Supplementary Information.

Two-photon uncaging of γ-aminobutyric acid in intact brain tissue.

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Supplementary Methods.

Synthesis.

Methyl {1-[4-(*tert*-butoxycarbonylamino)butanoyl]indolin-4-yloxy}acetate 8. To a solution of **7** (0.84 g, 4.06 mmol) dicyclohexylcarbodiimde (0.927 g, 4.50 mmol), N-BOC-4-aminobutyric acid (0.914 g, 4.5 mmol) and dimethylaminopyridine (catalytic) in acetonitrile were stirred for 18 h. The reaction mixture was filtered and the solvent removed to give a dark brown liquid that was purified by flash chromatography [hexanes-ethyl acetate, (1:1)] to give **8** (0.994 g, 59% yield) and recovered 7 (0.182 g). ^{1}H NMR: δ (300 MHz, $CDCl_3$) 7.88 (d, J=8.0 Hz, 1H), 7.14 (t, J=8.0 Hz, 1H), 6.43 (d, J=8.0 Hz, 1H), 4.8 (br s, 1H), 4.67 (s, 2H), 4.08 (t, J=8.5 Hz, 2H), 3.80 (s, 3H), 3.26-3.17 (m, 4H), 2.47 (t, J=7.0 Hz, 2H), 1.93 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (70 MHz, $CDCl_3$): 170.97, 169.53, 156.34, 154.20, 144.90, 129.24, 119.45, 111.33, 107.09, 79.48, 65.71, 52.62, 48.86, 40.65 (2C not resolved), 33.68, 28.82, 25.19. MS: 393.2017 (M+H observed).

1-[4-(*tert*-Butoxycarbonylamino)butanoyl]indolin-4-yloxy}acetic acid 9. To a solution of **8** (0.99 g, 2.40 mmol) in methanol (50 mL) was added sodium hydroxide (3.6 mL of a 1N solution). After 3 h at RT citric acid (3.6 mL of a 1N solution) was added and the methanol was removed by rotary evaporation and the reaction mixture was extracted with ethyl acetate. The organic solvent was removed to give **9** (0.78 g, 77% yield) as a foamy light yellow solid. ^{1}H NMR (some peaks appear as rotamers): δ (300 MHz, $CDCl_3$) 7.86 (d, J=8.0 Hz, 1H), 7.13 (t, J=8.0 Hz, 1H), 6.46 (d, J=8.0 Hz, 1H), 4.84 (br s, 1H), 4.65/4.61 (s, 2H) 4.01 (br s, 2H), 3.22 (br s, 2H), 3.14/3.07 (br t, 2H), 2.6-2.4 (m, 2H), 2.0-1.8 (m, 2H), 1.49/1.42 (s, 9H). This material was pure enough for the next step, but not for ^{13}C NMR or MS analysis.

(1-[4-Aminobutanoyl]-5/7-nitroindolin-4-yloxy)acetic acids (CNI-GABA 6 and 10). A solution of **3** (0.78 g, 1.85 mmol) in TFA was stirred at RT for 3 h, then sodium nitrate (0.19 g, 2.00 mmol) was added as solid, and after 1 h at RT an additional amount of nitrate was added. HPLC showed no SM. The TFA was removed with a stream of nitrogen and then a vacuum pump (0.01 torr) for 18 h (NMR of the reaction mixture showed equal amounts of the two products). The reaction mixture was dissolved in water, passed through a 0.45 mm filter and the two products were separated by preparative HPLC on an Alltech Altima

