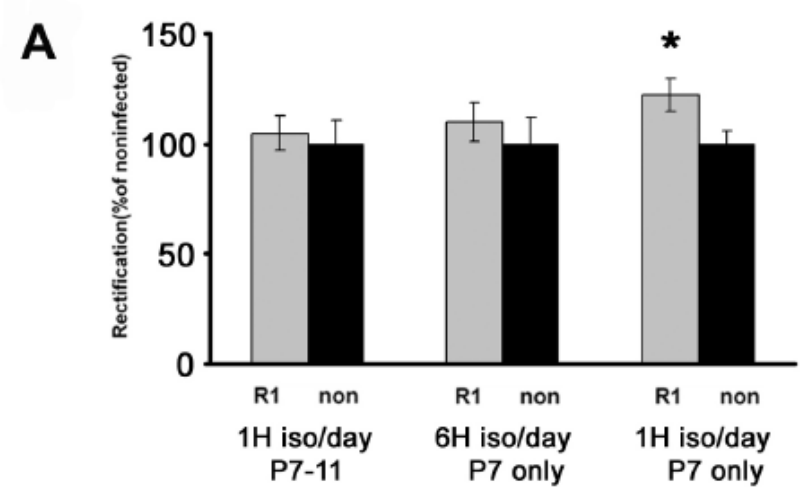
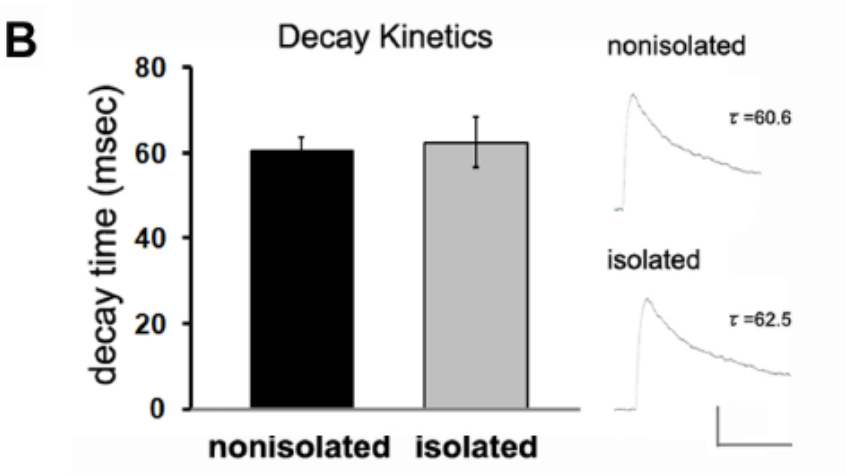
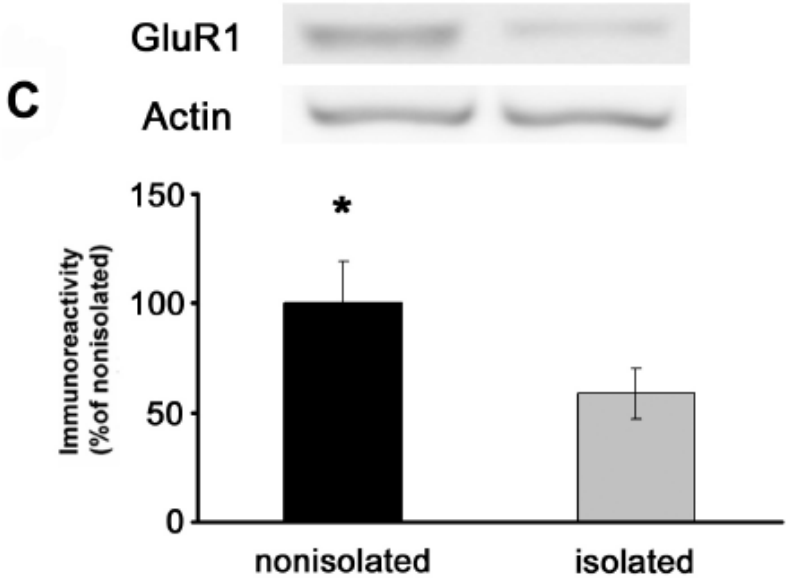
Supplemental information

