

Establishment of a *Drosophila* AD model

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Supplementary Information

Figure S1. APP protein expression level in larvae and flies.

Figure S2. Expression of APP induced aging-dependent locomotion defects.

Figure S3. Expression of APP induced immediate-recall memory defects.

Figure S4. Expression of APP induced no obvious morphological defects in the mushroom body.

Supplementary information of this article can be found online at <http://www.jbmethods.org/jbm/rt/suppFiles/61>.

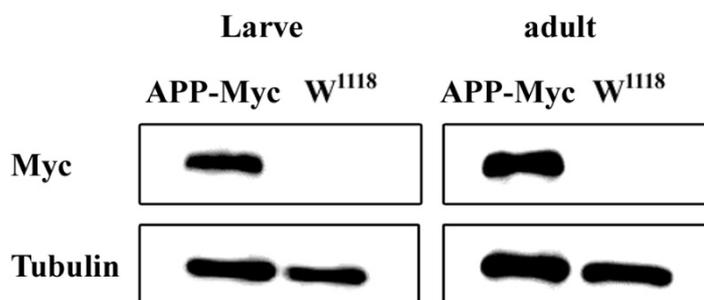


Figure S1. APP protein expression level in larvae and flies. Expression of APP protein were measured by western blot. APP protein is tagged by Myc and could be detected by anti-Myc antibodies in APP expressing larvae or adults, but not in the controls. Third instar larvae or adult flies were homogenized in RIPA buffer (CST) with PMSF and proceeded with the standard western blot protocol. In brief, lysates were separated on 10% SDS-PAGE gels and transferred to PVDF membranes. After being blocked with 5% nonfat milk dissolved in TBST buffer, the membranes were incubated with monoclonal antibodies against Myc (CST) or tubulin (CST) overnight and then checked with a secondary HRP-conjugated anti-mouse IgG or anti-Rabbit IgG (Jackson). Signals were detected with Super Signal West Pico Stable Peroxide Solution (Thermo Scientific).

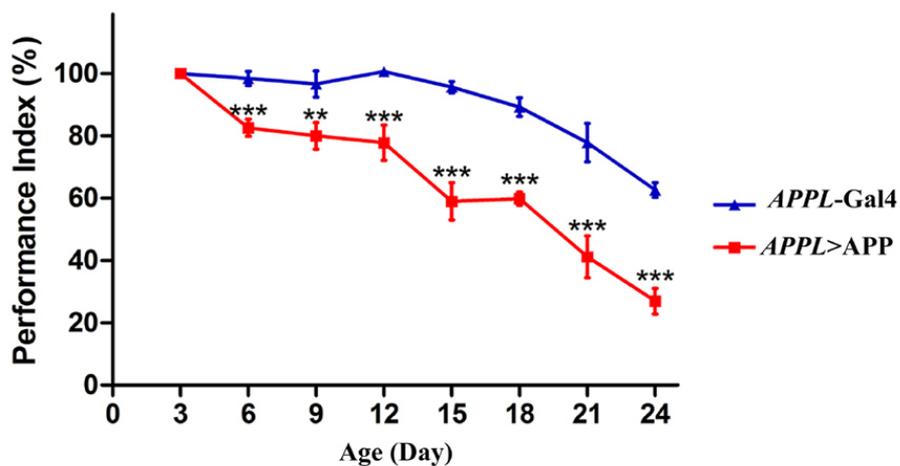


Figure S2. Expression of APP induced aging-dependent locomotion defects. Expression of APP driven by *APPL-Gal4* results in a more serious aging-dependent impairment compared with the *APPL-Gal4* alone. ***, $P \leq 0.001$. Genotypes: Control groups (*APPL-Gal4/+*), tested groups (*APPL-Gal4/+; UAS-APP/+*).

