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Original Research

Subregional Expression of Hippocampal Glutamatergic and GABAergic Genes in F344 Rats with Social Isolation after Weaning

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Many studies have shown that postweaning social isolation (pwSI) alters various behavioral phenotypes, including hippocampus-dependent tasks. Here, we report the comprehensive analysis of the expression of glutamatergic and GABAergic neurotransmission-related genes in the distinct hippocampal subregions of pwSI rats. Male F344 rats (age, 4 wk) experienced either pwSI or group housing (controls). At 7 wk of age, the hippocampus of each rat was removed and laser-microdissected into the CA1 and CA3 layers of pyramidal cells and the granule cell layer of the dentate gyrus. Subsequently, the expression of glutamatergic- and GABAergic-related genes was analyzed by quantitative RT-PCR. In the CA1 and CA3 pyramidal cell layers, 18 of 24 glutamate receptor subunit genes were at least 1.5-fold increased in expression after pwSI. In particular, the expression of several N-methyl-D-aspartate and kainate receptors (for example, *Grin2a* in CA1, *Grik4* in CA3) was significantly increased after pwSI. In contrast, pwSI tended to decrease the expression of $GABA_A$ receptor subunit genes, and *Gabra1*, *Gabra2*, *Gabra4*, *Gabra5*, *Gabrb2*, *Gabrg1*, and *Gabrg2* were all significantly decreased in expression compared with the levels in the group-housed rats. These results indicate a subregion-specific increase of glutamate receptors and reduction of $GABA_A$ receptors, suggesting that the hippocampal circuits of pwSI rats may be in more excitable states than those of group-housed rats.

Abbreviations: AMPA, α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid; LMD, laser microdissection; NMDA; N-methyl-D-aspartic acid; pwSI, postweaning social isolation; DG; dentate gyrus

Behavioral phenotypes differ among strains of laboratory animals, including mice and rats. The acquisition of strain-specific behavioral traits is governed not only by the genetic background but also by the postnatal rearing environment. The level of maternal care is a representative environmental factor. For example, inbred Fischer 344 pups raised by Wistar dams showed Wistar-like behavioral traits in adulthood in socially interactive situations.[35] Furthermore, the effect of environmental factors is not limited to neonatal periods. In animals that form a society among conspecifics, such as mice and rats, postweaning social environments have a marked effect on behavioral traits. Many studies have reported that postweaning social isolation (pwSI) alters various strain-specific behavioral phenotypes, including aggressiveness,[15,32,34] novelty preference,[22] locomotor activity,[29] anxiety-like behavior,[20] and learning and memory.[13,15,20,31] In other words, pwSI means the deprivation of several social interactions among conspecifics. The expression of social contact begins fundamentally in the postweaning periods, its frequency increases to a peak at 4 to 5 wk of age, and it declines thereafter until sexual maturity.[2,17,30] Therefore, rats were isolated from their conspecifics to deprive them of social contact during the current study.

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In the brain, the hippocampus is necessary for the acquisition of episodic and spatial memory,[28] but the influences of pwSI on hippocampal functions remain largely unclear. The hippocampus has a lamellar organization of neurons, and the intrinsic neuronal circuit of the hippocampus, termed the trisynaptic circuit, consists of 3 topographically and morphologically distinct neuronal layers: the pyramidal cell layer in subfields CA1 and CA3 and the granule cell layer in the dentate gyrus (DG). Sensory information is carried first to the DG by a perforant pathway that originates in the entorhinal cortex. DG granule cells project to the apical dendrites of the CA3 pyramidal cells through mossy fibers. In turn, CA3 pyramidal cells project to the CA1 layer through Schaffer collaterals.[1,18] Glutamate is a key excitatory neurotransmitter in the hippocampus and plays a central role in the activation of the trisynaptic circuit, whereas the inhibitory neurotransmitter GABA modulates the activated circuit. This excitatory–inhibitory balance is critical for the appropriate functioning of the hippocampal circuit. Here, we comprehensively investigated the effect of pwSI on the expression of glutamatergic and GABAergic neurotransmission-related genes in the 3 hippocampal subregions of inbred F344 rats.

