America

Abstract

Many antidepressants are cationic amphipaths, which spontaneously accumulate in natural or reconstituted membranes in the absence of their specific protein targets. However, the clinical relevance of cellular membrane accumulation by antidepressants in the human brain is unknown and hotly debated. Here we take a novel, evolutionarily informed approach to studying the effects of the selective-serotonin reuptake inhibitor sertraline/Zoloft[®] on cell physiology in the model eukaryote *Saccharomyces cerevisiae* (budding yeast), which lacks a serotonin transporter entirely. We biochemically and pharmacologically characterized cellular uptake and subcellular distribution of radiolabeled sertraline, and in parallel performed a quantitative ultrastructural analysis of organellar membrane homeostasis in untreated vs. sertraline-treated cells. These experiments have revealed that sertraline enters yeast cells and then reshapes vesiculogenic membranes by a complex process. Internalization of the neutral species proceeds by simple diffusion, is accelerated by proton motive forces generated by the vacuolar H⁺-ATPase, but is counteracted by energy-dependent xenobiotic efflux pumps. At equilibrium, a small fraction (10–15%) of reprotonated sertraline is soluble while the bulk (90–85%) partitions into organellar membranes by adsorption to interfacial anionic sites or by intercalation into the hydrophobic phase of the bilayer. Asymmetric accumulation of sertraline in vesiculogenic membranes leads to local membrane curvature stresses that trigger an adaptive autophagic response. In mutants with altered clathrin function, this adaptive response is associated with increased lipid droplet formation. Our data not only support the notion of a serotonin transporter-independent component of antidepressant function, but also enable a conceptual framework for characterizing the physiological states associated with chronic but not acute antidepressant administration in a model eukaryote.

Citation: Chen J, Korostyshevsky D, Lee S, Perlsstein EO (2012) Accumulation of an Antidepressant in Vesiculogenic Membranes of Yeast Cells Triggers Autophagy. *PLoS ONE* 7(4): e34024. doi:10.1371/journal.pone.0034024

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† These authors contributed equally to this work.

Introduction

Cationic amphiphilic/amphipathic drugs (CAD) represent a subset of Food and Drug Administration (FDA) approved compounds that promiscuously interact with both proteinaceous and non-proteinaceous targets, the latter being cellular membranes [1,2]. CAD association with cellular membranes depends on an ionizable amine that is positively charged at physiological pH and a lipophilic polycyclic scaffold, but does not depend on stereochemistry, as in the peculiar case of the antidepressant sertraline/Zoloft[®] moonlighting as a fungicide [3]. The primary protein target of sertraline is thought to be the human serotonin transporter (hSERT), which localizes to synaptic clefts and recycles the monoamine neurotransmitter serotonin after each burst of neurotransmission. According to the monoamine hypothesis of depression, antidepressants like sertraline bind hSERT and acutely block reuptake of serotonin in the brain [4]. However, a latency period whose molecular basis is unknown precedes the emergence of the actual antidepressant effect in humans, and in rodent behavioral models of depression, suggesting that antidepressants exert additional effects at targets besides hSERT. Given the well known and wide-ranging effects of CAD on cellular membrane homeostasis in the absence of specific protein targets [5,6], the

clinical relevance of antidepressant accumulation in neuronal cell membranes has been vigorously debated. For example, there is evidence that supports the existence of serotonin transporter-independent components of antidepressant function in vertebrate cellular models [7], some of which appears to involve membrane accumulation by antidepressants [8,9]. Yet a comprehensive model of antidepressant function that accounts for all drug-target interactions in the human brain has so far been elusive.

The goal of the present study is to begin developing and validating a comprehensive model of complex antidepressant function in humans. The first step in this arduous process is to reconcile two pharmacological perspectives that have historically dominated conventional thinking about CAD activity in cells lacking specific integral membrane protein targets. On the one hand, a molecular view of drug-membrane interactions derives from the seminal work of Singer and Sheetz on amphipath-induced morphological transformations of freshly isolated human erythrocytes, a cell-based model system superior to reconstituted liposomes but still lacking endomembranes. Singer and Sheetz proposed the bilayer couple/balance model, which states that a charged amphipath preferentially accumulates at equilibrium in the leaflet (monolayer) exhibiting the opposite net charge [10]. A