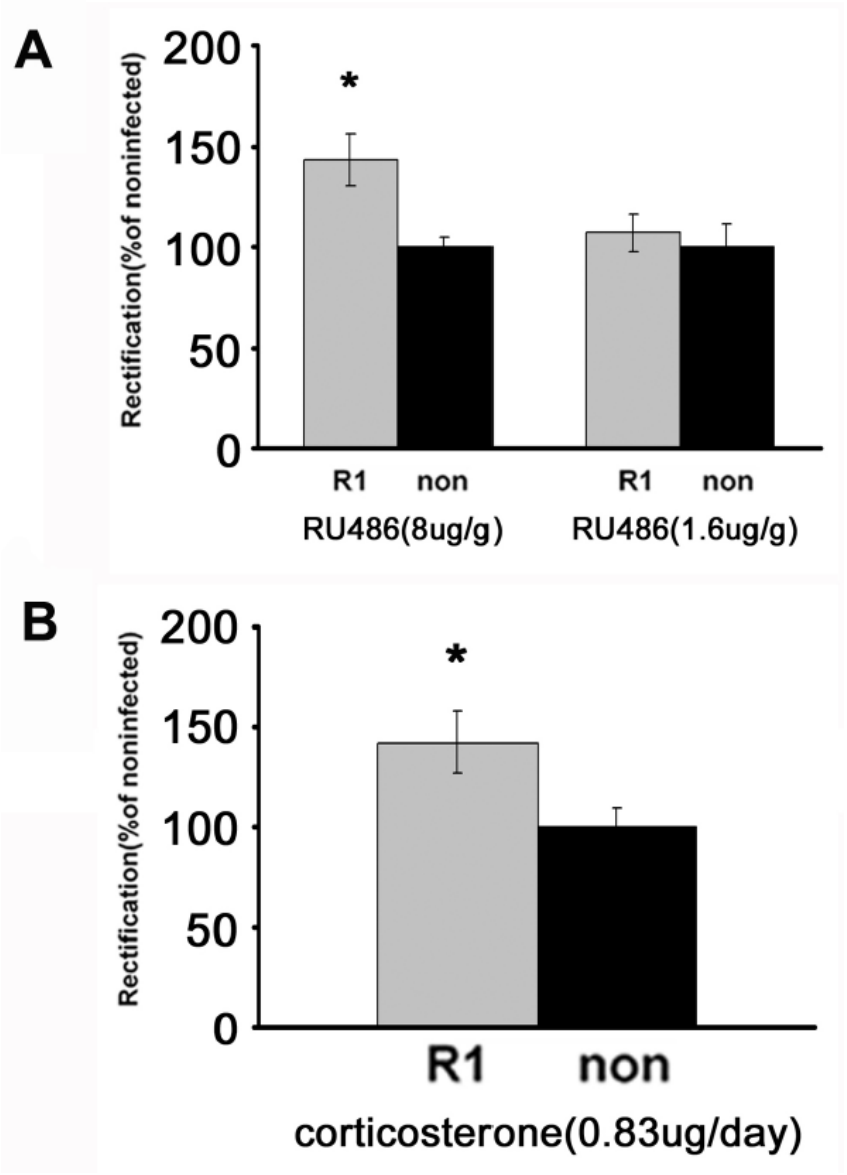






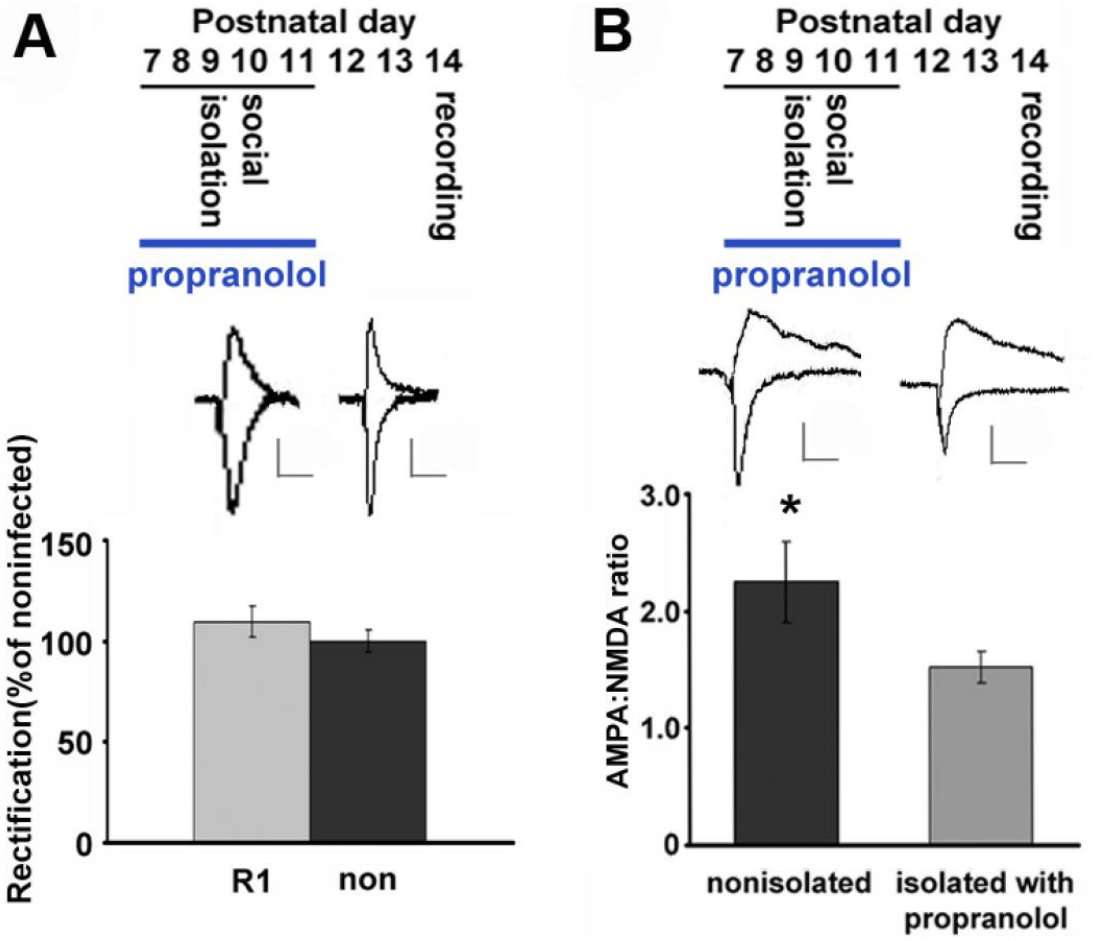
Supplemental Figure 1

Social isolation-induced disruption of experience-driven synaptic GluR1 delivery was social isolation-dose dependent. (A) RI values of infected neurons normalized to non-infected neurons. Social isolation for 1 hour (1H iso/day) during postnatal (P)7-P11 and for 6 hours (6H iso/day) just at P7 disrupted experience-driven synaptic delivery of GluR1 at P12-14 (n=7 animals with 1 hour isolation at P7-P11 and n=7 animals with 6 hours isolation at P7). Social isolation for 1 hour just at P7 (1H iso/day) did not disrupt experience-dependent synaptic GluR1 delivery (*P<0.05 compared with nonexpressing neurons, n=10). R1: GFP-GluR1 expressing neurons, non: nonexpressing neurons. (B) Social isolation does not affected decay time constants of NMDA receptor-mediated synaptic responses. Average decay time constants of NMDA receptor-mediated synaptic responses in nonisolated and isolated animals. A single exponential function fitted to the decay of the NMDA current between 15 and 165ms after the peak was used to estimate the time constant (n=14 per each group). Scale bar: 50pA/150ms. (C) Social isolation for 6 hours/day during P7-P11 exhibited reduced amount of GluR1 in the synaptosome fraction at P11 (*P<0.05 compared with isolated, n=6 per each group). The lanes were run on the same gel but noncontiguous.


