LETTER

Reply to Rubinsztein and Nixon: Potent and reliable autophagy induction in neurons

We thank Rubinsztein and Nixon (1) for their interest in our study (2). We have the greatest respect for their contributions to the field of autophagy.

In our paper, we reported the discovery of compounds with related structures that stimulate autophagy in primary neurons by a pathway that is independent of the mammalian target of rapamycin (mTOR) (2). The lead compound mitigates against toxicity in two models of neurodegeneration (2). Using one conventional assay, the most potent compound in that series induced autophagy at least fivefold. Subsequently, we found analogs that induce autophagy 10-fold. Although not the focus of our study, we offered an assessment of the efficacy of rapamycin and said that it induces autophagy weakly, if at all, in primary neurons.

Using similar assays of autophagy, Boland et al. (3) reported a twofold induction by rapamycin in neurons and described it as robust. The appropriate adjective to describe the strength of an induction is admittedly subjective. However, in light of the more potent induction of autophagy in primary neurons by mTOR-independent pathways (2), including work with rilmenidine by Rose et al. (4), we described the potency of rapamycin as relatively weak.

Perhaps even more interesting, however, is that we found conditions in which rapamycin failed to induce autophagy in neurons, although the same conditions supported potent induction by mTOR-independent pathway(s) (1, 3). Only one related supplemental figure appears in the study cited by Rubinsztein and Nixon (1, 2), but in other work published online in *Autophagy* and available from us (5), additional data are presented. Although rapamycin effectively induced autophagy in nonneuronal cell lines and inhibited mTOR-dependent p70S6K phosphorylation in neurons, autophagy was not induced by rapamycin in neurons. These neurons were competent to undergo autophagy induction, because inducers of mTOR-independent pathway(s) were positive controls. The failure to detect autophagic responses to rapamycin in neurons cannot be explained solely by accelerated basal flux, because we performed control flux assays with bafilomycin A, as suggested by Rubinsztein and Nixon (1), and failed to uncover latent autophagy induction.

Why rapamycin might induce autophagy variably in neurons is unclear. We observed that rapamycin induces autophagy in astrocytes similar to nonneuronal cells (5). Primary cultures include different proportions of astrocytes, and therefore, those differences might contribute to the variability in autophagy detection. Even in nonneuronal cells, the effects of rapamycin are complex. Nanomolar concentrations completely inhibit mTOR, but much higher concentrations are required to induce autophagy in nonneuronal cells. Perhaps rapamycin acts on additional cellular targets, such as protein synthesis (6). With the unusual susceptibility of neurons to disruption of autophagy and protein misfolding in neurodegenerative disease, autophagy in neurons might be regulated by mechanisms that differ, at least in part, from those in nonneuronal cells (reviewed in ref. 6). We are investigating this hypothesis.

Autophagy remains a very interesting therapeutic target for neurodegenerative disease. Indeed, the desire to find safe, effective, and potent inducers of autophagy in neurons was the main motivation for our study (2).

***Andrey S. Tsvetkov*[a] *and Steven Finkbeiner*[a,b,c,d,1]**

[a]*Gladstone Institute of Neurological Disease and the Taube-Koret Center for Huntington Disease Research, San Francisco, CA 94158; and* [b]*Neuroscience and* [c]*Biomedical Sciences Programs and* [d]*Departments of Neurology and Physiology, University of California, San Francisco, CA 94143*

1. Rubinsztein DC, Nixon RA (2010) Rapamycin induces autophagic flux in neurons. *Proc Natl Acad Sci USA* 107:E181.
2. Tsvetkov AS, et al. (2010) A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington disease model. *Proc Natl Acad Sci USA* 107:16982–16987.
3. Boland B, et al. (2008) Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *J Neurosci* 28:6926–6937.
4. Rose C, et al. (2010) Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease. *Hum Mol Genet* 19:2144–2153.
5. Tsvetkov AS, Mitra S, Finkbeiner S (2009) Protein turnover differences between neurons and other cells. *Autophagy* 5, in press.
6. King MA, et al. (2008) Rapamycin inhibits polyglutamine aggregation independently of autophagy by reducing protein synthesis. *Mol Pharmacol* 73:1052–1063.

Author contributions: A.S.T. and S.F. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. E-mail: sfinkbeiner@gladstone.ucsf.edu.

www.pnas.org/cgi/doi/10.1073/pnas.1014950107