Supplemental Figures

Figure S1. NO-LTD is induced at corticostriatal synapses by SNAP or 8-Br-cGMP. (Related to Figure 1). (A) An example recording from brain slices taken from a transgenic Thy1-ChR2 mice in which ChR2 is expressed in the cortex (see supplemental methods). Corticostriatal responses were evoked using full field stimulation with an LED light source (0.2ms stimulus duration). Similar to what was seen with electrical stimulation of cortex, transient perfusion of SNAP (100μM) induced depression of cortical EPSCs. (Control, min 1-5: -38.5 pA; Post SNAP, min 25-30: -24.0 pA, 55.89% of baseline) *$p<0.05$, Mann-Whitney nonparametric test. (B) NO-LTD induced by transient perfusion of SNAP (100μM) was occluded by pre-incubation with 8-Br-cGMP (500μM). 8-Br-cGMP was perfused for 10 minutes before, plus during SNAP application (n=5 cells). $p>0.5$, Wilcoxon signed rank test. (C) An example recording utilizing the perforated patch recording configuration demonstrating that NO-LTD induction using 8-Br-cGMP was not effected by cellular dialysis associated with the whole-cell recording configuration. Examples traces are shown to the right. Scale bars: (C) 50 pA, 20 ms.

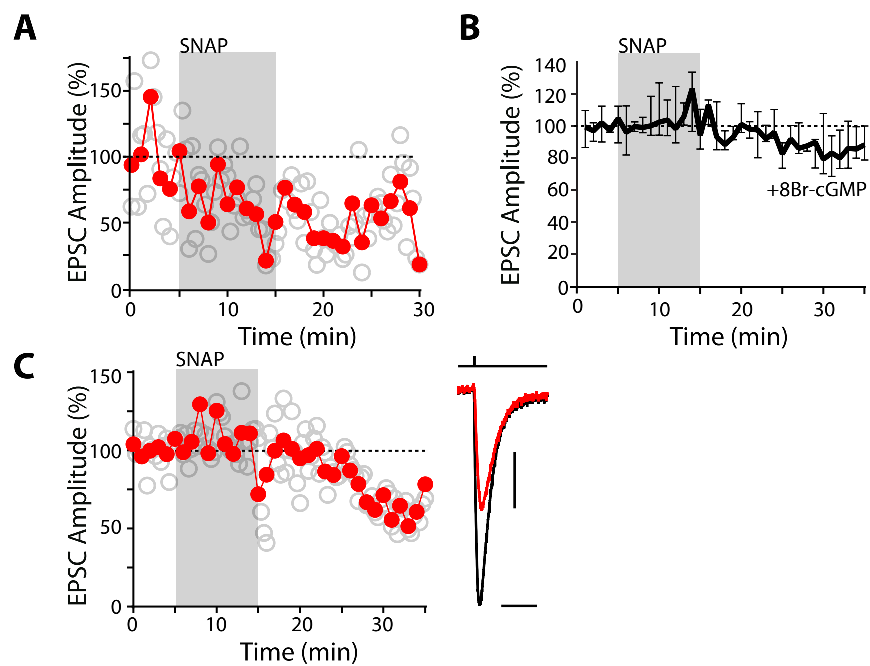
Figure S2. Local interneurons generate NO-LTD in a select population of SPNs. (Related to Figure 2). (A) A PLTSI in brain slices prepared from a SOM-Cre mouse in which a cre-dependent ChR2 construct has been stereotaxically delivered was patched using a Cs+ internal. The cell was subjected to the same 15Hz stimulation protocol that was used to induce NO-LTD in SPNs. The graph demonstrates the recorded PLTSI photocurrent over 1 minute of stimulation, normalized to the first 10% (6 s) of recording time. Circles are averages of responses within a 1 s window. (Avg 1-5 sec = 100.9% baseline; Avg 55-60 sec = 97.9% baseline; Q1=97.48%; Q3=99.6%). $p>0.05$, Mann-Whitney test. (B) Two SPNs from another SOM-cre::DIO-ChR2 mouse were patched. Approximately 5 minutes after break-in, both were briefly stepped from -70 to 0 mV. PLTSIs were stimulated via full field LED illumination for 1-5ms and then brought back down to -70 mV, GABAzine applied, and the LTD paradigm described in figure 2 was run. SPNs that exhibited GABAergic inhibitory postsynaptic currents (IPSCs) (top panel) exhibited NO-LTD whereas those that did not show an IPSC (bottom panel) did not exhibit LTD. (C) A summary plot average firing rates recorded from PLTSIs in SOM-Cre::DIO-ChR2 mice in the cell-attached recording configuration. PLTSI firing rates were consistently lower than the 15Hz stimulation paradigm (Mean firing frequency and standard deviation= 4.57±3.05 Hz; range: 0.5472 to 10.58 Hz, n=12 cells). (D) Summary data demonstrating the relationship between the amplitude of the GABAergic IPSCs and interneuron dependent LTD. Stimulating PLTSIs at lower frequencies (10 Hz) did not cause as robust LTD as higher (15 Hz) frequencies (For cells with IPSC > 10 pA, 10 Hz: depression in 3/5 cells, potentiation in 2/5 cells; 15 Hz: depression in 5/6 cells). (E) PLTSI induction of NO-LTD was occluded by pre-incubation with SNAP (100μM). (Average min 1-5 = 100.38% baseline; min 30-35 = 93.68% baseline; Q1=93.82%; Q3=96.22%. n=8 cells). $p>0.05$, Wilcoxon signed rank test. Scale bars: (A) 50 pA, 20 ms.

