

## Supplementary Data

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# Low Endogenous and Chemical Induced Heat Shock Protein Induction in a 0N3Rtau-Expressing *Drosophila* Larval Model of Alzheimer's Disease

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Supplementary Table 1  
Heat shock protein (HSP) modulating compounds linked to tau pathology and neurodegeneration

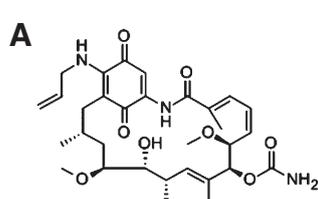
	HSPs	Link to Tau/Neuropathol/HSPs	Reference
17-AAG (hsp90)	27/40/70	reduces insoluble tau in mice*	4
		induces HSPs/reduces polyQ toxicity	5
		clears tau/p-tau in cells	6
		modulates tau kinase levels	2
Radicicol (hsp90)	40/70	reduces htt-polyQ agg	7
		induces hsp40/70 in motor neurons	8
Celastrol (hsp70/90)	27/70	induces HSP/protects vs cell stress	9
		reduces htt-polyQ agg	10
		reduces p-tau levels in mice	11
Simvastatin (HMG CoA reductase)	27/70	induces hsp27/reduces neuron death	12
		induces HSPs in smooth muscle cells	13
Withaferin-A (annexin II?)	27/70	reduces tau neurofibrillary tangle burden in mice	14
		increases htt-polyQ agg <sup>†</sup>	–

Candidate compounds that target the HSF-1 pathway to modulate HSP levels. Suspected molecular targets are listed in parentheses below the name of each chemical shown to induce expression of the listed HSPs in cell/animal models [1]. Chemical treatment also modulates molecular events associated with tauopathy and other neurodegenerative proteinopathies. 17-AAG, 17-allyl-amino-geldanamycin; p-tau, hyperphosphorylated tau. htt-polyQ, polyglutamine expanded huntingtin protein. Agg, protein aggregate. \*This effect, dependent upon *de novo* hsp70 induction, was for the 17-AAG parent compound geldanamycin. Other listed effects on tau pathology were shown to be independent of *de-novo* HSP induction [2, 3]. <sup>†</sup>Association between hsp27/70 induction and modulation of htt-polyQ aggregation *in vitro* (unpublished observations of M. Duennwald, personal communication, 2009). Withaferin-A is generally described and characterized in reference [15].

Supplementary Table 2  
Western blot kinesin band and coomassie lane densities, and RP49 RT-PCR Ct values, in drug treated versus control larvae

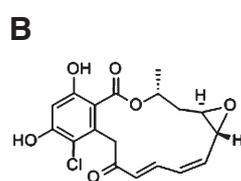
17-AAG ( $\mu$ M)	0	0.2	2	20
Kinesin	79.4	90.0	96.2	80.1
Coomassie	49.3	43.8	44.4	50.4
RP49 (Ct)	16.6	16.0	17.0	–
Radicicol ( $\mu$ M)	0	0.1	1	10
Kinesin	71.7	79.3	90.4	56.9
Coomassie	37.8	34.8	37.0	35.9
RP49 (Ct)	16.5	17.3	–	16.2

Values shown are band or lane densities from single representative western blots (kinesin band or coomassie whole lane) or average of  $n = 3$  samples in a single RT-PCR experiment (RP49 Ct values). No pattern for decreased kinesin band or coomassie lane density, nor increase in RP49 Ct value, was observed that resembled the observed reduction in total tau levels shown in Fig. 4.



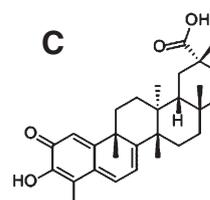
### 17-AAG

synonyms: -  
chem class: ansamycin  
MWt: 624.2  
H-Bond Don/Acc: 7/9  
Polarity: 173



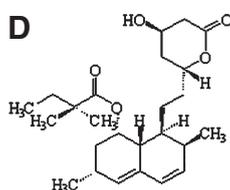
### Radicicol

synonyms: monorden  
chem class: mactrolide  
lactone  
MWt: 364.8  
H-Bond Don/Acc: 2/6  
Polarity: 96.4



