# Letter to the Editor

**The *Journal* does not ordinarily have a Letters to the Editor section. However, a paper published by Lazarov et al. in the March 2, 2005 issue directly challenged findings from previous work by Goldstein and colleagues. Thus in fairness, we asked Dr. Goldstein to reply. His response is published below without editing or review.**

We previously published evidence that the amyloid precursor protein (APP) and a protein called sunday driver (syd or JIP-3) have important functions as vesicular receptors for the proposed cargo-binding subunit of kinesin-1 [called kinesin light chain (KLC)] in axons and that APP function is important for axonal transport of a vesicular compartment containing APP, presenilin, and $\beta$-site APP cleaving enzyme (BACE) (Bowman et al., 2000; Kamal et al., 2000, 2001; Gunawardena and Goldstein, 2001). Lazarov et al. now report their inability to reproduce some of our published experiments and their failure to obtain data consistent with our proposals. We are not convinced by their data or by the sufficiency of their methods, because they vary significantly from ours in many cases, and in some cases are arguably inferior in quality or sensitivity. In addition, Lazarov et al. do not discuss/cite the many published papers that provide corroborating data for our experiments and proposals, including some published by coauthors of Lazarov et al.

Below we summarize the data reported in each of the five figures in Lazarov et al., noting our view of the technical differences/inadequacies as well as citing published papers that support our work.

*(1) Lazarov et al. conclude from Figure 1 that proteins such as APP, JIP, and syd bind to the tetratricopeptide repeat (TPR) regions of KLC as a result of nonspecific hydrophobic interactions.* Our primary concern is that their experiments use nonquantified methods to test binding of unknown amounts of proteins translated *in vitro* to unreported amounts of proteins made in bacteria. In contrast, our experiments quantified binding of syd and APP (Bowman et al., 2000; Kamal et al., 2000) to KLC using defined amounts of purified proteins produced in *Escherichia coli*; we observed saturable binding in both cases. We note that proteins translated *in vitro* are not pure, and thus their binding reactions contain many potentially competing proteins present in the well known high concentrations of unrelated proteins found in typical *in vitro* translation extracts. In addition, some of their proteins were insoluble and thus denatured before "renaturing/refolding." No evidence is provided that these proteins have a native conformation, and because inputs to binding reactions are not shown, relative proportions of control and experimental proteins bound cannot be evaluated. We find it intriguing that their Figure 1*B* (top two panels) confirms our report that glutathione *S*-transferase (GST)-KLC binds green fluorescent protein (GFP)-APP intracellular domain substantially more than GFP (Kamal et al., 2000). Their "nonspecific" binding only becomes prominent with deletion constructs, which may have refolding problems. Little data are provided to support their statements about binding of these proteins being mediated by "hydrophobic patches" (undefined, undocumented, and inconsistent with atomic structure data of TPR motifs). Finally, recent experiments (Verhey et al., 2001) provide strong functional evidence that binding of different JIP proteins to KLC is important for JIP transport *in vivo*.

*(2) Lazarov et al. conclude from Figure 2 that APP and KLC cannot be coimmunoprecipitated and that kinesin-1 and APP do not cofractionate.* In contrast, three other groups do report coimmunoprecipitation of APP and KLC, although two of these groups suggest that the APP–kinesin-1 complex contains and requires JIP-1 (Inomata et al., 2003; Matsuda et al., 2003) (V. Muresan, personal communication). We note that different groups sometimes obtain different results in coimmunoprecipitation experiments. However, usually when experiments are done multiple times in different groups, a consensus emerges. For example, a previously published paper from the senior author of Lazarov et al. (Thinakaran et al., 1998) reported their inability to repeat previously published coimmunoprecipitation of APP and presenilin (Weidemann et al., 1997). Subsequent work from other groups (Xia et al., 2000; Annaert et al., 2001; Kamal et al., 2001) fully supports the original coimmunoprecipitation data. Regarding cofractionation, differential pelleting as done by Lazarov et al. is inferior as a fractionation method to our flotation methods (Kamal et al., 2001). Lazarov et al. also state that ". . . kinesin-1 and APP do not fully cofractionate in rodent brain, as would be expected if APP acted as the only cargo receptor for kinesin-1." We never proposed that APP is the only cargo receptor for kinesin-1 and argue consistently in our papers that it is one of several. Thus, we expect incomplete cofractionation of APP and kinesin-1.