A
Rectification(%of noninfected)
200
150
100
50
0
*
R1
non
R1
non
RU486(8ug/g)
RU486(1.6ug/g)
B
Rectification(%of noninfected)
200
150
100
50
0
*
R1
non
corticosterone(0.83ug/day)

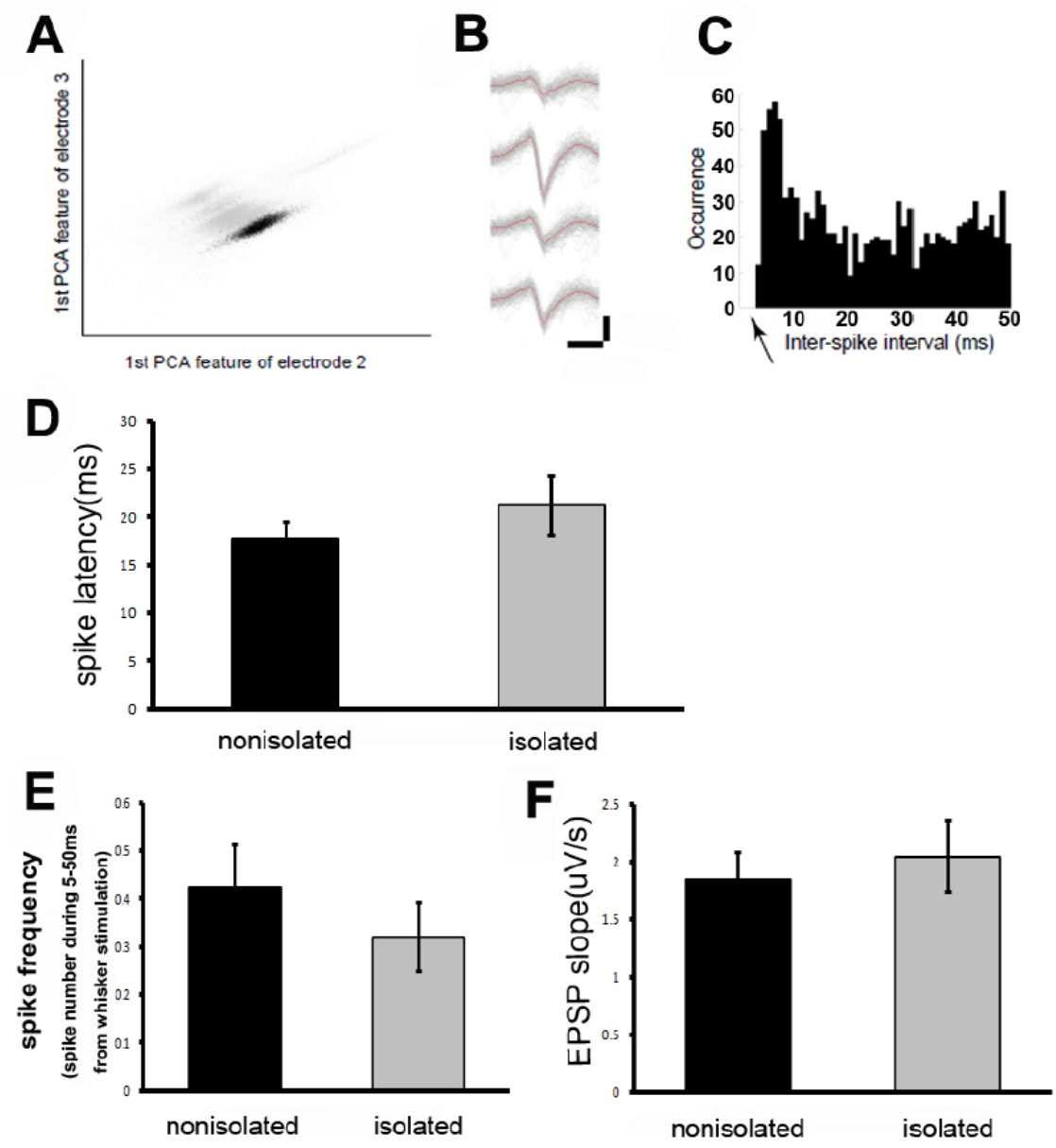
Supplemental Figure 2

Dose dependent effects of RU486 and corticosterone. R1: GFP-GluR1 expressing neurons, non: nonexpressing neurons. (A) The effect of RU486 on the social isolation-induced disruption of experience-driven synaptic GluR1 delivery was RU486-dose dependent. RI values of infected neurons normalized to uninfected neurons. While social isolation for 6hours/day at P7-P11 with RU486 (8μg/g of body weight) did not impair synaptic GluR1 delivery (*P<0.05 compared with nonexpressing neurons, n=6), social isolation for 6hours/day at P7-P11 in the presence of RU486 (1.6μg/g of body weight) disrupted synaptic GluR1 delivery (n=9). (B) The effect of corticosterone on the experience-driven synaptic GluR1 delivery was corticosterone-dose dependent. Injection of corticosterone (0.83μg per one day) did not disrupt experience-dependent synaptic GluR1 delivery (*P<0.05 compared with nonexpressing neurons, n=9).


A
Postnatal day
7 8 9 10 11 12 13 14
social isolation
recording
propranolol
Rectification(%of noninfected)
150
100
50
0
R1
non
B
Postnatal day
7 8 9 10 11 12 13 14
social isolation
recording
propranolol
AMPA:NMDA ratio
3.0
2.0
1.0
0
*
nonisolated
isolated with propranolol