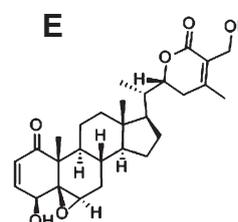
### Celastrol

synonyms: tripterine  
chem class: triterpene  
MWt: 450.6  
H-Bond Don/Acc: 2/4  
Polarity: 74.6



### Simvastatin

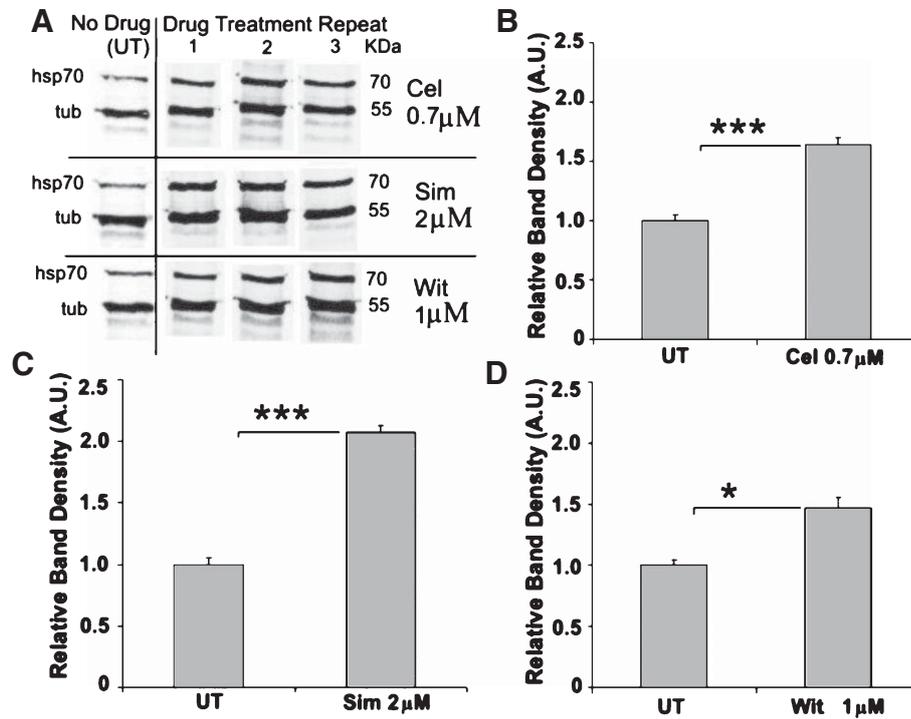
synonyms: zocor, synvinolin  
chem class: statin  
MWt: 418.6  
H-Bond Don/Acc: 1/5  
Polarity: 72.8