C_{18} column (22x250 mm) using isocratic elution (20% acetonitrile, 0.1% TFA, 10 mL/min). The combined yield of both isomers was 80% (1.48 mmol) of CNI-GABA **6** eluted at 15 min and **10** at 18 min. ^{1}H NMR: δ (300 MHz, CD_3OD) **11**, 7.90 (d, J=8.9 Hz, 1H), 7.80 (d, J=8.9 Hz, 1H), 4.66 (s, 2H), 4.19 (t, J=8.5 Hz, 2H), 3.28 (t, J=8.0 Hz, 2H), 3.08 (t, J=7.0 Hz, 2H), 2.68 (t, J=6.4 Hz, 2H), (2.03 (m, 2H). **CNI-GABA 6** 7.70 (d, J=8.9 Hz, 1H), 6.76 (d, J=8.9 Hz, 1H), 4.85 (s, 2H), 3.19 (t, J=8.2 Hz, 2H), 3.02 (t, J=7.1 Hz, 2H), 2.71 (t, J=6.8 Hz, 2H), 2.03-1.98 (m, 2H). ^{13}C NMR (70 MHz, CD_3OD): 172.99, 172.89, 159.56, 138.23, 137.54, 126.69, 125.96, 109.74, 52.01, 40.99, 34.03, 28.14, 24.63. MS: 324.1191 (M+H observed). ε = 4,200 M^{-1} cm^{-1}.

(1-[4-Aminobutanoyl]-5,7-dinitroindolin-4-yloxy)acetic acid (CDNI-GABA 3). A solution of **10** (0.351 g, 1.53 mmol) in TFA was stirred with sodium nitrate (31 mmol) for 3d. The solvent was removed under a stream of nitrogen and then under high vacuum. The reaction mixture was dissolved in water and purified by HPLC (as above for CNI-Glu) to give CDNI-GABA **3** (148 mg, 29% yield). ^{1}H NMR: δ (300 MHz, CD_3OD) 7.80 (s, 1H), 4.81 (s, 2H), 4.29 (t, J=7.1 Hz, 2H), 3.37 (t, J=7.1 Hz, 2H), 3.00 (t, J=6.8 Hz, 2H), 2.71 (t, J=6.3 Hz, 2H), 2.03-1.98 (m, 2H). ^{13}C NMR (70 MHz, CD_3OD): 173.26, 172.03, 153.08, 141.95, 140.09, 137.20, 133.72, 124.12, 71.47, 52.25, 40.90, 33.96, 28.67, 24.41. MS: 369.1038 (M+H observed). ε = 6,400 M^{-1} cm^{-1}.

Quantum yield determination.

The quantum yield for uncaging CDNI-GABA and CNI-GABA were measured by comparing the time of photolysis with the filtered (320-400 nm) output of a 500 W medium pressure Hg lamp of an equimolar solution (0.25 mM) of MNI-Glu and new cage. Inosine was used an inert internal standard. The path-length of the cuvette was 0.1 mm. The extent of reaction was monitored by HPLC[3,4].

Electrophysiology.

All experiments using animal tissue were performed according to protocols approved by the animal experimental committee of Faculty of Medicine, University of Tokyo.

Hippocampal slices with a thickness of 350 μm were obtained from 14-19 day old Sprague-Dawley rats as previously described[3]. The extracellular aCSF solution contained 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 20 mM glucose. This bathing solution also contained 1 μM tetrodotoxin (Nacalai Tesque, Kyoto, Japan) and 200 μM Trolox (Aldrich, Milwaukee, WI, USA). To block activation of non-*N*-methyl-D-aspartate receptors and GABA-A receptors, 10 μM CNQX (Tocris, Bristol, UK) or 50 μM picrotoxin (WAKO, Osaka, Japan), respectively, were included. The whole-cell patch pipette solution consisted of 140 mM CsCl, 2 mM NaCl, 2 mM MgATP, 0.5 mM NaGTP, 2 mM D-ascorbic acid, 10 mM Cs-HEPES, 10 mM EGTA, and 0.05-0.10 mM Alexa-594, adjusted to pH 7.3 with CsOH. All physiological experiments were performed at room temperature (23-25º C). To detect the currents, the membrane potential was held at -70 mV (Axopatch 200B, Axon Instrument, Foster City, California). Data were low-pass filtered at 2 kHz, sampled at 5-10 kHz, and recorded using FV1000-MPE software (Olympus, Tokyo, Japan).

Two-photon excitation imaging and uncaging of caged compounds.