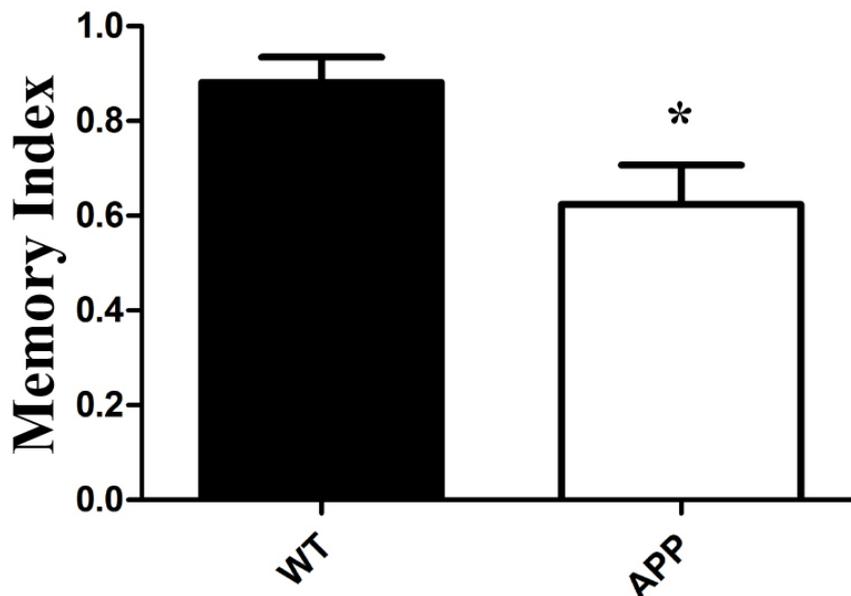


Figure S3. Expression of APP induced immediate-recall memory defects.

Expression of APP driven by *elav-Gal4* results in a moderate defect in the immediate-recall memory compared with the wild control. All females and males were collected within 4 h after eclosion and were raised on standard medium at 25°C in a 12-h light dark cycle. Males assayed were 3-day old and were kept in one single vial independently. Females used were 3-day old and were kept in groups of 20 per vial. Females were mated the day before experiment by mixing 20 females with >30 *Oregon R* males overnight. For training, a male was put into a round observation chamber (Diameter of 15 mm and depth of 8 mm) either with a predated female (Training group) or without any female (Sham group) and were trained for 1 h. Then the male was transferred to a new round observation chamber with a mated female within 2 min. All males were transferred without anesthesia. The courtship index (CI) was calculated as the percentage of time that a male displayed any of the courtship steps towards the females during the 10 min observation period. The CI_{sham} and CI_{test} were then calculated and used for generating MI as (mean CI_{sham} - CI_{test})/mean CI_{sham}. The MI=0 means no immediate-recall memory and MI=1 means normal immediate-recall memory. *, $P \leq 0.1$. Genotypes: WT groups (*elav-Gal4/+*), APP groups (*UAS-APP/+; elav-Gal4/+*)

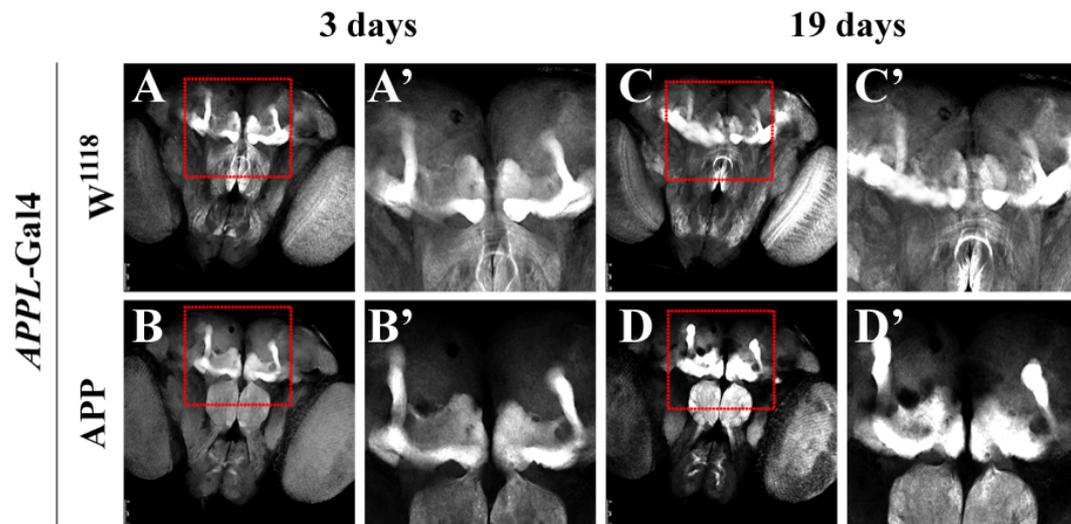


Figure S4. Expression of APP induced no obvious morphological defects in the mushroom body. Fluorescent images of the brain and mushroom body (MB) of the adult flies were shown. Compared with *APPL-Gal4* controls (A, A', C and C'), expression of APP (B, B', D and D') induced no evident morphological defects of MB in 3 days and 19 days old flies. Genotypes: *APPL-Gal4/+; UAS-GFP/+* (A, A', C and C'); *APPL-Gal4/+; UAS-GFP/UAS-APP* (B, B', D and D').