

There are some procedures where the fly lines obtained can be directly examined without having to make F1 generations with selective crosses.

The lines which are OK371-ChR2 homozygous line, which expresses the light-activated channelrhodopsin in motor neurons. This line is made by crossing $w^{1118}; P\{GawB\}VGlut^{OK371}$ (BDSC stock # 26160) with w^* ; $P\{UAS-H134R-ChR2\}2$ (BDSC stock # 28995 Pulver et al., 2011).

The homozygous line for both OK371-Gal4 and UAS-H134-ChR2 was used in this study. We used another recently created ChR2 line which is very sensitive to light called $y^1 w^{1118}; PBac\{UAS-ChR2.XXL\}VK00018$ (BDSC stock # 58374) (Dawydow et al., 2014). Virgin females from w^* ; $P\{UAS-H134R-ChR2\}2$ were crossed with males of D42-Gal4 (BDSC stock#8816), TRH-Gal4 (BDSC stock#38389), Gad1-Gal4 (BDSC stock# 51630), or ppk-Gal4 (BDSC stock# 32078) line to express ChR2-XXL variant in motor neurons, serotonergic neurons, GABAergic neurons or Type IV sensory neurons, respectively.

We also used UAS-H134R-ChR2;Trh-Gal4 (III) homozygous line which is kindly provided by Dr. Andreas Schoofs (Schoofs et al., 2014).

Table 1: Fly lines

Line	Sex	Outcome
$w^{1118}; P\{GawB\}VGlut^{OK371}$ Cross with line below opposite sex	Male or Female	ChR expressed in neurons which express vesicular transporter for glutamate. Motor neurons.
w^* ; $P\{UAS-H134R-ChR2\}2$	Male or Female	
$y^1 w^{1118}; PBac\{UAS-ChR2.XXL\}VK00018$ Cross with below lines	Virgin Female	
D42-Gal4	Male	ChR expressed in motor neurons.
TRH-Gal4	Male	ChR expressed in serotonergic neurons
Gad1-Gal4	Male	ChR expressed in GABAergic neurons
ppk-Gal4	Male	ChR expressed in Type IV sensory neurons

Can use male or female of UAS-H134R-ChR2;Trh-Gal4 (III) homozygous line. There is no need to make and crosses as this line is homozygous. All one has to do is feed one group ATR and a control group without ATR.

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