Imaging of CA1 hippocampal neurons was performed with an upright microscope (BX61WI; Olympus, Tokyo, Japan) equipped with a water immersion objective lens (LUMPlanFI/IR 60x, numerical aperture of 0.9) and with the FV1000-MPE software. Two mode-locked femtosecond pulsed Ti:sapphire lasers (MaiTaiHP; Spectra Physics, Mountain View, CA, USA) set at wavelengths of 720 and 830 nm were connected to the laser-scanning microscope via two independent scan heads. Both lasers were chirp-compensated before entering the scanheads. When imaging 0.1 μm fluorescent beads by the 720-nm laser, full-widths of half-maximum (FWHMs) of the intensity profiles of the beads along *X*- and *Z*-axis were 0.30 μm and 1.4 μm, respectively. The laser at the wavelength of 830 nm was used for imaging of the neuronal morphology. Immediately before 2P photolysis, one image was acquired at the wavelength of 720 nm for precise adjustment of the region of photolysis; each of caged compounds (CDNI-GABA or MNI-Glu) was then applied locally from a glass pipette positioned close to the selected dendrite. For mapping the current induced by 2P uncaging, a pseudorandom sequence of scanning in the region of interest was constructed as previously described[3]. We have custom-written software that divides a region of interest (ROI) into pixels to which it directs uncaging for a fixed period, followed by current recording through a patch clamp

amplifier. A temporal delay is selected until the next uncaging event, so the neuron has returned to the resting current. IPlab (BD Biosciences, Rockville, MD) and our own software programs, based on LabView (National Instruments, Austin, TX), were used for image processing.