*(3) Figure 3 from Lazarov et al. concludes that APP deletion mutants in mouse have normal axonal transport of kinesin-1 and TrkA.* Their ligation data have several problems, particularly the controls. For example, they do not observe the well documented accumulation of TrkA on the distal side of ligatures caused by retrograde transport of TrkA [Ehlers et al. (1995), which includes coauthors of Lazarov et al.]. In addition, unligated immunofluorescence controls are not shown, and the distal side of their ligations does not exhibit the reduced amounts of APP relative to intact nerve consistently observed by us (Kamal et al., 2000) and others (Koo et al., 1990) (because of transport of APP out of distal segments). Their gel controls are also generally overloaded, which would render it difficult to detect the ~70% decreases we reported (Kamal et al., 2001) (rather than complete loss as implied by Lazarov et al.). Finally, we wonder whether Lazarov et al. have inconsistent protein extraction from sciatic nerves; this material is difficult in our experience, and different isolation methods give variable levels of consistency. Supporting this concern, Lazarov et al. show obvious accumulation of kinesin-1 on the proximal side of ligatures by immunofluorescence, but not by Western blots in most of the samples shown.

Corroboration of our proposals comes from the finding that deletion of APPL in *Drosophila* (Gunawardena and Goldstein, 2001) or overexpression of APP in *Drosophila* (Torroja et al.,

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Correspondence should be addressed to Lawrence Goldstein, University of California San Diego, Howard Hughes Medical Institute 0683, 9500 Gilman Drive, La Jolla, CA 92093-0683. E-mail: lgoldstein@ucsd.edu.

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1999; Gunawardena and Goldstein, 2001) or mice (Stokin et al., 2005) induces axonal transport defects that are genetically enhanced by kinesin-1 mutations (Torroja et al., 1999; Gunawardena and Goldstein, 2001; Stokin et al., 2005), again consistent with the proposal that APP plays an important role in transport. Lazarov et al. state that ". . . *APP* KO mice are viable and phenotypically near normal (Zheng et al., 1995). . . " and note that this finding is inconsistent with a transport defect in APP deletion animals. These statements are not correct. APP deletion mice are obviously abnormal, because they are smaller than normal and have defects in behavioral tests, corpus callosum formation, long-term potentiation (LTP), and cortical synaptic density (Muller et al., 1994; Zheng et al., 1995; Perez et al., 1997; Dawson et al., 1999; Magara et al., 1999; Seabrook et al., 1999; White et al., 1999). These phenotypes are consistent with the proposed partial reductions in transport function in APP deletion animals (Kamal et al., 2001). Lazarov et al. also state that ". . . knock-outs of specific KHC (Tanaka et al., 1998; Kanai et al., 2000) or KLC1 (Rahman et al., 1999) genes are embryonic lethal, with neurological defects." This is also incorrect. The KHC and KLC1 mutants reported by Kanai et al. (2000) and Rahman et al. (1999) are fully viable and exhibit adult phenotypes of varying severity, some reminiscent of the APP deletion.

