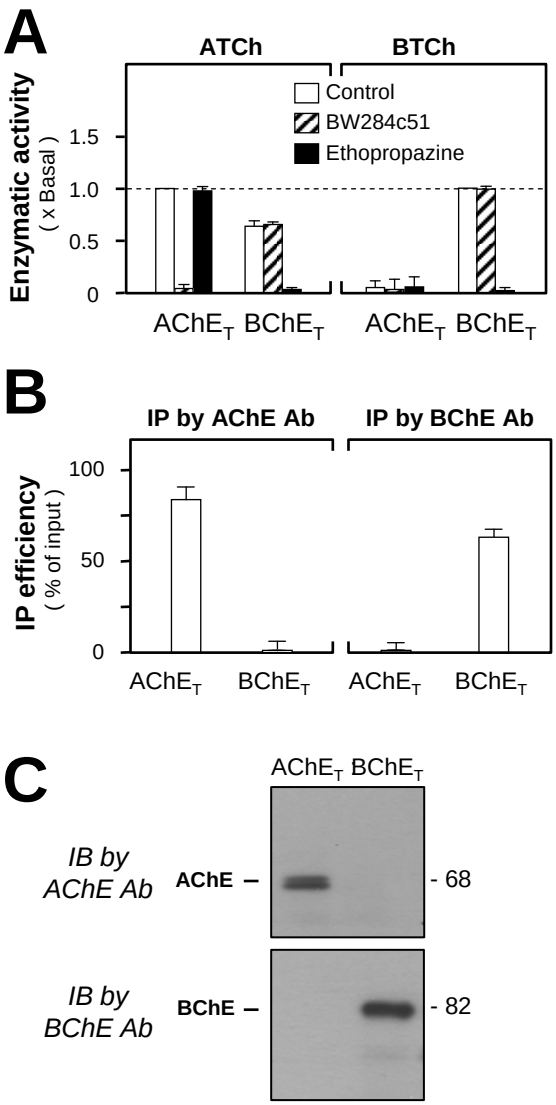


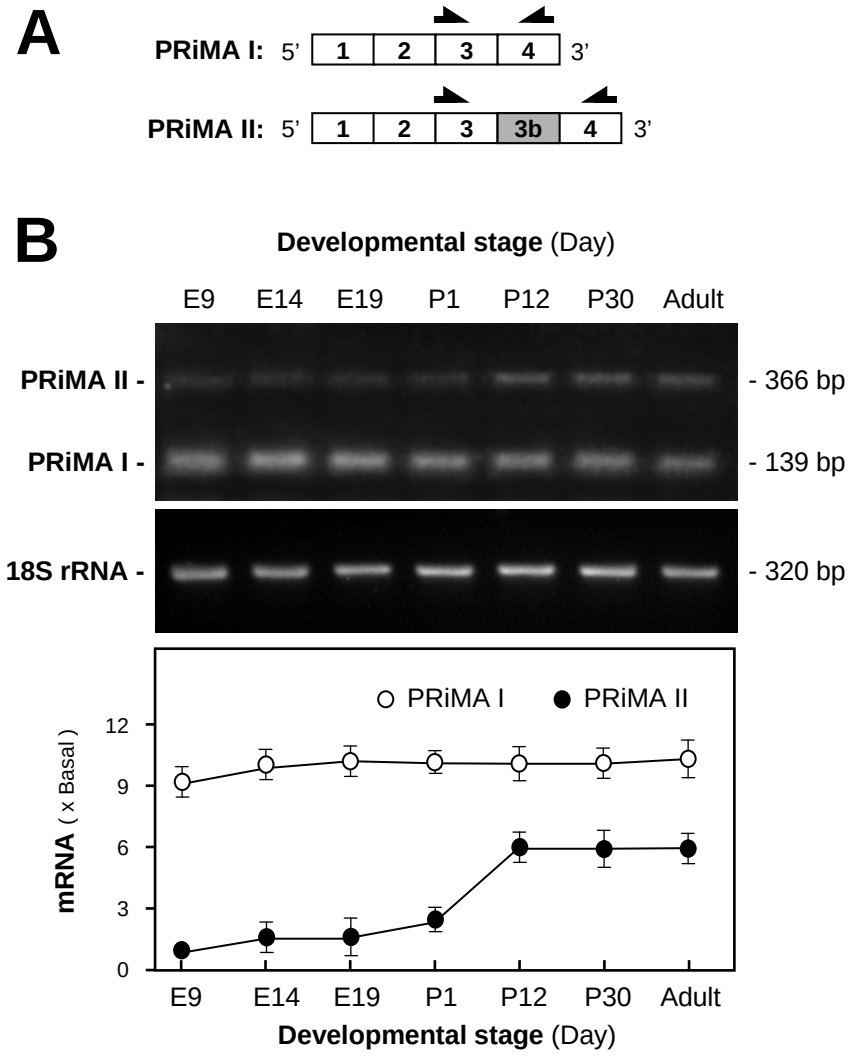
Supplementary Figure 1. **Specificity of enzymatic activity assays for AChE and BChE, and specificity of anti-AChE and anti-BChE antibodies.**

(A): HEK293T cells were transfected with cDNAs encoding $AChE_T$ and $BChE_T$ for two days. Cell extracts containing AChE activity (100 mOD/min) and BChE activity (100 mOD/min) were incubated for 10 min with 20 μM of AChE inhibitor, BW284c51, or 40 μM of BChE inhibitor, ethopropazine, before addition of the substrates, 0.625 mM of ATCh (left panel) or BTCh (right panel), to detect the enzymatic activities. The absorbance at 410 nm was taken, and the enzyme activities were calculated in units of absorbance / min / g of protein. Data are normalized to the control which was without inhibitor. All values are means ±SEM, each with triplicate samples (*n*=3).

