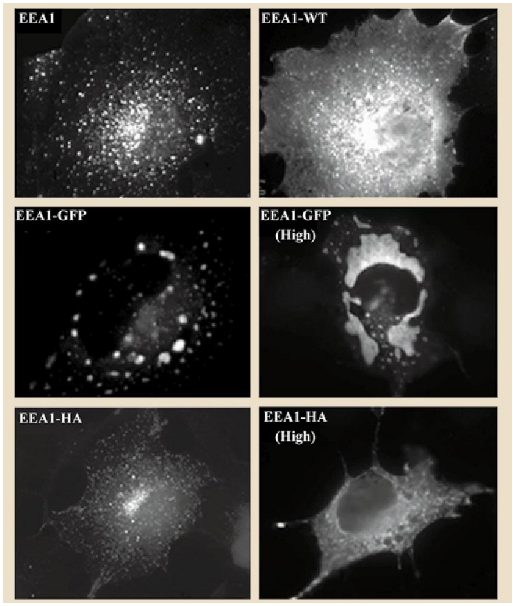

Figure 2 EAA1-GFP prevents the correct formation of early endosomes. Cos-1 cells (control is shown at top left) were transfected with wild-type EEA1 (top right), EEA1-GFP (middle panels) or EEA1-haemagglutinin (bottom panels). Cells were stained with anti-EEA1 antiserum (top panels) or with anti-haemagglutinin antiserum (bottom panels). EEA1-GFP intrinsic fluorescence was visualized in unstained cells (middle panels). Examples of cells expressing relatively low (left panels) or high (right panels) levels of each construct are shown.

135 amino acids of EEA1 bound increasingly to liposomes containing increasing amounts of PtdIns(3)P (Fig. 1a). Chelation of zinc inhibited binding, indicating that the interaction between the fusion protein and PtdIns(3)P was through a RING zinc-finger domain present in this carboxy-terminal region (Fig. 1b). We saw no specific binding to liposomes containing other polyphosphoinositides (Fig. 1b).

A fusion protein containing GST and the pleckstrin-homology domain of GRP-1, which binds phosphatidylinositol-3,4,5-trisphosophate with high affinity ($K_d < 1$ μM) (ref. 10), bound to liposomes containing phosphatidylinositol-3,4,5-trisphosphate, but not to liposomes containing PtdIns(3)P (Fig. 1b). Thus, the binding of the EEA1 RING finger to PtdIns(3)P is specific and of high affinity. Furthermore, the homologous domain in Hrs has been reported[11] to bind specifically to PtdIns(3)P. Thus this 'FYVE' domain seems to constitute a conserved binding motif for PtdIns(3)P.

A chimaera containing green fluorescence protein (GFP) at the carboxy terminus of EEA1 (EEA1–GFP) bound to liposomes containing PtdIns(3)P, but, in contrast to wild-type EEA1, this chimaera also bound to liposomes composed of phosphatidylserine or phosphatidylserine and phosphatidylinositol (Fig. 1c).

When transfected into Cos-1 cells, EEA1–GFP caused the formation of lagoon-like structures (Fig. 2), which contained transferrin internalized from outside the cells (not shown), indicating that the structures may represent fused endosomes. Overexpression of wild-type EEA1 (Fig. 2, top), or of another EEA1 chimaera containing nine amino acids of the haemaglutinnin protein of influenza virus, did not produce this phenotype. Thus, the loss in binding specificity caused by GFP correlates with the formation of abnormal endosomal structures, indicating that the specific interaction between EEA1 and PtdIns(3)P may be vital in the control of endosome fusion.

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Does practice shape the brain?

Pantev *et al.* (*Nature* **392,** 811–814; 1998) suggest that the degree of cortical reorganization and enhancement of the cortical response to musical notes depends on the age at which musicians first begin learning to play an instrument. Specifically, the younger the subjects were when they started to play, the larger was their cortical reorganization in recognition of piano tones. In addition to its biological interest, such a finding, if true, would have great implications for musical education. But we believe that the evidence presented by Pantev *et al.* is equally consistent with other interpretations.

Pantev *et al.* based their report on suggested correlations between the age at which musicians first began to play an instrument and the strength of cortical activation in response to piano tones when tested as adults. But the evidence for such correlations in their data is weak.

They present three correlations (for musicians with absolute pitch or with relative pitch, and for both groups combined). The (marginally significant) probability levels quoted are for one-tailed tests, used because 'negative correlations were predicted'. But it is possible that the cortical response to sensory experience might initially increase with age in young children, and so a positive correlation would be predicted. As both negative and positive correlations are possible, two-tailed tests should have been used in this case. Two-tailed tests show none of the reported correlations to be statistically significant at the traditional 5% level, and the confidence intervals for the correlation coefficients all include zero.

Even if more extensive experiments were to find a significant correlation, correlation does not indicate causation. The main contributors to the trends (if they exist) shown in Pantev *et al.*'s Fig. 2 are the musicians who began learning their instrument by 3–5 years of age. Musical tuition in such very young children is almost certainly associated with a high degree of parental musical skill or interest. At least two other explanations for the correlation would therefore be possible. First, perhaps only children with a particular type of cortical response to musical sounds are capable of learning an instrument from a very early age. Such a cortical response may have an inherited component. Second, it is possible that children brought up in musical families hear more music at a very young age, inducing more cortical reorganization. Neither explanation requires the children to have practised an instrument.

Pantev *et al.*'s comment that there was a "dependence of auditory [cortical]… representations on practice beginning before the age of about 10 years" ignores genotypic and environmental influences. It will be possible to establish the true nature of any relationship only by controlling for these effects. In the meantime, given our current state of knowledge, those wanting musical children might be well advised to examine carefully the musical abilities and compact-disc collections of potential mates, rather than investing in expensive music lessons for reluctant three-year-olds.

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