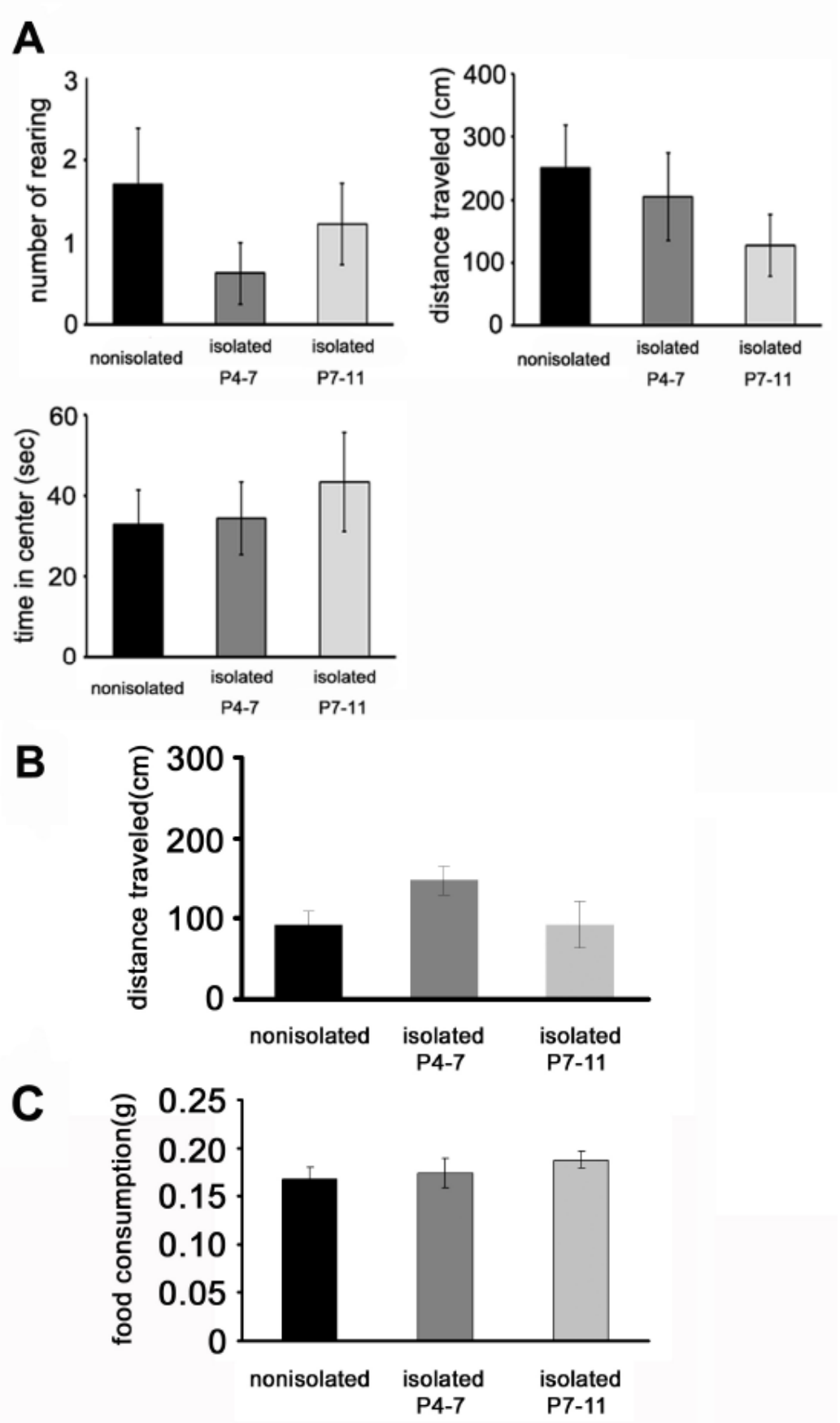
Supplemental Figure 3

NE pathway does not mediate social isolation-induced disruption of synaptic delivery of GluR1. (A) Administration of propranolol, an antagonist of β adrenergic receptor, did not prevent the disruption of AMPA receptors delivery into synapses by social isolation during postnatal (P)7-P11 (n=24). Scale bar: 10pA/20ms. R1: GFP-GluR1 expressing neurons, non: nonexpressing neurons. (B) A/N ratio at P14 was lower with rats isolated in the presence of propranolol during P7-P11 than nonisolated rats (*$P<0.05$ compared with isolated with propranolol, n=25 per each group). Note that A/N ratio at P14 of isolated rats in the presence of propranolol is comparable to isolated rats in the absence of propranolol (see Figure 1D). Scale bar: 20pA/20ms.


A
1st PCA feature of electrode 3
1st PCA feature of electrode 2
B
C
Occurrence
60
50
40
30
20
10
0
10
20
30
40
50
Inter-spike interval (ms)
D
spike latency(ms)
30
25
20
15
10
5
0
nonisolated
isolated
E
spike frequency
(spike number during 5-50ms from whisker stimulation)
0.6
0.5
0.4
0.3
0.2
0.1
0
nonisolated
isolated
F
EPSP slope(uV/s)
2.5
2
1.5
1
0.5
0
nonisolated
isolated