(B): Cell extracts from (A) containing AChE activity (50 OD/min) and BChE activity (50 OD/min) in 500 μl were used for immuno-precipitation (IP) by E-19 anti-AChE antibody (1:200, left panel) or N-15 anti-BChE antibody (1:200, right panel). Fifty μl of protein G beads were added to each sample. After incubation and washing, the supernatants and protein G beads were subjected to Ellman assay. The sum of enzymatic activities remaining in the supernatants and associated with protein G beads was equal to the activity of the total input. The IP efficiency was expressed as the percentage decrease of enzymatic activity in the supernatant from the input. All values are means ±SEM, each with triplicate samples (*n*=3).

(C): Cell extracts from (A) containing equal amounts of protein (40 μg) were subjected to 8% SDS-PAGE. The specificity of E-19 AChE antibody and N-15 BChE antibody were tested by Western blot analysis to recognize AChE (68 kDa) and BChE (82 kDa), respectively.

Supplementary Fig. 1
Chen et al 2010



Supplementary Figure 2. The expression profile of PRiMA mRNA during chicken brain development.

(A): Splice variants of chicken PRiMA mRNA (PRiMA I and II) are illustrated. PRiMA I contains exon 1, 2, 3 and 4, while PRiMA II contains an additional exon 3b. Arrows show the location of primers used for semi-quantitative RT-PCR analyses.

(B): Total RNAs were extracted from chicken cerebrums at different developmental stages to perform RT-PCR for PRiMA I (amplified fragment of 139 bp) and PRiMA II (amplified fragment of 366 bp). 18S ribosomal RNA (320 bp) served as an internal control. One representative result is shown. The bottom panel shows quantitation of the PCR fragments from gel by calibrated densitometry. Data are normalized by the level of 18S ribosomal RNA and expressed as a ratio to the value obtained at E9 (basal). All values are means ±SEM, each with triplicate samples (n=3).

Supplementary Fig. 2
Chen et al 2010

	AChE		BChE	
	FHB-1	FHB-2	FHB-1	FHB-2
	352 362	506 520	390 401	540 561
Human	D**L**AAE**AV**V**L**H**Y**	A**Q**ACA**FW**NR**FLPKLL**	**S**EFGK**E**SIL**F**H**Y**	T**KLRA**Q**QC**R**FW**TS**FFPKVLE**M**T**
Mouse	D**L**AAE**AV**V**L**H**Y**	A**Q**TCA**FW**NR**FLPKLL**	**S**RLGK**E**AVL**F**Y**Y**	S**KLRA**P**QC**Q**FW**RL**FFPKVLE**M**T**
Rat	D**L**AAE**AV**V**L**H**Y**	A**Q**T**C**A**FW**NR**FLPKLL**	**S**SLGK**E**AIL**F**Y**Y**	S**KLRA**P**QC**Q**FW**RL**FFPKVLE**I**T**
Chicken	E**L**AAE**AV**V**L**H**Y**	T**Q**I**C**A**FW**TR**FLPKLL**	**S**KLAI**E**SII**F**Q**Y**	T**KLRA**Q**QC**R**FW**NM**FFPKVLE**M**T**
Torpedo	D**L**GLD**AV**T**L**Q**Y**	V**Q**M**C**V**FW**NQ**FLPKLL**		

Supplementary Figure 3. Predicted sequence of four-helix bundle (FHB) of AChE and BChE among different species.

The amino acid sequences of human, mouse, rat, chicken and *Torpedo* AChE catalytic subunits were deduced from nucleotide sequences accessed from GeneBankTM AAA68151, CAA39867, EDM13278, P36196, and CAA27169, respectively. The amino acid sequences of human, mouse, rat and chicken BChE catalytic subunits were deduced from nucleotide sequences accessed from GeneBankTM AAA99296.1, AAH99977, NP_075231, and NP_989977, respectively. The sequences of the two alpha helices (FHB-1 and FHB-2) forming the dimeric contact zone («four helix bundle») of AChE and BChE are shown. The residues conserved across species are highlighted in bold. It is noteworthy that the two FHB domains are highly conserved across different species for each enzyme, AChE and BChE. On the other hand, the similarity between FHB domains of AChE and BChE is very low, which may explain our finding that AChE and BChE cannot form an AChE-BChE hybrid dimer.

Supplementary Fig. 3
Chen et al 2010