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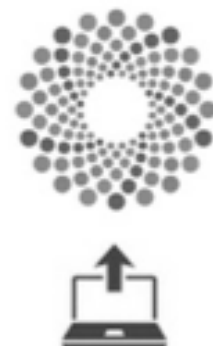
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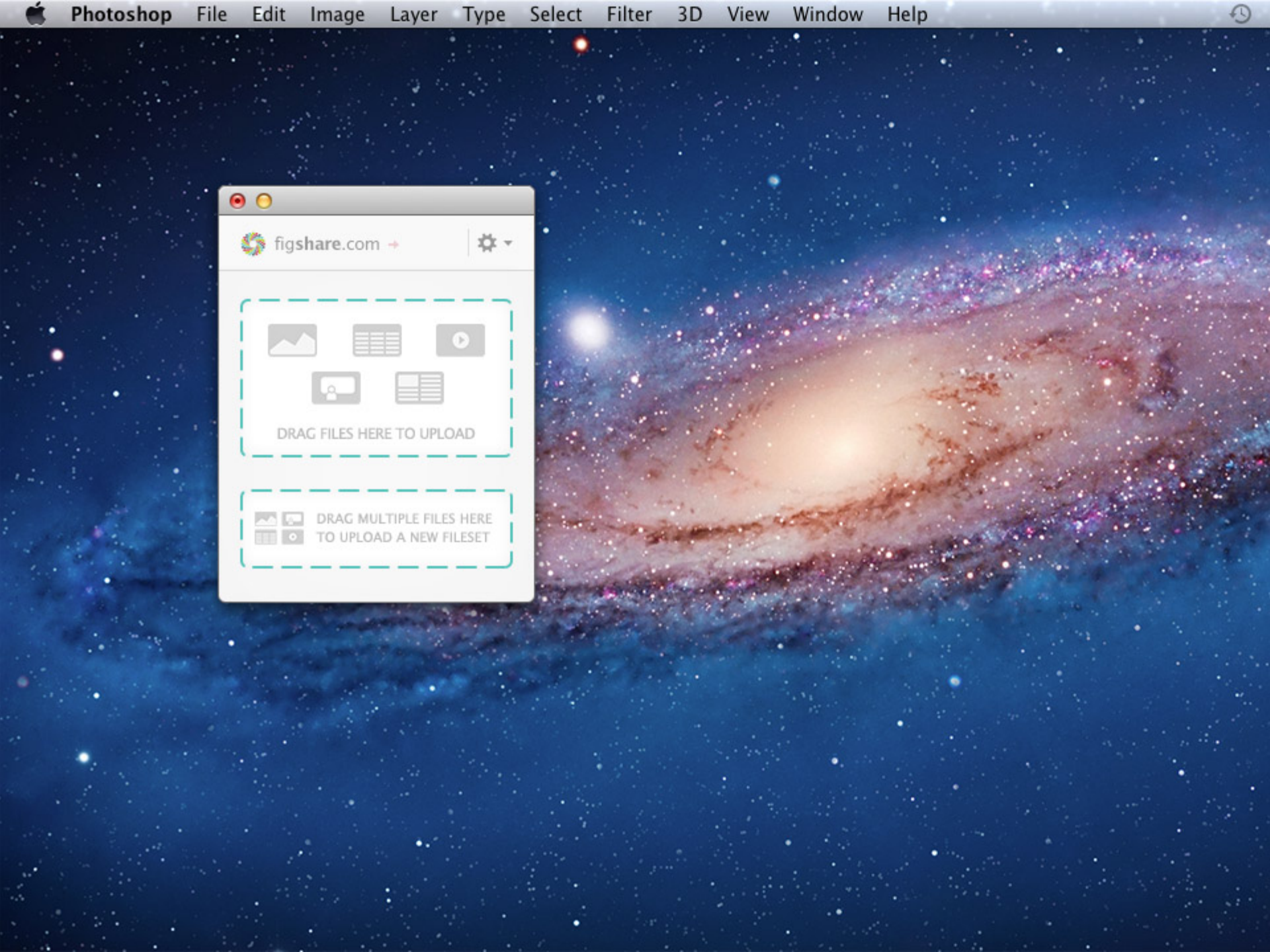
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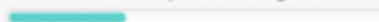
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








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<input type="checkbox"/>			03/11/11 12:15	<input type="button" value="PUBLIC"/>	123 views ● 25 shares ● 4 cites ●
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Detailed actions

Dataset metrics should:

- a. Ensure the default approach is that datasets which underpin submitted scientific articles are accessible and usable, at a minimum by scientists in the same discipline.
- b. Give credit by using internationally recognised standards for data citation.
- c. Provide standards for the assessment of datasets, metadata and software that combines appropriate expert review with quantitative measures of citation and reuse.
- d. Offer clear rules on the delineation of what counts as a dataset for the purposes of review, and when datasets of extended scale and scope should be given increased weight.

- e. Seek ways of recognising and encouraging and novel ways of communal work and appropriately validated social metrics.

UK-specific

- a. These principles should be adopted by the UK Higher Education Funding Councils as part of their Research Excellence Framework (REF). The REF is a powerful driver for how universities evaluate and reward their researchers. Use in the REF of metrics that record citable open data deposition would be a powerful motivation for data release.
- b. JISC's Managing Research Data programme, or a similar initiative, should be expanded beyond the pilot 17 institutions within the next five years. The aim of any initiative should be to support a coordinated national move towards institutional data management policies.



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