*(4) Lazarov et al. conclude from Figure 4 that PS1 is not transported by fast axonal transport and therefore that it cannot be in the same vesicular carrier as APP.* Although Lazarov et al. dismiss our, and independent, work (Kamal et al., 2001, Kasa et al., 2001), we think our data from Western blots and immunofluorescence are better controlled and more convincing. Lazarov et al. are missing nonligated controls, and gel bands are overexposed and outside the linear range. Our experience also suggests that Lazarov et al. could fail to see accumulation if they have not maintained antibody excess in their experiments. Corroborating our proposals are reports of presenilin accumulation at damaged axonal endings, the generation/accumulation of amyloid $\beta$ (A$\beta$) at damaged axonal endings, the presence of multiple $\gamma$-secretase subunits in axons (Pigino et al., 2003; Chen et al., 2004; Siman and Salidas, 2004), the finding of APP and presenilin in chromaffin vesicles (post-Golgi secretory vesicles that may be homologous to axonal transport vesicles) of adrenal medulla cells (Efthimiopoulos et al., 1998), and the presence of $\gamma$-secretase activity (Lazarov et al., 2002; Sheng et al., 2002) at axonal termini in mature neurons in the CNS, where it appears to process APP to A$\beta$. To be transported to axonal termini, presenilin must almost certainly be packaged into post-Golgi vesicles transported by the fast transport system. We note that several of the authors of Lazarov et al. recently published (Sheng et al., 2003) support for our proposal that PS1 is transported in axons.

*(5) Lazarov et al. conclude from Figure 5 that BACE is not transported by fast axonal transport and therefore that it cannot be in the same vesicular carrier as APP. They also report that A$\beta$ cannot be detected in sciatic nerve.* First, the BACE antibody they show binds a cross-reacting protein with the same mobility as BACE in sciatic nerve. Thus, their antibody is not specific, and the comigrating, cross-reacting band detected may obscure the behavior of BACE itself. Second, they change their experimental paradigm to use crushed nerves instead of ligations, making it difficult to compare with their previous figures or our experiments. Indeed, the APP accumulation they observe on the proximal side of the crushed nerve is almost undetectable, suggesting that crushing the nerve has a different impact on the axonal transport of APP and other proteins. Third, their loading control is overloaded, exacerbating again the issue of accumulation and linearity of response. For A$\beta$ in sciatic nerve, we cannot evaluate their data, because they report no measures of sensitivity of their methods. Using ELISAs, we confirmed recently that A$\beta$ is readily detectable in sciatic nerves of Tg-swAPP$^{PrP}$ mice (Stokin et al., 2005). Other recent papers (Capell et al., 2002) report that BACE can be found in axons of cultured neurons, at axonal termini, and at axonal growth cones (Lee et al., 2003). Further corroboration of our work comes from a recent report that A$\beta$, APP, kinesin-1, BACE, and presenilin accumulate at damaged axonal endings in a CNS damage model (Chen et al., 2004); other workers have reported comparable phenomena (Efthimiopoulos et al., 1999; Smith et al., 1999; Capell et al., 2002; Hook et al., 2002; Roher et al., 2002). The presence of A$\beta$, BACE, and presenilin in regulated secretory vesicles in chromaffin cells, which as noted above may be homologous to axonal transport vesicles, has also been reported (Hook et al., 2002). Finally, several of the authors of the Lazarov et al. paper recently published support for our proposal that BACE is transported in axons (Sheng et al., 2003).

In closing, although we do not know with certainty why Lazarov et al. fail to reproduce some of our findings, there are many papers reporting data that do support our findings and hypotheses (Muller et al., 1994; Zheng et al., 1995; Perez et al., 1997; Efthimiopoulos et al., 1998; Dawson et al., 1999; Magara et al., 1999; Seabrook et al., 1999; Smith et al., 1999; Torroja et al., 1999; White et al., 1999; Gunawardena and Goldstein, 2001; Kasa et al., 2001; Capell et al., 2002; Hook et al., 2002; Kaether et al., 2002; Lazarov et al., 2002; Roher et al., 2002; Sheng et al., 2002; Inomata et al., 2003; Lee et al., 2003; Matsuda et al., 2003; Pigino et al., 2003; Chen et al., 2004; Siman and Salidas, 2004; Stokin et al., 2005). To extend and test our and others' data on these issues, we are currently using more rigorous methods with transgenic mice expressing tagged versions of some of these proteins to purify these vesicles and evaluate their content definitively.

**Lawrence Goldstein, Angels Almenar, Adeela Kamal, and Gorazd Stokin**

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