Materials and Methods

All experiments were performed in accordance with the Guidelines for Animal Experiments (College of Bioresource Sciences,

Nihon University) and Nihon University's Animal Care and Use Committees. Male F344/DuCrlCrlj rats (age, 3 wk; SPF, viral antigen-free) were purchased from Charles River Laboratories Japan (Yokohama, Japan). Rats were housed in opaque polypropylene cages (345 × 403 × 177 mm) and allowed free access to food (Labo MR Stock, Nosan, Kanagawa, Japan) and water. The rats were housed in an animal room maintained at 22 ± 1 °C with a humidity of 555 ± 10% and a 14:10-h light:dark cycle (lights on, 0600). When rats had reached 4 wk of age, they were allocated to 1 of 2 rearing conditions: isolated (pwSI; 1 rat per cage, $n = 3$) or group-housed (3 rats / cage, $n = 3$, with 1 rat randomly obtained from each cage) rearing. Rats were euthanized at 7 wk of age by CO_2, and whole brains were removed. The trimmed brains were embedded in OCT compound and stored at –80 °C.

To separate the 3 hippocampal subregions, we used a laser microdissection system (LMD; model LMD7000-4, Leica Microsystems, Tokyo, Japan). First, coronal sections including the hippocampus (30 µm) were cut by using a cryostat (HM550; Thermo Fischer Scientific, Yokohama, Japan) at –10 °C and mounted on membrane slides (Membrane Slides Nuclease and Human Nucleic Acid Free PEN-Membrane, Leica Microsystems). According to the manufacturer's (Leica Microsystems) recommendations, the sections were fixed in a mixture of acetate and ethanol (1:19, v/v), and briefly stained with toluidine blue. The slides were air-dried and set within the LMD system. The CA1 and CA3 pyramidal cell layers and DG granule cell layers were cut according to the Allen Brain Atlas (http://mouse.brain-map.org/static/atlas) and collected separately into the caps of 0.2-mL microtubes (BT-02LC; Ina-Optica, Osaka, Japan) supplied with the LMD system.

The quantitative RT-PCR protocol was modified from previous methods.[36] The collected specimens were completely dissolved in 9.5 mL of the lysis buffer provided with CellAmp Direct RNA Prep Kit (Takara Bio, Ohtsu, Japan) supplemented with proteinase K (0.3 U, Takara Bio). DNA in the lysate was digested by adding 1 mL of DNase provided with the kit and incubating at 37 °C for 5 min. Then, distilled water (UltraPure DNase/RNase-Free Distilled Water; Life Technologies, Carlsbad, CA) was added to the lysate, which was incubated at 75 °C for 5 min to inactivate DNase. The cDNA was synthesized by using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA) according to the manufacturer's instructions. Real-time PCR was performed by using Mini Opticon (BioRad) with SsoFast EvaGreen qPCR SuperMix (BioRad). Gene-specific primers were designed by using Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), and all primer sequences are listed in Table 1.

Statistical significance was determined by using 2-way ANOVA, and differences between control and pwSI groups when comparing the same region were tested in subsequent planned comparisons, contrasting the mean comparisons of selected levels of a factor, by using the software package Super ANOVA (Abacus Concepts, Berkeley, CA). The criterion for significance was a P value less than 0.05 in all cases.

Results

The glutamatergic neurotransmission-related genes measured in the present study were α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid (AMPA) receptor subunits (*Gria1–Gria4*), NMDA receptor subunits (*Grin1–Grin3b*), kainate receptor subunits (*Grik1–Grik5*), metabotropic receptor subunits (*Grm1–Grm8*), vesicular glutamate transporters (*Slc17a6–Slc17a8*), membrane glutamate transporters (*Slc1a1*, *Slc1a6*), and glutamate synthases (*Gls*, *Gls2*). These results are shown in Table 2.