Figure S3. NO-LTD is independent of conventional determinants of eCB-LTD. (Related to Figure 3). (A) Application of 8-Br-cGMP (500μM) induced LTD in the presence of the D_2R antagonist sulpiride (1

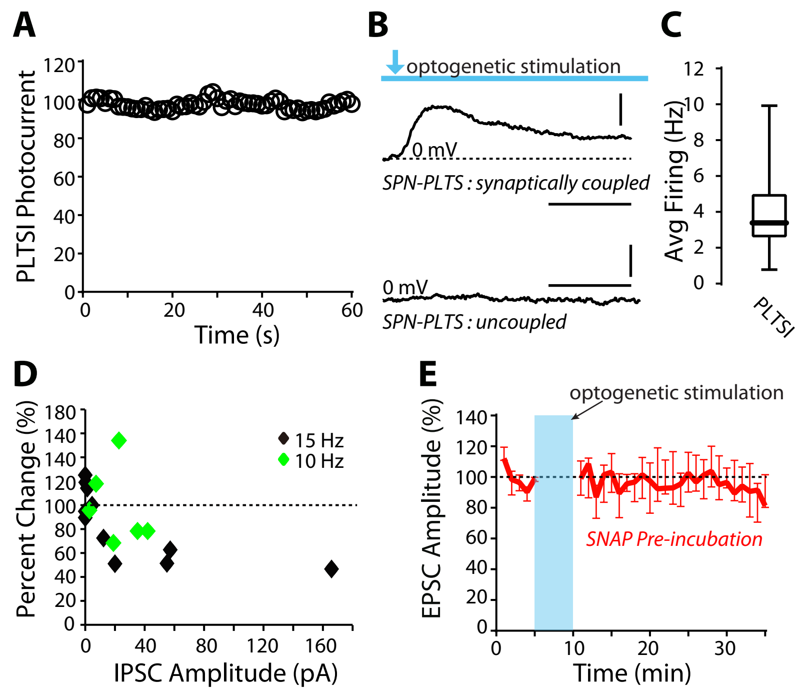
μM) and in the presence of CB1 receptor antagonist AM251 (2μM) in both dSPNs and iSPNs (Sulpiride: dSPNs: 74.72% baseline; Q1=68.53%; Q3=79.93%, n=7 cells; iSPNs: 77.95% baseline; Q1=69.12%; Q3=82.54%, n=8 cells; AM251: dSPNs: 63.27% baseline; Q1=49.12%; Q3=78.36%, n=8 cells; iSPNs: 58.69% baseline; Q1=45.79%; Q3=69.96%, n=7 cells). * $p<0.05$, signed rank test. (B) Activation of PKG by 8-Br-cGMP (500 μM) induced LTD in the presence of 20 mM BAPTA, loaded via the recording electrode (Post 8Br, min 25-30: 77.0% baseline; Q1=72.0%; Q3=79.7%; n=7 cells). *$p<0.05$, signed rank test. (C) Photomicrograph depicting the location of virally delivered ChR2 in the thalamus. (D) Activation of PKG by 8-Br-cGMP (500 μM) induced LTD at optically stimulated thalamic synapses (top panel, 61.78% baseline; Q1=51.72%; Q3=65.71%, n=6 cells). *$p<0.05$, Wilcoxon signed rank test. (E) NO-LTD induced by 8-Br-cGMP did not significantly alter the paired pulse ratio of SPNs (left panel, Pre-LTD: 1.01±0.27, average and standard deviation; Post-LTD, 8Br-cGMP: 1.16±0.33, n=5 cells). $p>0.05$, Wilcoxon signed rank test. Population time courses are shown at left. Traces above demonstrate EPSC depression and no change in PPR in one cell. (F) Cessation of cortical stimulation during 8-Br-cGMP application had no effect on LTD induction (75.21% baseline; Q1=66.45%; Q3=84.61%, n=6 cells). *$p<0.05$, signed rank test. Scale bars: (C) 1 mm; (D) 10 pA, 10 ms (top), 20 pA, 10 ms (bottom); (E) 50 pA, 20 ms.