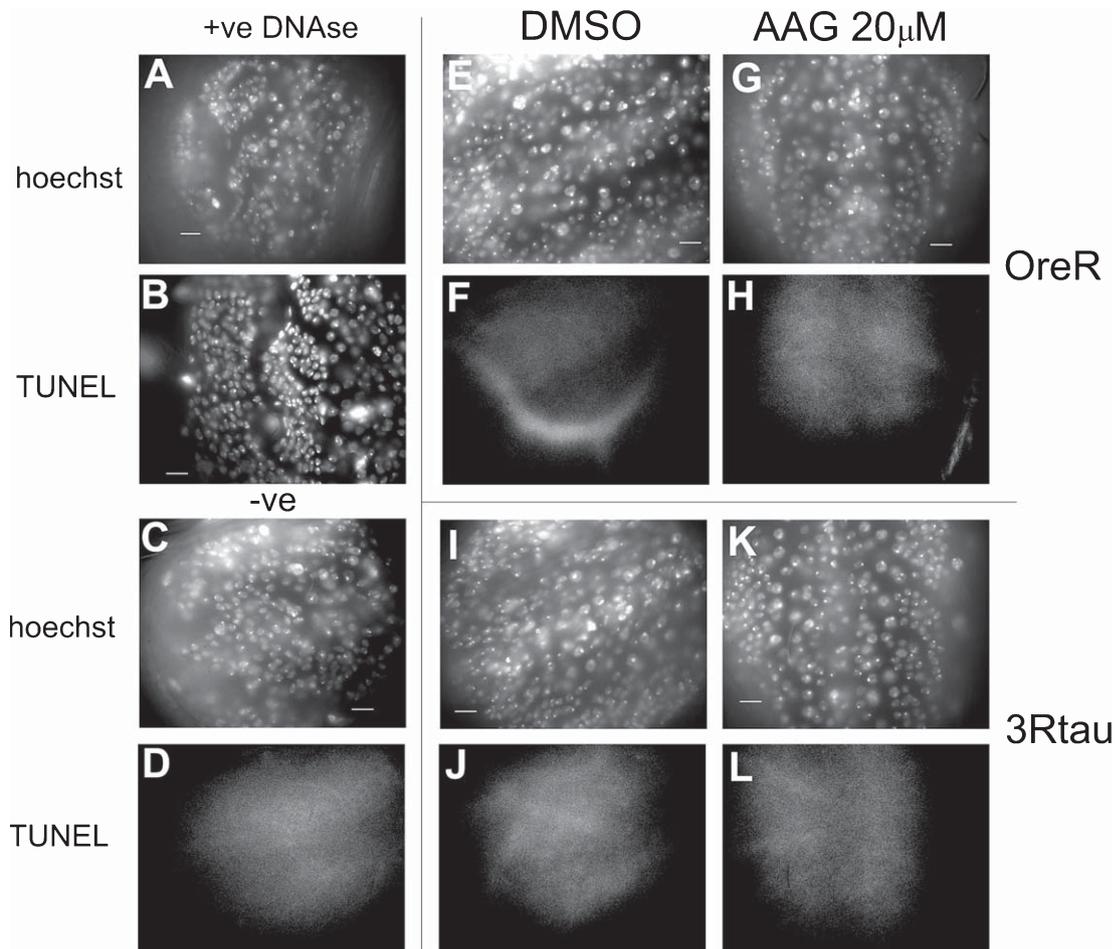
### Withaferin-A

synonyms: -  
chem class: withanolide  
MWt: 470.6  
H-Bond Don/Acc: 2/6  
Polarity: 96.4

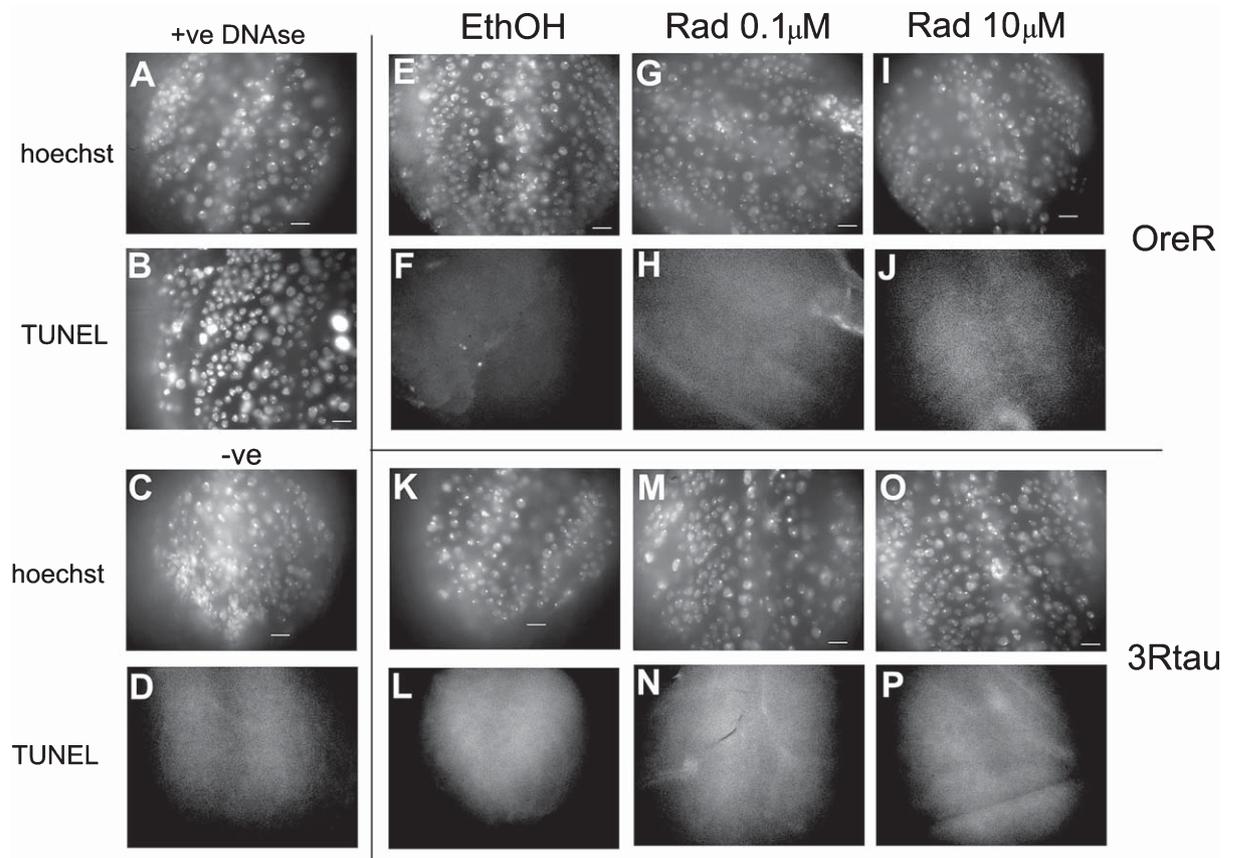
Supplementary Figure 1. Chemical structure and properties of candidate HSP-inducing chemicals. Structural information is from the online NCBI Pubchem Compound database. MWt (average calculated molecular weight) given to 4sf. No. of H-Bond Donors/Acceptors is also given. Polarity score is based upon 'topological polar surface area', which is calculated based upon the number of N- or O-containing moieties, taking hybridizations, charges, and participation of aromatic systems into account but ignoring 3D coordinates of each group. It has been estimated from empirical analyses that compounds with a polarity of less than 60 are best absorbed across intestinal epithelia and the human blood-brain barrier by passive diffusion. Conversely, compounds with a polarity above 120 or 140 are poorly absorbed across the blood-brain barrier and intestinal epithelia respectively [16, 17]. Examples of receptor or carrier-mediated processes that allow passage of more polar molecules across these barriers are known, however.