In CA1 and CA3 pyramidal cell layers, 18 of the 24 glutamate receptor subunit genes evaluated were at least 1.5-fold increased ($P < 0.05$) in expression in pwSI rats compared with group-housed rats, and only Gria3 was decreased significantly ($P < 0.05$) by pwSI. In particular, *Grin2a* ($P = 0.0003$) in CA1 and *Grik4* ($P = 0.0065$) in CA3 were significantly ($P < 0.05$) increased by pwSI. In contrast, pwSI had little effect on the expression of the glutamate receptor subunits in the DG granule cell layer, whereas the vesicular transporters, membrane transporters, and synthases tended to increase. The GABAergic neurotransmission-related genes analyzed were: $GABA_A$ receptor α subunits (*Gabra1–Gabra6*), β subunits (*Gabrb1–Gabrb3*), γ subunits (*Gabrg1–Gabrg3*), δ subunit (*Gabrd*), $GABA_B$ receptor subunits (*Gabbr1, Gabbr2*), vesicular GABA transporter (*Slc32a1*), membrane GABA transporter (*Slc6a1*), and GABA synthases (*Gad1*, *Gad2*), and the results are shown in Table 3. Compared with group housing, pwSI had little effect on the expressions of these genes in the CA1 and CA3 regions. In contrast to its effect in the pyramidal cell layers, pwSI tended to decrease the expression of 8 of the 13 $GABA_A$ receptor subunit genes, especially 4 of the 5 α-subunit genes and 3 of 3 γ-subunit genes. Among them, the expression of *Gabra1*, *Gabra2*, *Gabra4*, *Gabra5*, *Gabrb3*, *Gabrg1*, and *Gabrg2* was significantly ($P < 0.05$) lower in the DG of pwSI rats than in their group-housed counterparts.

Discussion

The present study showed that the gene expression of $GABA_A$ receptor subunits, except for the $GABA_B$ receptor, in the DG granule cell layer of pwSI rats largely tended to be lower than that in group-housed rats. The $GABA_A$ receptor is a ligand-operated chloride channel composed of 5 subunits. Sixteen subunit isoforms have been identified in mammalian brains:[26] 6 α subunits, 3 β subunits, 3 γ subunits, and single δ, ε, θ, and π subunits. The predominant $GABA_A$ receptors consist of 2 α, 2 β, and a single γ or δ subunit.[25,31] The physiologic properties and intracellular localization of $GABA_A$ receptors are governed by the subunit composition of the pentamer.[24] For example, $GABA_A$ receptors containing α1 subunits, which were reduced in expression by pwSI in the present study, show postsynaptic localization and mediate phasic synaptic inhibition.[12] In contrast, the receptors that contain α4 or α5, which also showed decreased expression in pwSI rats, are extrasynaptic and induce tonic conductance.[10] Irrespective of these effects, pwSI might reduce the expression of functional $GABA_A$ receptors in the DG granule cell layer. Moreover, our finding that the α6 subunit was not expressed in either the pyramidal cell layer or the granule cell layer is consistent with a previous report.[20] Several reports describe the functional consequences of the various $GABA_A$ receptors. A study using sections from adult rat brain revealed that $GABA_A$ receptors containing the α5 subunit are the predominant extrasynaptic inhibitory receptor.[3] Rendering this receptor dysfunctional rescues the learning impairment induced by anesthetics[5] and enhances the learning ability analyzed by a variety of behavioral tasks, including fear conditioning.[4,6,9] Therefore, the functional consequence of $GABA_A$ receptors continues to become clear.

The expression of NMDA and kainate receptors, rather than AMPA receptors, tended to increase in CA1 and CA3 pyramidal