Figure S4. NO-LTD is amplified by BAY-41. (Related to Figure 4). (A) SNAP induced, NO-LTD (grey trace) is enhanced in the presence of the NO-independent activator of sGC, BAY41-2272 (black trace, control data set from figure 1C. SNAP Ctrl: 79.24% baseline, Q1=70.46%, Q3=89.50%, n=14 cells. SNAP+BAY: 53.74% baseline, Q1=38.36%, Q3=66.46%, n=5). *$p<0.05$, Mann Whitney U test. Like SNAP induced NO-LTD, this enhancement can be blocked by inclusion of an inhibitor of PKG in the recording pipette (red trace, 144.6% baseline; Q1=88%; Q3=184%, n=5 cells). *$p<0.05$ Mann Whitney U test comparing SNAP/BAY to SNAP/BAY+PKG inhibitor. (B) Summary data demonstrate the effects of SNAP, SNAP + BAY41-2272, and inhibition with Rp-8-Br-PET-cGMPs. *$p<0.05$, Mann Whitney nonparametric test. (C) 8-Br-cGMP inhibited eCB-LTD, similar to SNAP mediated inhibition (88.51% baseline; Q1=77.29%; Q3=107.44%, n=8 cells). $p>0.05$, Wilcoxon signed rank.

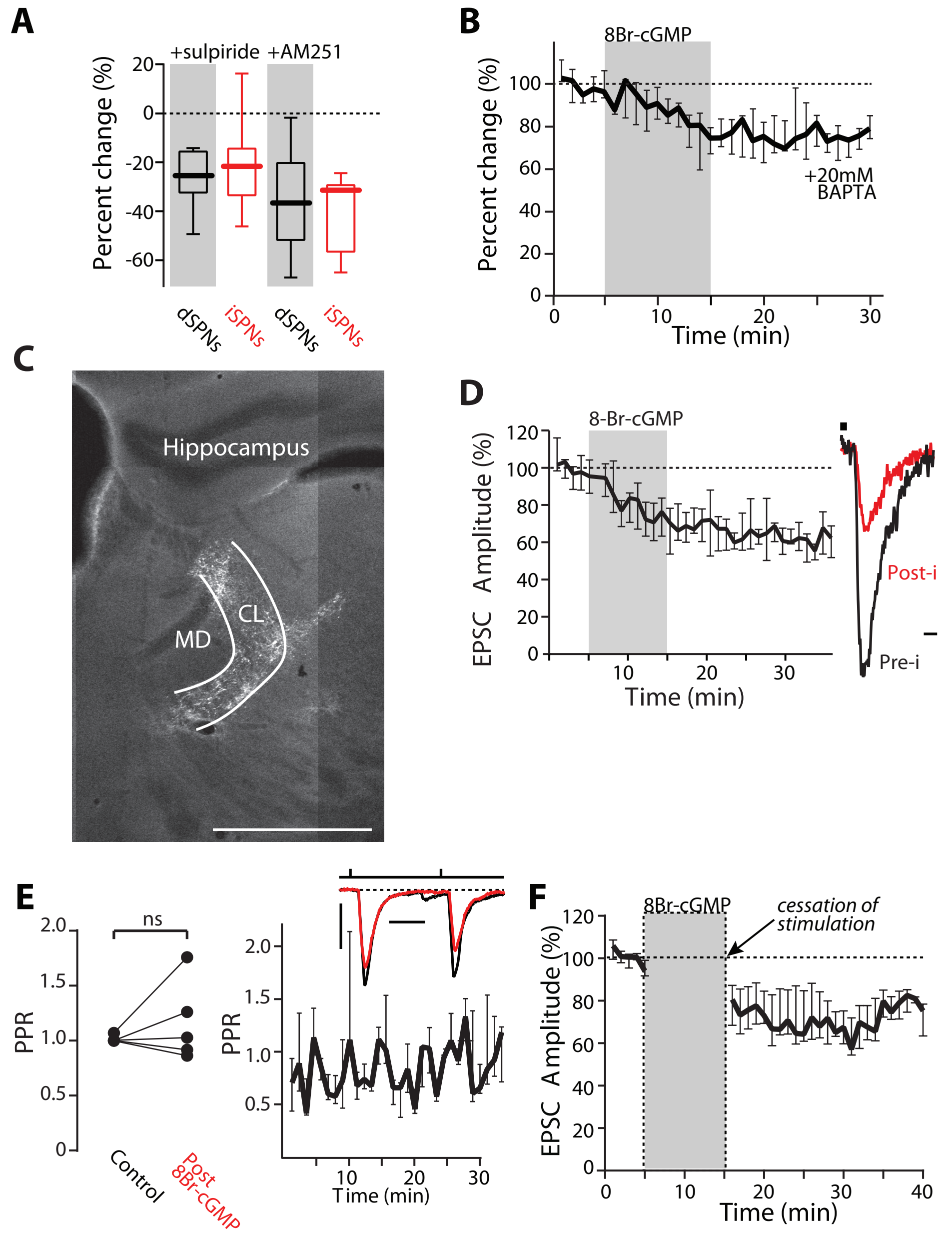
Supplemental Figure 1: Rafalovich et. al.



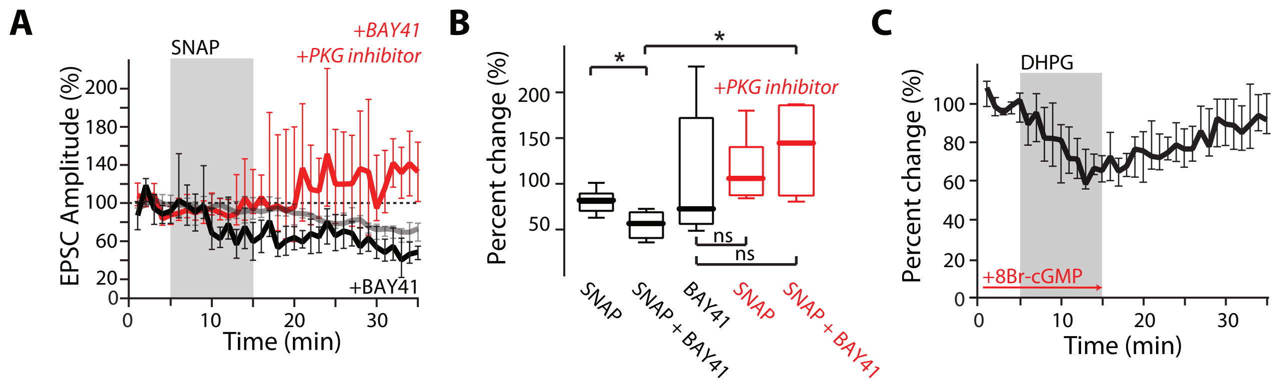
Supplemental Figure 2: Rafalovich et. al.



Supplemental Figure 3: Rafalovich et. al.



Supplemental Figure 4: Rafalovich et. al.