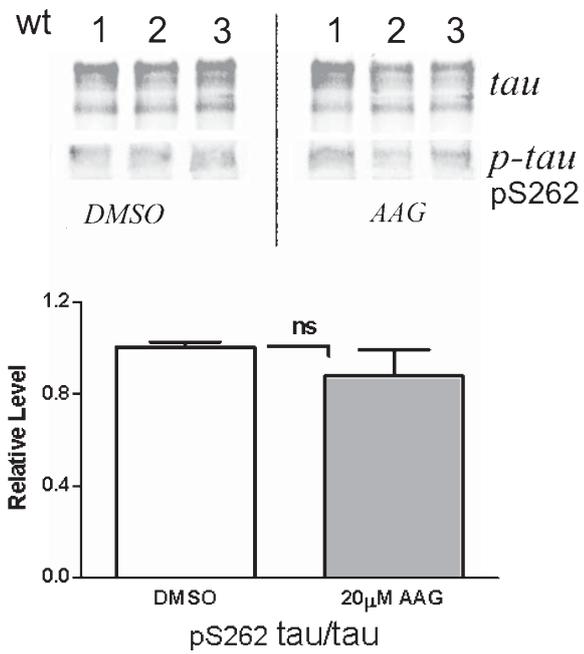
Supplementary Figure 2. Hsp70 protein levels in HeLa cells treated with HSP-inducing chemicals. A) Human HeLa cells were exposed to culture media containing the indicated concentration of HSP-inducing chemical for 24 h and lysed for western blot analysis. Hsp70 protein band densities were corrected to  $\beta$ -tubulin band (tub) densities, assessed in triplicate (drug treatment repeats 1–3) using cell lysate from a single experiment, and expressed relative to untreated cells (UT) for celastrol (Cel, B), simvastatin (Sim, C), and withaferin-A (Wit, D). Relative band density equates to fold change for drug-treated samples relative to UT. \*\*\* $p < 0.001$ , \* $p < 0.05$ . Error bars for chemical treated samples = SEMs.