Table 1. Sequences of the primers used in quantitative RT-PCR

Primer	Forward (5′→3′)	Reverse (5′→3′)	Accession no.
Gria1	CTTCATGCAGCAAGGATGTG	TGGCTGTGTACGAGGAGATG	NM_031608
Gria2	AGGTGGCAAAGAATCCACAG	GCCTTGCTCCTCATGACATC	NM_017261
Gria3	GGGAAGCATTCAAGAGGCTA	CTGCTGAACCATTGGGTTTT	NM_032990
Gria4	TTTGCAGGCAGATTGTCTTG	TGGTCTGTGTTTCGGATGAA	NM_017263
Grin1	TCCAGCTTCAAGAGACGTAGG	CTCTCCCTATGACGGGAACA	NM_017010
Grin2a	CTGGAAGAGGCAGATTGACC	TCTTCTCGTTGTGGCAGATG	NM_012573
Grin2b	GCATGCCTACATGGGAAAGT	GTTGAGCACAGCTGCATCAT	NM_012574
Grin2c	GACTTCCTGCTGGCTTTCAG	CTGCAGCATCTTCAGCACAT	NM_012575
Grin2d	TCTTCGCCACCACTGGTTAT	ATTGTGGCAGATCCCTGAAA	NM_022797
Grin3a	GAAGAAAAGCAGCCACGTTC	GGTTTTGTCCTTCCTCGTCA	NM_138546
Grin3b	AGGACCTGGCCTTTGACTTT	TTGATGCTGAAGCTGGTCAC	NM_133308
Grik1	TGGCAAATATGGAGCACAGA	GTCATGAAGGGCTTGGAGAA	NM_017241
Grik2	TGGAGGATGGGAAATATGGA	CGATGACCTTCTCACGAACA	NM_019309
Grik3	CGGAGTCTGGTTTGGGAATA	CGTCCGCGTACTCAAAGATT	NM_181373
Grik4	GCTCCAGCATGACCTTCTTC	CCCTCCTCTGTGCTCTTCAC	NM_012572
Grik5	TTCTCCTTCCTGGACCCTTT	GAAGACACGGGTGAGGGTTA	NM_031508
Grm1	GGTCCCTTCTGACACTTTGC	CATTCCACTCTCGCCGTAAT	NM_017011
Grm2	GGTAACTCACGGGGACAGAA	TCACCAGCCCACTGATGTAA	NM_001105711
Grm3	CCAAGCTCTGTGATGCAATG	CATCCCGTCTCCAAAAGTGT	NM_001105712
Grm4	TCAAGAAGGGAAGCCACATC	CACAGCGTCAATCACGAACT	NM_022666
Grm5	GTTTGCACAGGAGAACAGCA	AGGGACATCTGCATATTGTGG	NM_017012
Grm6	GAGGCCTTTGTGCAGATTTC	CCGGATCACTTTGTGGAACT	NM_022920
Grm7	CCAGGGCGTTATGACATCTT	AGGATGGGATCTCTCGGACT	NM_031040
Grm8	CGAGCAAGGGAAGAAATCTG	CCACGACAAACCACACAAAC	NM_022202
Slc17a6	TCAACCAGCTTATTTCGAGGA	ATTGTCATGACCAGGTGTGG	NM_053427
Slc17a7	CTGGTGGTCGGATACTCACA	ATTGGAAATGCCCATCAAGA	NM_053859
Slc17a8	ACGGGATGTGGAGTAAGTGG	TACTCCTGCAAGGGGCATAG	NM_153725
Slc1a1	TTCATAGTCGTGCGGAAGAA	CACAGCGGAATGTAACTGGA	NM_013032
Slc1a6	CATGCTGGTGGTGTGTTACC	AAGAGGTACCCATGGCTGTG	NM_032065
Gls	CTTGACAAGATGGGCAACAG	TCCTTCTCTCCGAGGATCAA	NM_012569
Gls2	AACAAGACCGTGGTGAACCT	TGCGGGAATCATAGTCCTTC	NM_138904
Gabra1	TCTGAGCACTCTCTCGGGAA	AGTTACACGCTCTCCCAAGC	NM_183326
Gabra2	CGTATGGTTTCCGCTGCTTG	GTAATGCTGTCTCCCAGTCCT	NM_001135779
Gabra3	CCAAGGGGAGTCAAGACGAC	AGTCACTGCATCTCCAAGCC	NM_017069
Gabra4	TGTGCCTGGCGACTTGTTTA	CCAAATCCAGGACGCAGTCT	NM_080587
Gabra5	TGTCCGACACGGAAATGGAAT	TGTTGAGAGGGAGACGTTGC	NM_017295
Gabra6	AACGCTGACTGTCCGATGAG	TCGGATAGGCATAGCTCCCA	NM_021841
Gabrb1	ACAACAGGGGCATATCCACG	CAGTGTGGAGGGCATGTAGG	NM_012956
Gabrb2	TGTCAACAAGATGGACCCACA	ATGCTGGAGGCATCATAGGC	NM_012957
Gabrb3	CTGTACGGGCTCAGGATCAC	CGATCCTTTCCACGCCAGTA	NM_017065
Gabrg1	AAACATGGGTCTTGGCACCT	ATTACTGTGGGTCTCACGCC	NM_080586
Gabrg2	GAAAAACCCTGCCCCTACCA	TGCGAATGTGTATCCTCCCG	NM_183327
Gabrg3	GAGGCTCACTGGATCACCAC	GGGGTAGCCATAGCTAGAGA	NM_024370
Gabrd	GCGCCAGAGCAATGAATGAC	GCGTAGCCCTCCATTAGTCC	NM_017289
Gabbr1	CTCTCGGGCTGGATGGTTAC	GGCTCTAGGGTCTTCCTCCA	NM_031028
Gabbr2	CAGGGAAGACTCCACAGCAG	AGGCGTACCCATGGAACTTG	NM_031802
Slc32a1	CCGTCGAGGGAGACATTCATT	CACGAACATGCCCTGAATGG	NM_031782
Slc6a1	ACAGCCAGTTCTGTACCGTG	CACACGGCAGCAATGAAGAG	NM_024371
Gad1	GATGGTTTTCGATGGT	CCATGGTTGTTCCTGA	NM_008077
Gad2	GTGTTCGATGGGAAGCCTCA	TAATCACTGGCGCCACCTTT	NM_012563
Gapdh	ATGACTCTACCCACGGCAAG	TACTCAGCACCAGCATCACC	NM_017008