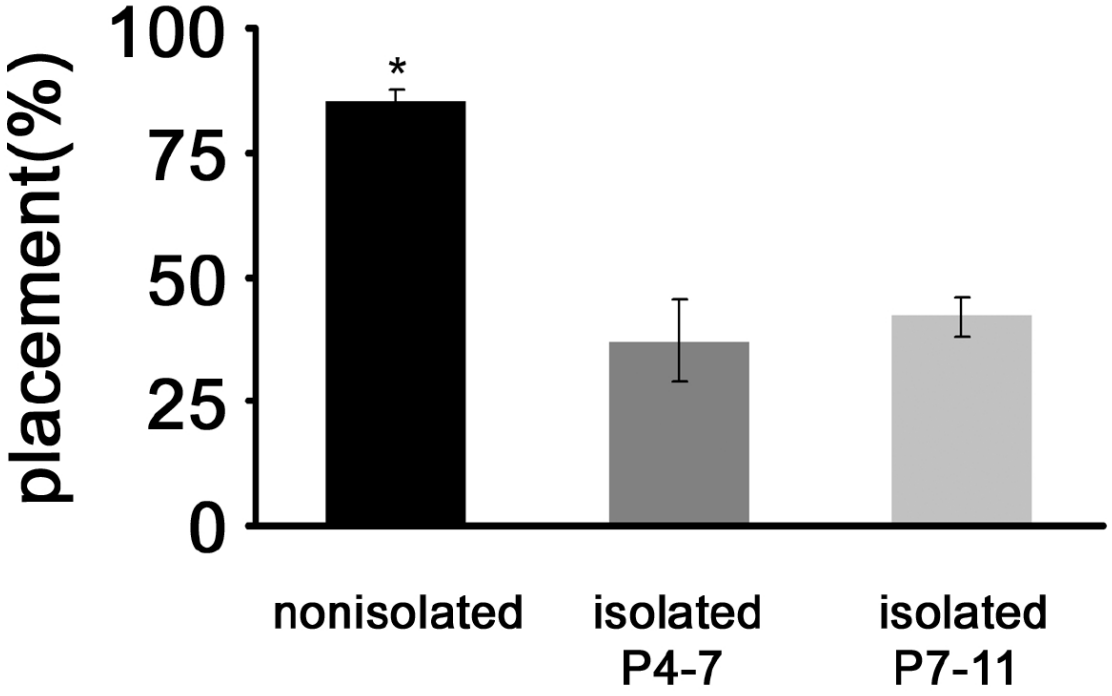
Supplemental Figure 4

Social isolation attenuates whisker-sensitivity at the layer2/3 of the barrel cortex. (A-C) Isolation of single unit activity in the somatosensory cortex by tetrode recording. After spike detection, spike waveform features from each of the electrodes were computed by the principal component analysis (PCA). (A) The spike features computed by the first principal components of electrode 2 and 3 are plotted in a scattergram. The scales of the abscissa and ordinate are arbitrary. An isolated cluster by use of the KlustaKwik program and subsequent manual isolation procedures is labeled in black dots. (B) Waveforms of individual spikes of the isolated cluster in A are shown. Red traces indicate the average waveforms. Scale bar: 100μcale ba. (C) Inter-spike interval histogram of the isolated cluster is plotted. Note the clear 2-3 ms refractory period (arrow). (D) Absolute value of latency of spike onset. Note that there is no difference in spike latency by the deflection of the principal whisker between animals with social isolation during postnatal (P)4-P7 and control nonisolated rats at P23 (n=10 per each group). (E-F) Spike frequency (spike number during 5-50ms from whisker stimulation, E) and field EPSP slopes (F) recorded from layer 4 of the barrel cortex by the deflection of the principal whisker. Note that there is no difference in spike frequency and EPSP slopes by the deflection of the principal whisker between animals with social isolation during P4-P7 and control nonisolated rats at P23. (E: n=8 isolated and n=7 non-isolated, F: n=4 isolated, n=5 non-isolated).