Supplemental Experimental Procedures

Tissue preparation

All experiments were performed in accordance with the Northwestern University ACUC and NIH guidelines. Unless otherwise specified, we used 6-12 week old, hemizygous, male C57Bl/6 mice expressing enhanced green fluorescent protein (eGFP) under control of either the *Drd1a* or *Drd2* receptor regulatory elements. In some experiments, BAC transgenic mice were crossed with a transgenic line expressing ChR2 in the cortex (Thy1-ChR2). For PLTSI stimulation experiments, male and female SOM-IRES-Cre mice ($Sst^{tm2.1(cre)Zjh}$/J) were used (The Jackson Laboratory). For optogenetic experiments probing thalamostriatal synapses, male Tg(Grp-Cre)KH288 mice developed by the GENSAT Project were utilized, in which cre expression is limited to the central lateral (CL) nucleus of the thalamus. Mice were anesthetized with a ketamine/xylene mixture, perfused with ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) and decapitated. The ACSF contained (in mM): 124 NaCl, 3.5 KCl, 2$CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose. Para-sagittal brain slices, 275-300μM thick, containing striatum were prepared and, after cutting, the slices were transferred to a holding chamber containing ACSF and bubbled continuously with 95% O_2 and 5% CO_2 at 30-33°C.

Electrophysiology

Individual slices containing dorsal lateral striatum were transferred from the holding chamber to a submersion-style recording chamber. A variable flow mini-pump was used to continuously perfuse ACSF over the slice. All electrophysiological recordings were obtained at elevated temperature (30-33°C) with 10μM gabazine in the ACSF to block $GABA_A$ mediated currents. Whole-cell recordings were performed from D1 or D2 SPNs of the dorsolateral striatum, identified under differential contrast microscopy and via their fluorescence. Glass electrodes (3-6 MΩ) were filled with a cesium-based solution and cells allowed to fill for 10 minutes before starting experiments. The internal solution contained (in mM): 120 $CsMeSO_3$, 5 NaCl, 10 TEA-Cl, 10 Hepes, 5 QX-314, 4 ATP-Mg, .3 GTP-Na, and .25 EGTA. This internal solution was brough to pH 7.2-7.3 and 270-280 mOsm/l. EGTA concentration was increased to 5mM for experiments in which PLTSIs were optically stimulated. For evoked EPSC recordings, SPNs were voltage-clamped at -70 mV and stimuli delivered at 0.1Hz through a concentric bipolar electrode placed between layer V and VI of the cortex(Frederick haer& Co, ME). For sequential LTD experiments (Figure 4B-C) an attempt was made to normalize the amplitude of the baseline EPSCs as the pre-incubation with SNAP/BAY41 would have been expected to induce NO-LTD and depress cortical EPSCs compared to the control data set. For optogenetic PLTSI LTD experiments, SPNs were first briefly held at +10 mV in the absence of gabazine to ascertain the relative connectivity of the SPN to ChR2-infected PLTSIs by measuring the postsynaptic inhibitory current (IPSC) that resulted from a full-field (1-5ms) light stimulus from the LED light source. Cells were then brought back down to -70 mV, and gabazine perfused for the duration of the experiment. For perforated-patch recordings, the internal solutions contained (in mM):

126 KMeSO4, 14 KCl, 3 MgCl2, 0.5 CaCl2, 5 EGTA, 10 HEPES; pH was adjusted to 7.25 with NaOH and osmolarity to 275-280 mOsm. Electrical access was obtained using a stock solution of gramicidin (2mg ml-1) in dimethyl sulfoxide (DMSO) which was prepared daily and diluted in the cording solution before use to a final concentration of 2 ug ml-1. Access resistance was monitored throughout recording and cells in which a change >20% baseline was observed were thrown out. In experiments where drugs were transiently applied, application times were 10 minutes following 5 minutes of baseline. In experiments where drugs were perfused throughout the length of the recording, cells were first exposed to drugs at break in for a period of 10-20 minutes before collecting data. The EPSC amplitude was analyzed using custom scripts written in Matlab (Mathworks, Natic, MA).

Viral gene delivery

For PLTSI and thalamostriatal optogenetic experiments we used an adeno-associated virus (AAV) vector carrying a ChR2-eYFP expression construction (AAV2/9-EF1a-double floxed-hChR2(H134R)-eYFP-WPRE-HGHpA, supplied by University of Pennsylvania Vector Core; Addgene 20298). We injected 0.3-0.4 µL of virus into SOM-Cre or KH288-Cre mice, respectively, at a rate of ~100 nL/minute. Mice were sacrificed 2-3 weeks post infection and brain slices used for electrophyiological recordings. Coordinates for dorsolateral striatum injections into SOM-Cre mice were (relative to bregma): 1.10 mm anterior, -2.00 mm lateral, -3.00 mm ventral; to target CL of KH288-Cre mice, coordinates were (relative to bregma): 1.34 mm posterior, 0.78 mm lateral, 3.1 mm ventral.