Table 2. Effect of pwSI on glutamatergic neuron-related gene expressions in distinct hippocampal regions of F344 rats

	CA1		CA3		DG	
	Group	pwSI	Group	pwSI	Group	pwSI
Gria1	1.7E+00 ± 3.0E–01	1.8E+00 ± 3.0E–01	5.3E–01 ± 4.9E–02	7.5E–01 ± 1.2E–02	1.6E+00 ± 2.7E–01	1.9E+00 ± 3.5E–01
Gria2	6.1E–02 ± 1.5E–02	5.9E–02 ± 8.6E–03	1.7E–02 ± 1.3E–03	1.9E–02 ± 5.7E–04	7.2E–02 ± 5.6E–03	5.5E–02 ± 5.7E–03
Gria3	*4.8E–02 ± 9.5E–03*	*3.1E–02 ± 7.1E–03*[a]	1.2E–02 ± 1.1E–03	1.1E–02 ± 4.9E–04	3.4E–02 ± 5.7E–03	4.3E–02 ± 4.1E–03
Gria4	3.4E–01 ± 7.5E–02	4.7E–01 ± 8.8E–02	**9.8E–02 ± 1.1E–02**	**1.6E–01 ± 2.2E–02**	6.4E–01 ± 1.7E–01	8.1E–01 ± 1.6E–01
Grin1	6.6E–02 ± 1.4E–02	8.7E–02 ± 1.9E–02	3.4E–02 ± 5.6E–03	4.7E–02 ± 1.5E–03	6.9E–02 ± 1.0E–02	6.7E–02 ± 4.7E–03
Grin2a	**3.0E+00 ± 1.4E–02**	**5.0E+00 ± 4.7E–01**[c]	**9.6E–01 ± 1.6E–01**	**1.5E+00 ± 8.4E–02**	2.5E+00 ± 3.4E–01	2.4E+00 ± 3.3E–01
Grin2b	3.1E+00 ± 7.8E–01	3.9E+00 ± 7.3E–01	**1.1E+00 ± 4.9E–02**	**1.7E+00 ± 2.5E–01**	3.4E+00 ± 4.1E–01	3.7E+00 ± 2.5E–01
Grin2c	9.2E–02 ± 1.6E–02	1.1E–01 ± 1.5E–02	3.6E–02 ± 6.4E–03	4.3E–02 ± 5.1E–03	6.4E–02 ± 8.2E–03	8.4E–02 ± 2.4E–02
Grin2d	**5.1E–03 ± 4.7E–04**	**9.1E–03 ± 1.6E–03**	3.3E–03 ± 4.1E–04	3.3E–03 ± 4.0E–04	1.3E–02 ± 2.7E–03	1.2E–02 ± 1.4E–03
Grin3a	9.0E–03 ± 2.7E–03	1.3E–02 ± 1.3E–03	2.5E–03 ± 3.8E–04	2.6E–03 ± 2.3E–04	*1.0E–02 ± 1.5E–03*	*6.3E–03 ± 4.7E–04*
Grin3b	nd	nd	nd	nd	nd	nd
Grik1	**1.6E–01 ± 7.1E–02**	**2.8E–01 ± 7.6E–02**	**6.8E–02 ± 5.6E–03**	**1.9E–01 ± 2.7E–02**	**4.0E–01 ± 7.6E–02**	**5.9E–01 ± 8.6E–02**
Grik2	**4.9E–01 ± 8.0E–02**	**7.5E–01 ± 9.4E–02**	**4.9E–01 ± 7.4E–02**	**8.5E–01 ± 2.0E–01**	2.6E+00 ± 3.8E–01	2.6E+00 ± 3.8E–01
Grik3	5.3E–04 ± 1.2E–04	6.3E–04 ± 1.2E–04	2.9E–04 ± 1.8E–05	3.7E–04 ± 4.2E–05	2.9E–03 ± 3.7E–04	2.8E–03 ± 3.5E–04
