Supplemental methods

Spontaneous locomotor activity

Lyn KO and Lyn WT mice were placed in an open-field (43cm x 43cm x 31cm; Med Associates) for a 1-hr session. The total distance travelled during the session was recorded using 2 sets of 16 photobeams placed on the sides of the cage. At the end of the session mice were placed back in their home cage.

Loss of righting reflex

Mice were injected i.p. with ethanol (3.2, 3.6 and 4.0 g/kg, 20% v/v solution) (Yaka et al., 2003; Sharko and Hodge, 2008), and immediately after were placed in a plexiglass cage until the end of the experiment. After the mice lost their righting reflex (LORR), they were placed on their back and the duration of the LORR was recorded. The recovery from the LORR was defined as the time at which the mice could stay on their 4 paws 3 times within 1 min.

Blood ethanol concentration

Trunk blood was collected in heparinized capillary tubes from Lyn WT and Lyn KO mice 15, 40 and 60 min after an i.p. injection of a dose of ethanol (2 g/kg, 20% v/v). Serum was extracted with 3.4% trichloroacetic acid followed by a 5-min centrifugation at 420 g and assayed for ethanol content using the nicotinamide adenine dinucleotide (NAD^+-NADH) enzyme spectrophotometric method (Zapata and Shippenberg, 2006). Blood ethanol concentrations (BEC) were determined by using a standard calibration curve.

Supplemental Figure 1: Schematic drawings of coronal sections of the rat brain showing the placement of the microdialysis in the NAc. Only data from animals in which the histologically reconstructed sites of infusions were localized in the NAc were included in the analysis of each experiment. The drawings are taken from the Paxinos and Watson brain atlas (2001).

Supplemental Figure 2: Global deletion of the Lyn gene does not induce alterations in the expression of the Src and Fyn, in the metabolism of ethanol, locomotor activity or loss of righting reflex

a. Western blot analysis of whole brain from Lyn WT and KO mice at the age of 8 weeks. The membranes were probed with antibodies against Lyn, Src and Fyn with Actin used as a loading control. (n = 3). ***b.*** Lyn KO and WT mice were placed in an open-field for 1 hr and the spontaneous locomotor activity in a novel environment was measured. The results are expressed as mean ± SEM distance travelled by blocks of 5 min. WT = 14; KO n = 15. ***c,*** Mice received an i.p. injection of 2 g/kg of a 20% solution of ethanol (v/v) and blood was collected 15, 40 or 60 min after the injection. Blood samples were analyzed using the NAD^+-NADH enzymatic method. Results are expressed as mean ± SEM BEC (mM). WT and KO, n = 4. ***d***, Mice received i.p. injections of ethanol (3.2, 3.6 and 4.0 g/kg of a 20% solution, v/v), and the duration of LORR was recorded. Results are expressed as mean ± SEM duration of LORR (min). WT, n = 7; KO, n = 6.

References

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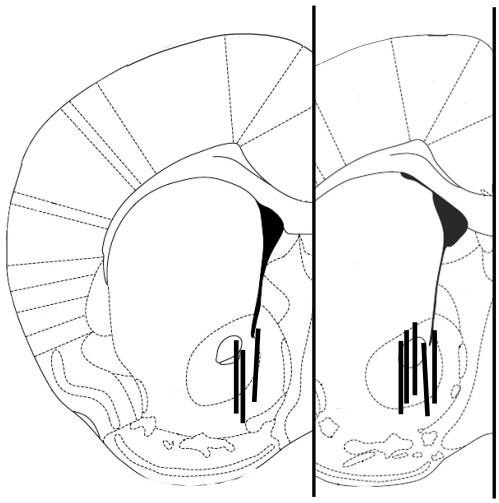
Sharko AC, Hodge CW (2008) Differential modulation of ethanol-induced sedation and hypnosis by metabotropic glutamate receptor antagonists in C57BL/6J mice. Alcohol Clin Exp Res 32:67-76.

Yaka R, Tang KC, Camarini R, Janak PH, Ron D (2003) Fyn kinase and NR2B containing NMDA receptors regulate acute ethanol sensitivity but not ethanol intake or conditioned reward. Alcohol Clin Exp Res 27:1736-1742.

Zapata A, Shippenberg TS (2006) Endogenous kappa opioid receptor systems modulate the responsiveness of mesoaccumbal dopamine neurons to ethanol. Alcohol Clin Exp Res 30:592-597.