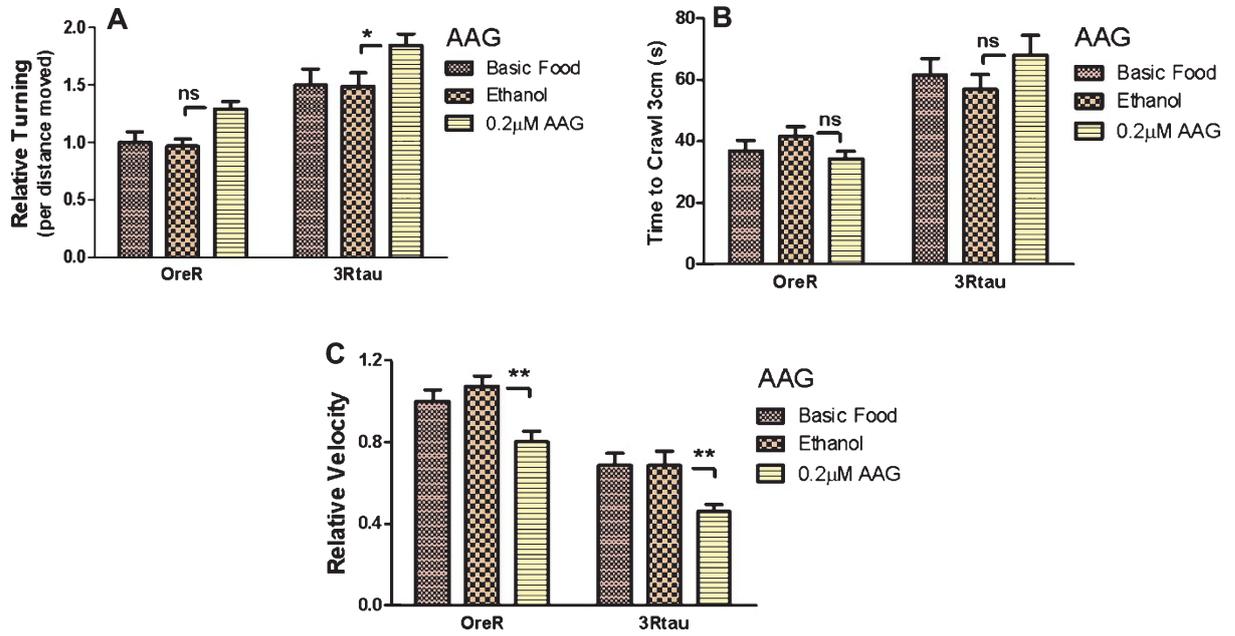
Supplementary Figure 3. TUNEL apoptotic cell death analysis in the CNS of larvae treated with 17-AAG. Chemical-treated larvae were dissected to reveal nervous tissue, fixed, and processed for TUNEL staining of apoptotic cell nuclei in the ventral nerve cord and central brain. Hoechst stain demonstrates numerous cell nuclei in the nervous system of all tissues, as visualized by epifluorescence microscopy. Unlike in positive control tissues exposed to DNase (A & B), and like negative controls that were not exposed to the active TUNEL enzyme (C & D), vehicle treated OreR (E & F) and 3Rtau (I & J) larvae did not show any detectable apoptotic nuclei by this method. This was also true in tissues from larvae treated with 20  $\mu$ M 17-AAG (G, H, K & L).