Grik4	4.7E–02 ± 1.7E–02	4.6E–02 ± 5.3E–03	**1.1E+00 ± 9.4E–02**	**1.7E+00 ± 2.2E–01**[b]	7.3E–01 ± 1.6E–01	1.0E+00 ± 2.6E–01
Grik5	1.1E+01 ± 2.2E+00	1.3E+01 ± 2.3E+00	**6.5E+00 ± 2.8E–01**	**1.0E+01 ± 1.0E+00**	1.3E+01 ± 2.0E+00	1.7E+01 ± 1.4E+00[a]
Grm1	**1.4E–03 ± 2.7E–04**	**2.3E–03 ± 3.7E–04**	**1.3E–02 ± 1.4E–04**	**2.2E–02 ± 3.1E–03**	6.4E–02 ± 7.2E–03	7.9E–02 ± 1.6E–03[a]
Grm2	nd	nd	nd	nd	9.2E–03 ± 1.1E–03	7.6E–03 ± 3.0E–04
Grm3	1.4E–02 ± 1.2E–03	1.4E–02 ± 4.0E–03	6.7E–03 ± 8.0E–04	7.7E–03 ± 7.3E–04	2.6E–02 ± 4.1E–03	3.6E–02 ± 7.9E–03
Grm4	**6.8E–03 ± 3.0E–03**	**1.1E–02 ± 3.5E–03**	1.3E–02 ± 1.0E–03	1.5E–02 ± 5.1E–03	1.6E–02 ± 3.5E–03	1.7E–02 ± 1.4E–03
Grm5	**1.1E–01 ± 7.9E–03**	**2.6E–01 ± 2.2E–01**	1.3E–02 ± 1.6E–03	**4.9E–02 ± 1.9E–02**	1.9E–02 ± 5.7E–04	2.2E–02 ± 3.0E–03
Grm6	**2.9E–03 ± 1.3E–03**	**4.4E–03 ± 2.3E–03**	1.0E–03 ± 5.0E–04	**1.8E–03 ± 1.8E–04**	nd	nd
Grm7	1.2E–02 ± 1.7E–03	1.5E–02 ± 3.1E–03	9.3E–03 ± 1.9E–04	1.2E–02 ± 2.0E–03	2.2E–02 ± 4.4E–03	2.8E–02 ± 2.1E–03
Grm8	nd	nd	nd	nd	**2.5E–03 ± 6.9E–04**	**5.1E–03 ± 1.1E–03**
Slc17a6	2.4E–03 ± 3.4E–04	2.4E–03 ± 7.0E–04	7.6E–04 ± 1.5E–04	*4.3E–04 ± 1.0E–04*	nd	nd
Slc17a7	5.9E–02 ± 6.1E–03	5.9E–02 ± 6.1E–03	5.5E–02 ± 3.4E–03	4.2E–02 ± 4.3E–03	4.4E–02 ± 3.7E–03	6.0E–02 ± 6.4E–03
Slc17a8	**2.1E–04 ± 6.8E–05**	**4.1E–04 ± 1.7E–04**	2.9E–04 ± 1.4E–04	**4.3E–04 ± 2.5E–04**	**5.6E–04 ± 2.1E–04**	**9.4E–04 ± 2.1E–04**
Slc1a1	3.4E–02 ± 3.2E–03	4.4E–02 ± 6.8E–03	1.5E–02 ± 3.8E–04	1.4E–02 ± 2.8E–03	**3.1E–02 ± 3.8E–03**	**4.7E–02 ± 1.0E–02**[a]
Slc1a6	nd	nd	nd	nd	**1.2E–03 ± 1.8E–04**	**2.1E–03 ± 5.9E–04**
Gls	*1.7E–02 ± 8.2E–04*	*1.1E–02 ± 1.7E–03*[a]	1.9E–02 ± 2.8E–03	1.8E–02 ± 3.2E–03	**1.4E–02 ± 1.4E–03**	**2.1E–02 ± 2.7E–03**
Gls2	1.6E–02 ± 3.1E–03	2.2E–02 ± 6.7E–03	7.4E–03 ± 8.0E–04	1.0E–02 ± 2.1E–03	1.1E–02 ± 5.1E–03	1.1E–02 ± 7.3E–04