2-photon laser scanning microscopy (2PLSM)

iSPNs were identified by somatic eGFP expression using 2-photon excited fluorescence induced and detected with a Prairie Ultima laser scanning microscope system (Prairie Technologies). Fluorescent and bright-field images were viewed in register using a Dodt contrast detector system. Cells were patched using video microscopy with a Hitachi CCD camera and an Olympus 60X/1.0 NA lens. Green (EGFP) and red (Alexa 568) signals were acquired using 810 nm excitation (Chameleon-XR laser system, Coherent Laser Group, Santa Clara, CA) pulsed at 90 MHz (~250 fs pulse duration). Power attenuation was achieved with two Pockels cells electro-optic modulators (models 350–80 and 350–50, Con Optics, Danbury, CT). The two Pockels cells were aligned in series to provide an enhanced modulation range for fine control of the excitation dose (0.1% steps over four decades). Following patch rupture the internal solution was allowed to equilibrate for 10-15 minutes before imaging. High magnification z-series of dendrite regions ~40-100 µm from the soma were acquired with 0.072 μm^2 pixels with a 4 µs dwell time and 0.5µm z-steps. At the end of each experiment whole cell z-series were acquired with 0.36 μm^2pixels with a 10 µs dwell times and 1 µm z-steps.

Ca^{2+} imaging

SPNs were loaded with 25 μM Alexa 568 and 600 μM Fluo 5F via the recording electrode, which also contained (in mM): 120 $CsMeSO_3$, 5 NaCl, 10 TEA-Cl, 10 HEPES, 5 QX-314, 4 ATP-Mg, 0.3 GTP-Na. Green fluorescent line scan signals were acquired from dendritic spines at 6 ms per line and 512 pixels per line with 0.08 μm pixels and a 10 μs pixel dwell time. The line scan was started 200 ms before a 300 ms voltage step from -70 to 0 mV and continued for 1.5 s to obtain the background fluorescence and to record the decay of the optical signal after stimulation. To reduce photo- damage and photo-bleaching, the laser was fully attenuated using the second Pockels cell at all times during the scan except for the period directly flanking the voltage step. Changes in Fluo 5F fluorescence were measured as $\Delta F/Fo$.

2-photon laser uncaging of glutamate (2PLUG)

Glutamate uncaging was achieved using a Verdi/Mira laser system. 5 mM MNI-glutamate (Tocris, Cookson, Ellisville, MO) was superfused over the slice at 0.4 ml/hr using a syring pump and mulit-barreled perfusion manifold (Cell MicroControls, Norfolk,VA). Glutamate was uncaged adjacent to individual spines using 1 ms pulses of 725 nm light typically 10-20 mW in power at the sample plane. Photolysis power was tuned via a third Pockels cell modulator (Con Optics, Danbury, CT) to achieve uncaged-EPSCs (uEPSCs) ≤5 pA.uEPSC amplitudes were measured from averaged (5 repetitions) traces.

Chemical and Reagents

Gabazine (SK95531), 8-Br-cGMP, SNAP, AM251, sulpiride, Bay-41, and CSST were obtained from Tocris. RP-8-Br-PET-cGMP was obtained from Biolog Life Science Institute. L-NAME was obtained from Sigma Aldrich. Custom peptides were synthesized by the Stanford peptide facility and used at a concentration of 1-2 mM.

Data Analysis and Statistics

Data analysis was performed using Clampfit 9.2 (Molecular Devices, Inc., Sunnyvale, CA), Igor Pro 6.0 (WaveMetrics, Lake Oswego, OR), and Matlab. For plasticity experiments, the magnitude of LTD was calculated as a percentage of the baseline where the baseline is defined as the average of minutes 0-5 of recording time. T he average of minutes 30-35 of each recording was used to calculate effects post drug application except for Figure 1E where minutes 25-30 were used for consistency between cells. Statistical analysis was performed using Matlab and Prism (GraphPad Software). Data are reported as Median, 1st quartile, 3rd quartile, whiskers represent maximum and minimum of data set.