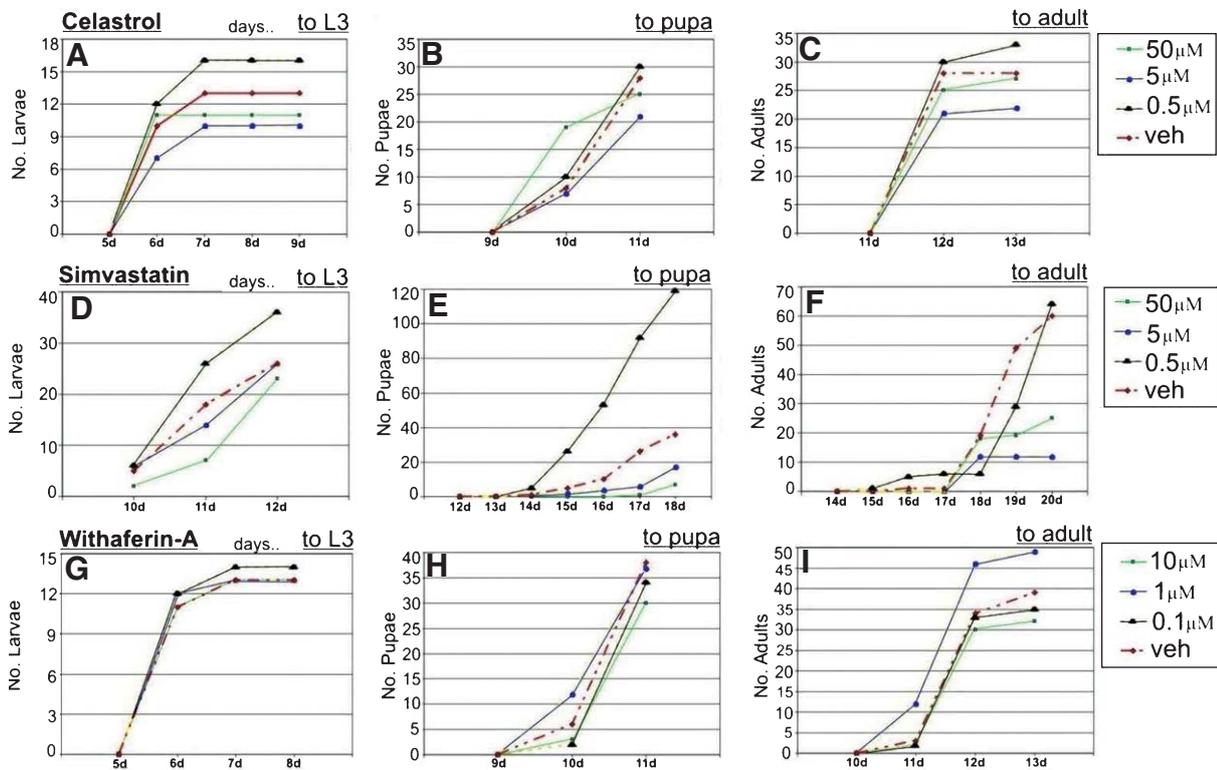
Supplementary Figure 4. TUNEL apoptotic cell death analysis in the CNS of larvae treated with radicicol. Chemical-treated larvae were dissected to reveal nervous tissue, fixed, and processed for TUNEL staining of apoptotic cell nuclei in the ventral nerve cord and central brain. Hoechst stain demonstrates numerous cell nuclei in the nervous system of all tissues, as visualized by epifluorescence microscopy. Unlike in positive control tissues exposed to DNase (A & B), and like negative controls that were not exposed to the active TUNEL enzyme (C & D), vehicle treated OreR (E & F) and 3Rtau (K & L) larvae did not show any detectable apoptotic nuclei by this method. This was also true in tissues from larvae treated with 0.1 µM (G, H, M & N) and 10 µM (I, J, O & P) radicicol.



Supplementary Figure 5. pS262 phospho-tau levels relative to total tau after 17-AAG treatment. Larval samples were probed by western blot with pS262 antibody specific for hyperphosphorylated human tau. pS262 tau band densities were corrected to total tau bands in a parallel blot, and the level of corrected pS262 tau in larvae treated with 20  $\mu$ M 17-AAG expressed relative to vehicle treated control animals. Ns, not significant. Error bars are SEMs.



Supplementary Figure 6. Effect of 17-AAG in ethanol vehicle on 3Rtau larval locomotion. 17-AAG (AAG) was delivered to fly food in ethanol vehicle at a final concentration of 0.2 µM in food. Primary behavioral readouts for the effects of the chemical were open field turning rate (A) and lane crawl time (B) at three different concentrations of each chemical. Ethovision 4 plate assay recordings were also used to measure open field velocity (C). For post-test comparisons between treatments, \*\* $p < 0.01$ , \* $p < 0.05$ , ns, not significant. Error bars are SEMs.



Supplementary Figure 7. Development of wild-type *Drosophila* raised on food treated with HSP-modulating chemicals. OreR development was monitored on food treated with celastrol (A–C), simvastatin (D–F), and withaferin-A (G–I) at the final concentrations indicated. The cumulative number of wandering L3 larvae (A, D, G), darkly pigmented late stage pupae (B, E, H) and newly eclosing adults (C, F, I) was measured at the times indicated after the seeding of newly-laid OreR/D42 embryos onto treated food. Results shown are totals from a single experiment. veh, vehicle for chemical delivery (ethanol at 0.2% v/v in food).

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