A
number of rearing
3
2
1
0
nonisolated
isolated P4-7
isolated P7-11
distance traveled (cm)
400
300
200
100
0
nonisolated
isolated P4-7
isolated P7-11
time in center (sec)
60
40
20
0
nonisolated
isolated P4-7
isolated P7-11
B
distance traveled(cm)
300
200
100
0
nonisolated
isolated P4-7
isolated P7-11
C
food consumption(g)
0.25
0.20
0.15
0.10
0.05
0
nonisolated
isolated P4-7
isolated P7-11

Supplemental Figure 5

Social isolation does not affect general anxiety on open field apparatus (A), on GAP crossing test apparatus (B) and motivation on GAP cross test apparatus (C). (A) The number of rearing (top left; ANOVA, $F_{(2,24)} = 0.913$), distance traveled (top right; ANOVA, $F_{(2,24)} = 1.000$), mean time spent in the central area (bottom; ANOVA, $F_{(2,24)} = 0.323$). There was no significant difference among animals with non-isolation, isolation at P4-P7, and isolation at postnatal (P)7-P11 (n=10, 8, 9 respectively). (B) Distance traveled during gap crossing task (ANOVA, $F_{(2,24)} = 2.094$). There was no significant difference among animals with non-isolation, isolation at P4-P7, and isolation at P7-P11 (n=10, 8, 9 respectively). (C) Food consumption during gap crossing test. There was no significant difference among animals with non-isolation, isolation at P4-P7, and isolation at P7-P11 (ANOVA, $F_{(2,11)} = 0.743$, n=5, 5, 4 respectively).


