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 w1118;P{GawB}VGlutOK371 (BDSC stock # 26160) with w\*; [P{UAS-H134R-ChR2}2](http://flybase.org/reports/FBti0116127.html) (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 y1 w1118; PBac{UAS-ChR2.XXL}VK00018 (BDSC stock # 58374) (Dawydow et al., 2014). Virgin females from w\*; [P{UAS-H134R-ChR2}2](http://flybase.org/reports/FBti0116127.html)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

w1118;P{GawB}VGlutOK371 Male or Female ChR expressed in neurons which express vesicular

Cross with line below opposite sex transporter for glutamate. Motor neurons.

w\*; [P{UAS-H134R-ChR2}2](http://flybase.org/reports/FBti0116127.html) Male or Female

y1 w1118; PBac{UAS-ChR2.XXL}VK00018 Virgin Female

Cross with below lines

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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