nd, not detected
Data are shown as the mean ± SEM (n = 3) expression level relative to that of *Gapdh*.
Bold values indicate that the mean of pwSI/group > 1.5
Italicized values indicate that the mean of pwSI/group < 0.7
Values differ significantly ([a]P < 0.05, [b]P < 0.01, and [c]P < 0.001) between group-housed and pwSI rats.

cell layers. In particular, the expression of *Grin2a* in CA1, and of *Grik4* in CA3 was markedly increased by pwSI. Seven NMDA receptor subunits are known: *Grin1*, 4 *Grin2* isoforms (*Grin2a*, *Grin2b*, *Grin2c*, and *Grin2d*), and 2 *Grin3* isoforms (*Grin3a*, *Grin3b*). The functional NMDA receptors are either di- or triheteromeric assemblies, and their physiologic properties are dependent on the subunit composition.[19] In particular, the NMDA receptors that contain GRIN2A subunits play an important role in hippocampal synaptic plasticity. For example, mice lacking *Grin2a* showed attenuated long-term potentiation in CA3–CA1 and impairments in spatial learning and contextual fear conditioning.[23,27] Unlike AMPA and NMDA receptors, which are predominantly involved with excitatory neurotransmission, the kainate receptors are thought to function mainly as modulators of excitatory signaling.[7] In fact, administration of the selective GRIK1 inhibitor UBP304 attenuates coordinated neuronal activity, that is, θ oscillations, in the hippocampus.[13] Therefore, the increased expression of glutamate receptors in CA1 and CA3 pyramidal cell layers may be involved in the behavioral alterations by pwSI, particularly in hippocampus-dependent tasks.

Taken together, the present results suggest that pwSI reduced $GABA_A$ receptor expression in the DG granule cell layer and increased the gene expression of several NMDA and kainate receptors in the CA1 and CA3 pyramidal cell layers. Although both hippocampal pyramidal cells and DG granule cells receive projections from inhibitory GABAergic interneurons,[11] the GABAergic inputs to DG granule cells are more important for filtering excitatory inputs from the entorhinal cortex to hippocampal circuit.[8]

Table 3. Effect of pwSI on GABAergic neuron-related gene expression in distinct hippocampal regions of F344 rats