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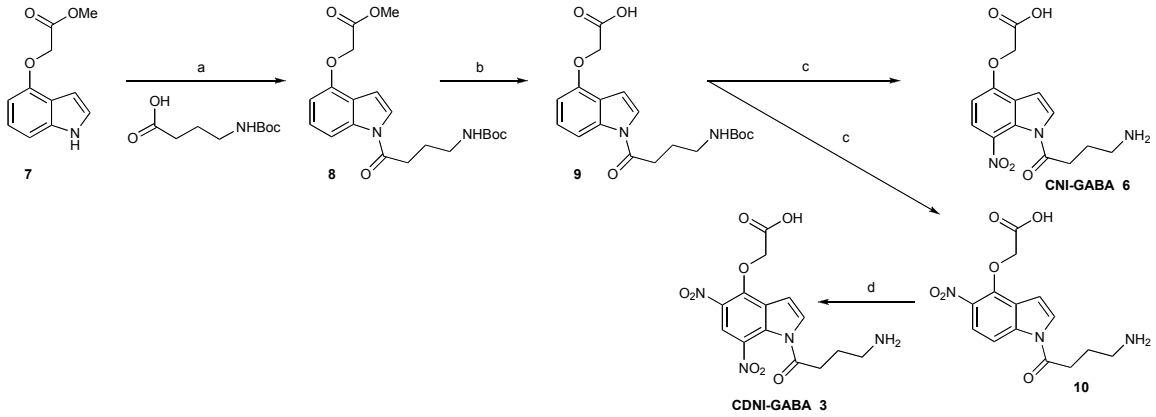
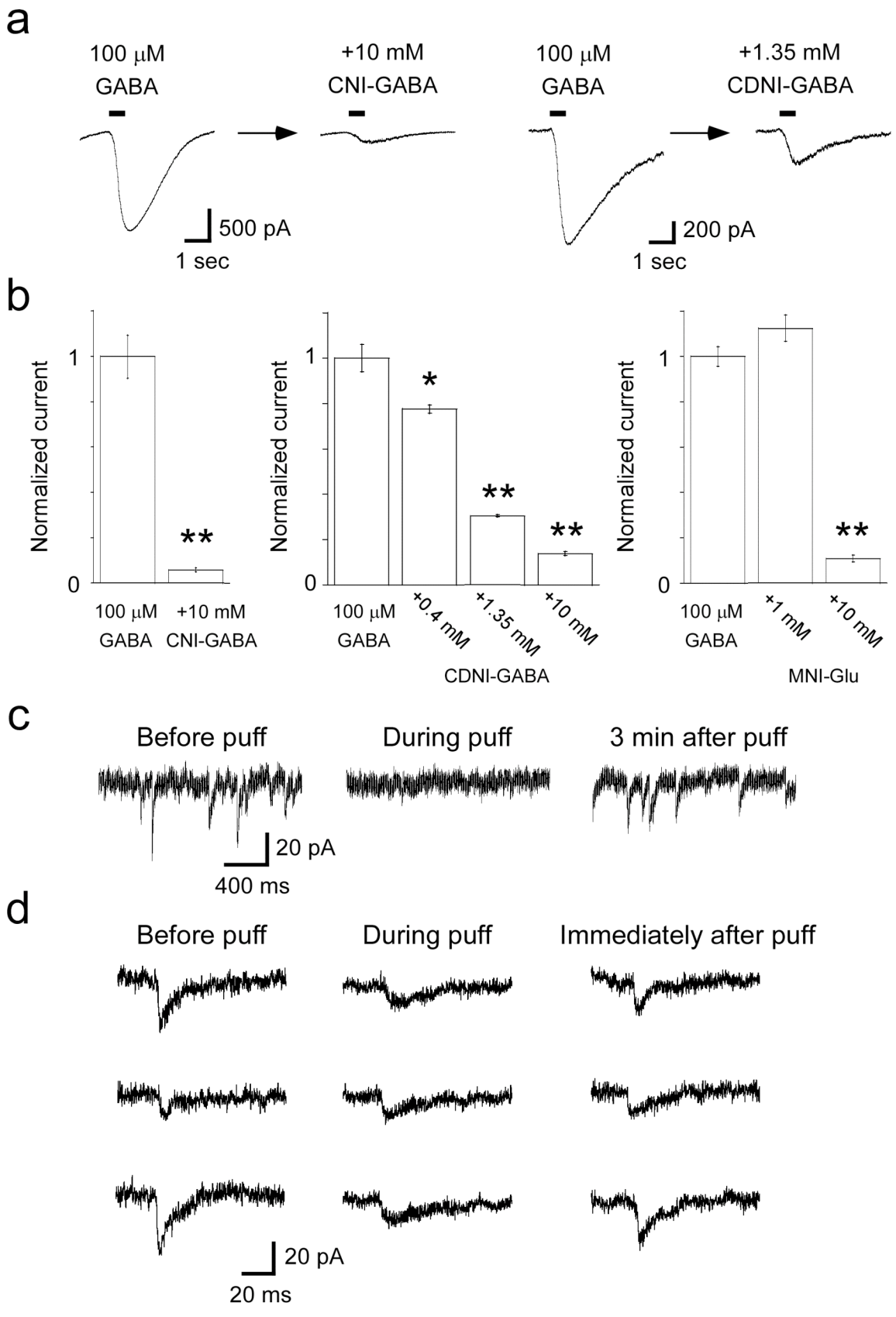
Supplemental figures and legends.

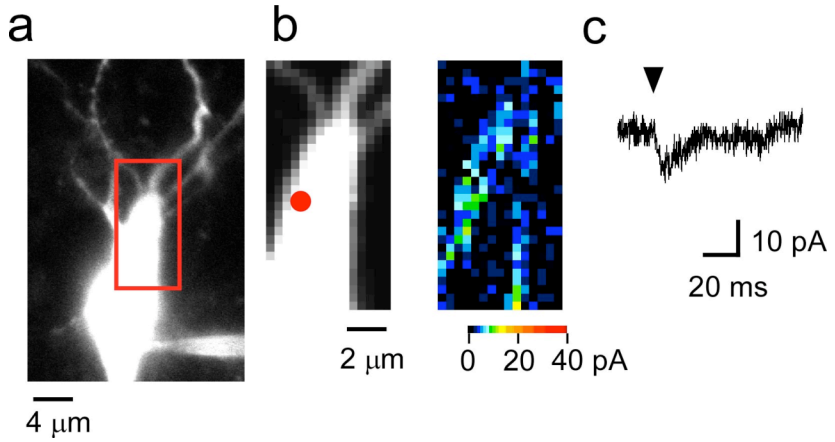


Supplementary Figure 1. Scheme of synthesis of CDNI-GABA.

