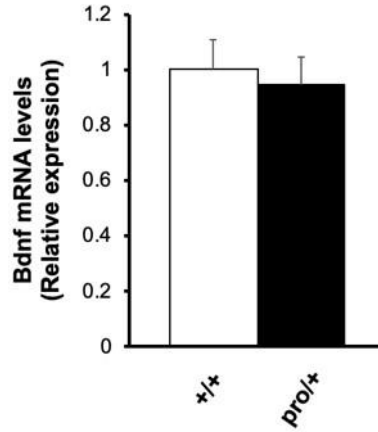


**IP: mouse anti-proBDNF monoclonal antibody clone 3C10H**  
**IB: mouse anti-BDNF monoclonal antibody, clone 3C11 (Icosagen)**

**Figure 1.** Western blotting was performed using hippocampal lysates prepared from 8-week-old *BDNF<sup>pro/pro</sup>* mice and 10-day-old *BDNF KO<sup>-/-</sup>* and *BDNF KO<sup>+/+</sup>* animals. For immunoprecipitation, hippocampal lysates containing 500  $\mu$ g protein were incubated with the 3C10H antibody. Next, the immunoprecipitates were electrophoresed on a 20% SDS-polyacrylamide gel. Immunoblotting was performed with a rabbit anti-pan-BDNF monoclonal antibody (EPR1292; Abcam). \* indicates light chain of IgG. Note that since the monoclonal antibody recognizing the BDNF pro-domain (3C10H) was used for immunoprecipitation, the proBDNF band was detected but mBDNF was not.



**Figure 2.** Quantitative real-time PCR analysis. The levels of *Bdnf* mRNA in the hippocampi prepared from BDNF<sup>pro/+</sup> and BDNF<sup>+/+</sup> mice (8 weeks old) were measured. All procedures of quantitative real-time PCR analysis are described in the Materials and Methods. Quantitative RT-PCR analyses revealed that there was no significant difference in *Bdnf* expression between the genotypes ( $n = 3$  mice/group).  $p = 0.30$  by  $t$  test.

**Table 1.** Mendelian inheritance frequencies of the animals.

Parameter	BDNF Genotype		
	+/+	pro/+	pro/pro
No. of pups	16	31	16
Frequency (%)	25.4	49.2	25.4

The frequencies were determined on the basis of genotyping of 63 pups from six independent colonies.