Suppl. Figure 1



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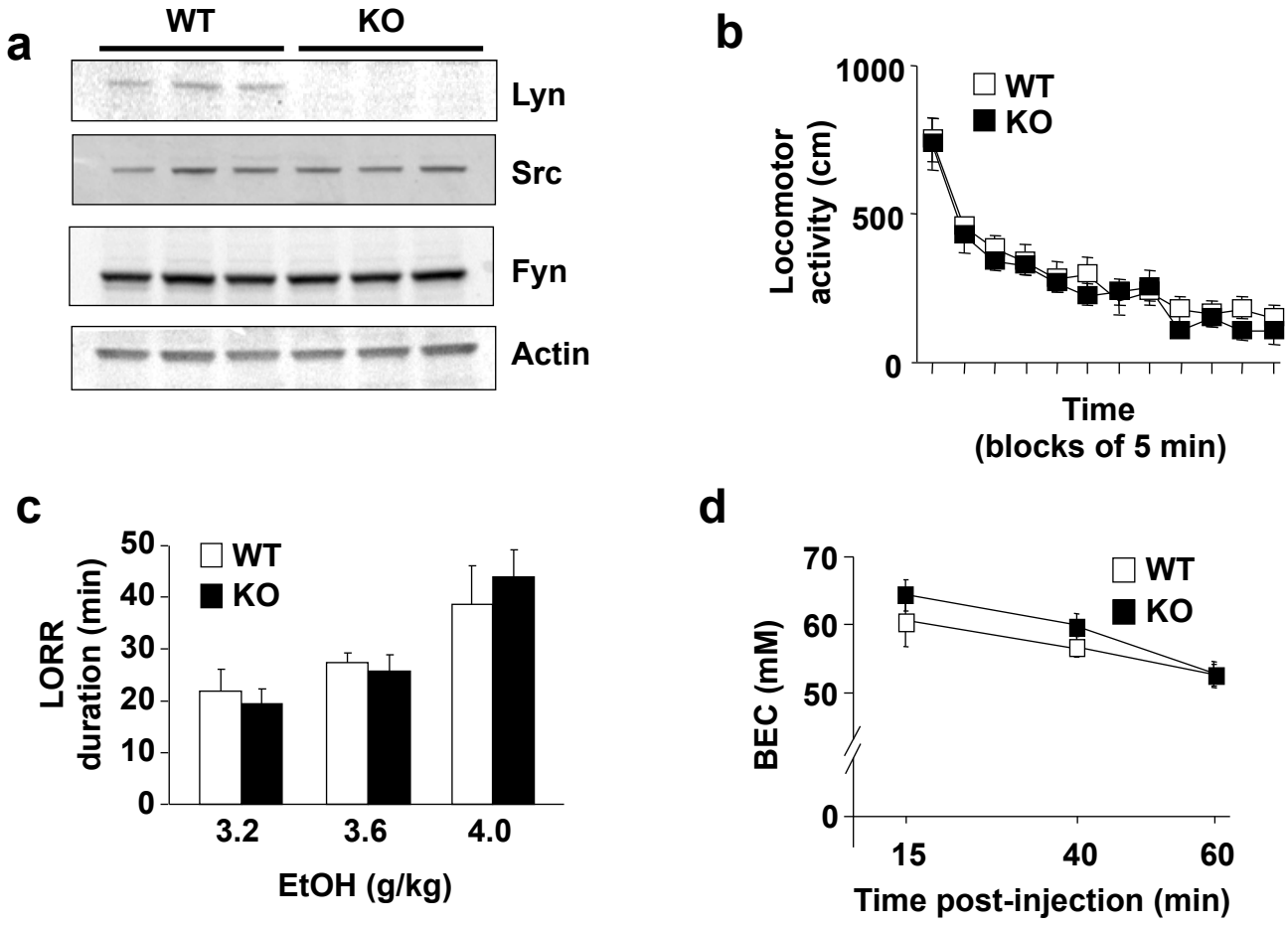
Suppl. Fig. 2


a
WT
KO
Lyn
Src
Fyn
Actin
b
1000
500
0
WT
KO
Locomotor activity (cm)
Time
(blocks of 5 min)
c
50
40
30
20
10
0
WT
KO
LORR duration (min)
3.2
3.6
4.0
EtOH (g/kg)
d
70
60
50
0
WT
KO
BEC (mM)
15
40
60
Time post-injection (min)