Conditions: (a) DCC with catalytic DMAP; (b) NaOH (1.5 equ.), MeOH, RT; (c) TFA, RT, 3h, then $NaNO_3$ (1.2 equ), RT, 5 min; then HPLC separation; (d) $NaNO_3$ (20 equ), in TFA, 3d, RT.


a
100 μM
GABA
+10 mM
CNI-GABA
100 μM
GABA
+1.35 mM
CDNI-GABA
500 pA
1 sec
200 pA
1 sec
b
Normalized current
1
0
**
100 μM
GABA
+10 mM
CNI-GABA
*
**
**
+0.4 mM
+1.35 mM
+10 mM
CDNI-GABA
+1 mM
MNI-Glu
c
Before puff
During puff
3 min after puff
20 pA
400 ms
d
Before puff
During puff
Immediately after puff
20 pA
20 ms

Supplementary Figure 2. GABA-A receptor antagonism of CNI-GABA, CDNI-GABA and MNI-Glu. (**a**) Representative current traces from puffer application around the soma of GABA, followed by GABA co-applied with CNI-GABA and CDNI-GABA at the concentrations indicated. (**b**) Summary of the amplitudes of the current evoked by puff of 100 μM GABA and caged compounds around the soma. The amplitude was normalized to the mean current that was induced by GABA application alone. (left) Control (n = 7); +10 mM CNI-GABA (n = 7). (center) Control (n = 10); +0.4 mM CDNI-GABA (n = 10), +1.35 mM CDNI-GABA (n = 14); +10 mM CDNI-GABA (n = 10). (right) Control (n = 9); +1 mM MNI-Glu (n = 4); +10 mM MNI-Glu (n = 9). ** $p < 0.0001$, * $p < 0.01$. (**c**) Representative spontaneous mIPSCs before, during, and after the local application of CDNI-GABA (1.35 mM). Sponntaneous mIPSCs were blocked by puffer application, but recovered after the cessation. (**d**) Representative spontaneous mIPSCs before, during, and after the local application of low concentrations of CDNI-GABA (0.4 mM). Sponntaneous mIPSCs were partially blocked by the local perfusion of 0.4 mM CDNI-GABA, but recovered rapidly after the cessation.



Supplementary Figure 3. Functional mapping of GABA-A receptor responses by 2P photolysis of low concentration of CDNI-GABA on the soma of a hippocampal CA1 neuron. (**a**) A *Z*-stacked image of a CA1 pyramidal neuron filled with Alexa-594 dye. CDNI-GABA at 0.4 mM was locally perfused onto the cell and uncaging was effected at the laser power of 11 mW for 1 ms in the region of the cell shown in the red box. (**b**) Left: the mapping of response values of the photomultiplier tube during 2P illumination at each pixel. Right: the mapping of induced GABA currents. The pseudo-color scale at the bottom indicates the amplitude of the GABA currents. The induced current at the red point is shown in (**c**). Arrow indicates the time of illumination.

	Rise time (20-80%, ms)	Decay half-time (ms)	Peak current (pA)	
2pIPSCs	2.0 ± 0.2	24.7 ± 2.5	15.3 ± 1.0	6 cells
mIPSCs	1.5 ± 0.2	10.5 ± 1.1	25.9 ± 3.4	6 cells
Paired t test	$p = 0.075$	$p < 0.005$	$p < 0.05$	

Supplementary Table 1. Comparison of of the properties of 2pIPSCs and spontaneous mIPSCs. 2pIPSCs with amplitudes > 10 pA were averaged for each cell and three parameters for each cell were measured and averaged. mIPSCs with amplitudes > 10 pA were averaged for each cell and the parameters for each cell were measured and averaged. Each parameter was statistically compared between the averages for the 2pIPSCs and mIPSCs recorded from the same cell.