placement(%)
100
75
50
25
0
*
nonisolated
isolated
P4-7
isolated
P7-11

Supplemental Figure 6

Social isolation attenuated whisker-function dependent forelimb test. Social isolation during postnatal (P)4-P7 or P7-P11 disrupted whisker function-dependent forelimb test at P14. Probability of normal forelimb response (see text) was shown. Nonisolated pups (n=10) exhibited significantly higher normal response than animals with isolation during P4-P7 and P7-P11 (ANOVA, $F_{(2,24)} = 30.065$, *P<0.05 compared with isolated at P4-P7 (n=7) and isolated at P7-P11 (n=10) animals).

		spike number	resting potential (mV)	input resistance (mOhm)
nonisolated	**infected**	2.5±0.2	67.5±1.1	451.2±17.9
	noninfected	2.7±0.3	69.0±2.1	482.7±24.2
isolated	**infected**	2.7±0.2	69.1±1.2	483.8±18.1
	noninfected	2.5±0.3	69.5±0.8	444.3±25.2

Supplemental Table 1

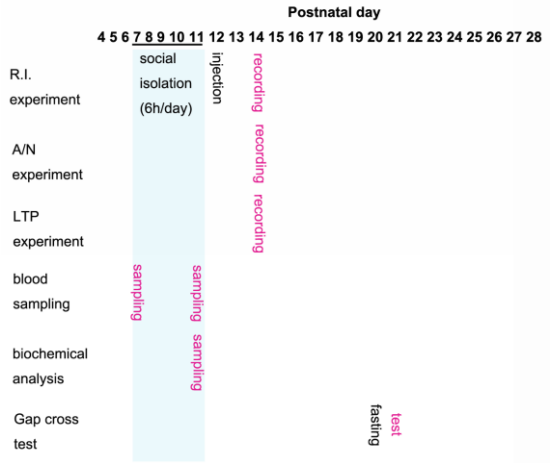
Social isolation does not affect electrophysiological properties of infected and non-infected neurons compared with nonisolated animals. Social isolation did not affect spike number(current injection;150pA 300ms), resting potential and input resistance.

	average weight (g)
nonisolated	29.0±1.0
isolated	29.1±1.8

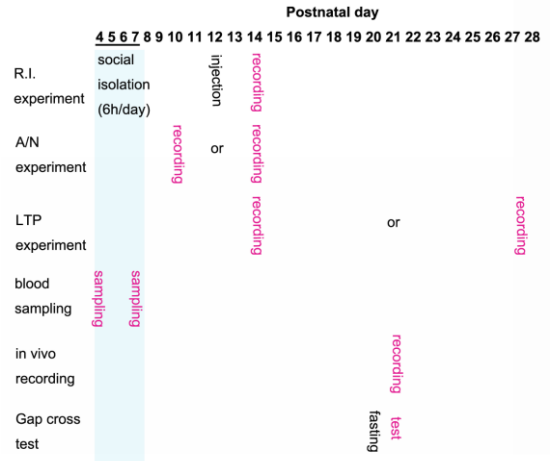
Supplemental Table 2

Social isolation does not affect pups body weight.

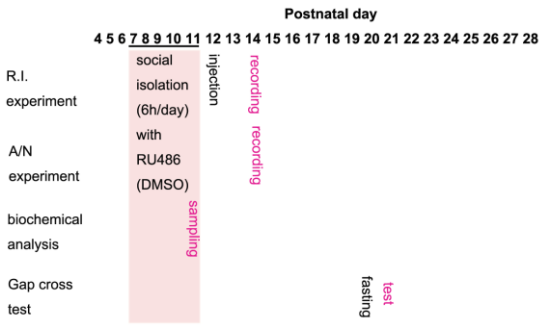
Experiment 1. isolated P7-11 (also mother removed and whisker mask)



Experiment 2. isolated P4-7



Experiment 3. isolated P7-11 with RU486 (DMSO)



Experiment 4. corticosterone P7-11

Supplemental chart

Summary chart of experimental procedures (except local RU486 injection experiment presented in Figure 4C)