	CA1		CA3		DG	
	Group	pwSI	Group	pwSI	Group	pwSI
Gabra1	1.4E-02 ± 1.7E-03	1.4E-02 ± 2.2E-03	4.3E-03 ± 4.2E-04	3.9E-03 ± 3.2E-04	*1.3E-02 ± 9.6E-04*	*9.0E-03± 3.2E-04*[c]
Gabra2	4.4E-03 ± 8.0E-04	4.0E-03 ± 4.3E-04	3.8E-03 ± 2.2E-04	3.7E-03 ± 2.0E-04	*9.6E-03 ± 9.2E-04*	*6.7E-03 ± 7.8E-04*[b]
Gabra3	nd	nd	**9.7E-05 ± 1.7E-05**	**1.6E-04 ± 8.9E-06**	3.0E-04 ± 3.0E-05	2.6E-04 ± 3.6E-05
Gabra4	1.1E-02 ± 2.5E-03	1.1E-02 ± 3.2E-03	3.1E-03 ± 3.3E-04	2.8E-03 ± 7.2E-05	*2.6E-02 ± 1.4E-03*	*1.9E-02 ± 9.2E-04*[c]
Gabra5	4.1E-02 ± 8.9E-03	3.9E-02 ± 1.3E-02	2.7E-02 ± 3.1E-03	3.4E-02 ± 3.9E-03	*3.5E-02 ± 1.1E-03*	*2.7E-02 ± 2.4E-03*[a]
Gabra6	nd	nd	nd	nd	nd	nd
Gabrb1	1.1E-02 ± 2.4E-03	1.2E-02 ± 2.6E-03	6.5E-03 ± 6.3E-04	8.0E-03 ± 6.1E-04	1.8E-02 ± 1.9E-04	1.5E-02 ± 1.7E-03
Gabrb2	1.2E-02 ± 3.0E-03	1.1E-02 ± 3.7E-03	3.0E-03 ± 1.1E-04	2.9E-03 ± 1.5E-04	1.2E-02 ± 1.4E-03	8.8E-03 ± 6.9E-04
Gabrb3	4.7E-03 ± 8.8E-04	5.4E-03 ± 9.7E-04	2.7E-03 ± 7.6E-05	2.6E-03 ± 3.5E-04	*1.1E-02 ± 1.6E-03*	*7.6E-03 ± 5.9E-04*[a]
Gabrg1	3.1E-03 ± 8.5E-04	2.9E-03 ± 7.7E-04	1.4E-03 ± 1.5E-04	1.2E-03 ± 2.0E-04	*4.4E-03 ± 6.2E-04*	*2.5E-03 ± 2.6E-04*[b]
Gabrg2	2.9E-03 ± 9.0E-04	2.3E-03 ± 6.0E-04	1.1E-03 ± 2.0E-05	1.1E-03 ± 7.4E-05	*4.8E-03 ± 1.2E-03*	*3.1E-03 ± 2.8E-05*[a]
Gabrg3	2.0E-04 ± 5.1E-05	2.4E-04 ± 2.1E-05	*1.2E-04 ± 3.3E-05*	*8.0E-05 ± 1.4E-05*	*5.9E-04 ± 1.7E-04*	*3.3E-04 ± 4.8E-05*
Gabrd	2.6E-03 ± 5.1E-04	2.5E-03 ± 9.0E-04	4.0E-04 ± 8.7E-05	3.3E-04 ± 1.5E-05	1.9E-02 ± 2.4E-03	1.4E-02 ± 1.7E-03
Gabbr1	2.1E-02 ± 2.3E-03	2.9E-02 ± 6.3E-03	7.9E-03 ± 8.6E-04	1.0E-02 ± 9.8E-04	2.1E-02 ± 1.9E-03	1.8E-02 ± 2.1E-04
Gabbr2	2.5E-02 ± 3.9E-03	2.5E-02 ± 4.5E-03	2.2E-02 ± 2.6E-03	2.6E-02 ± 1.1E-03	4.9E-02 ± 2.6E-03	4.3E-02 ± 3.1E-03
Slc32a1	4.5E-04 ± 1.0E-04	4.7E-04 ± 1.4E-04	2.8E-04 ± 4.9E-06	2.3E-04 ± 1.4E-05	**4.1E-04 ± 4.2E-05**	**6.8E-04 ± 1.2E-04**
Slc6a1	4.1E-03 ± 1.3E-03	5.3E-03 ± 2.1E-03	**2.3E-03 ± 2.9E-04**	**3.4E-03 ± 6.6E-04**	7.5E-03 ± 1.1E-03	1.0E-02 ± 1.6E-03
Gad1	3.1E-03 ± 5.6E-04	2.9E-03 ± 7.2E-04	1.1E-03 ± 4.6E-05	1.1E-03 ± 1.2E-04	5.6E-03 ± 3.7E-04	6.4E-03 ± 5.2E-04
Gad2	**2.8E-03 ± 6.5E-04**	**4.0E-03 ± 1.4E-03**	1.2E-03 ± 9.9E-06	1.6E-03 ± 2.7E-04	5.2E-03 ± 9.4E-04	5.6E-03 ± 5.6E-04

nd, not detected
Data are shown as the mean ± SEM (n = 3) expression level relative to that of *Gapdh*.
Bold values indicate that the mean of pwSI/group >1.5
Italicized values indicate that the mean of pwSI/group < 0.7
Values differ significantly ([a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$) between group-housed and pwSI rat

Therefore, the decreased expression of $GABA_A$ receptor genes in DG by pwSI could contribute to attenuated susceptibility to inhibitory inputs in granule cells and to enhanced excitability in downstream circuits. In addition, the expression of synthases and transporters that participate in glutamatergic neurotransmission in presynaptic cells was increased in DG, and glutamate receptor expression downstream of the circuits, that is, in CA3 and CA1, was upregulated, although the expression of GABA receptors was unaltered in both layers of pyramidal cells. In addition, these alterations in the pyramidal cell layer might contribute to enhanced hippocampal excitability. Therefore, the hippocampal circuits of pwSI rats may be in a more excitable state than that of group-housed rats.

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