Supplementary Materials

A feedforward inhibitory circuit mediated by CB1-expressing fast-spiking interneurons in the nucleus accumbens

William J. Wright, Oliver M. Schlüter, and Yan Dong

Department of Neuroscience, University of Pittsburgh, Pittsburgh PA 15260

Supplementary Methods

Animals

Animals used were CB1-tdTomato knock-in mice (~25g; 50-90d old). These mice express the soluble fluorophore tdTomato (tdT) under the endogenous CB1 promoter as previously described (Winters *et al*, 2012), allowing for the visualization of $CB1^+$ neurons in brain slices. Male and female mice were used. We examined potential sex differences for the basic functional properties of $CB1^+$ FSI-to-MSN and MSN-to-MSN synapses and found no differences between sexes (CB1-to-MSN or MSN-to-MSN, $p>0.05$ for all measures, t-test; **Fig. 1**). Therefore, for all subsequent experiments, data from both sexes were combined for final data analysis. All mice used were single housed on a 12h light/dark cycle (light on/off 7:00/19:00) with food and water available *ad libitum*. Animals were sacrificed for electrophysiology at the beginning of the light cycle. All animals were used in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Preparation of NAc acute slices

Mice were decapitated after isofluorane anesthesia. Coronal slices (250μm) were prepared on a VT1200S vibratome (Leica, Germany) in 4ºC cutting solution containing (in mM) 135 N-methyl-D-glutamine, 1 KCl, 1.2 KH_2PO_4, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 20 choline-HCO_3, and 11 glucose, saturated with 95% O_2/5% CO_2, pH 7.4 (305-310mM mOsm). Slices were incubated in artificial

cerebrospinal fluid (aCSF) containing (in mM) 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose, saturated with 95% O_2/5% CO_2 (285-290mM mOsm), for 30min at 37ºC. Slices in aCSF were then allowed to recover for 30min at room temperature before electrophysiological recordings.

Electrophysiology recordings

All recordings were made in the medial NAc shell. During recordings, slices were superfused with aCSF, heated to 29-31ºC by passing solution through a feedback controlled in-line heater (Warner, CT). Cells were visually targeted using DIC/fluorescence microscopy (Olympus, BX-51). $CB1^+$ cells were identified by tdTomato fluorescence (**Fig. 1A,B**). Whole-cell patch clamp recordings were made using a Multiclamp 700B amplifier and Digidata 1440A digitizer (Molecular Devices) through boroscilicate glass electrodes (2-5MΩ). Recordings were filtered at 3kHz, amplified 5 times, and digitized at 20kHz using Clampfit 10.2 (Molecular Devices). Series resistance was 8-23MΩ, uncompensated, and monitored throughout the recordings. Cells with a change in series resistance >15% were excluded for analysis.

Paired recordings were used to assess the properties of unitary inhibitory postsynaptic currents (uIPSCs) from $CB1^+$ FSI to MSN synapses and synapses between MSNs. To record connected pairs, postsynaptic MSNs were randomly sampled within a ~100µm radius from the presynaptic cell, within the approximate axonal arbor of FSIs and MSNs (~200-300µm) (Kawaguchi, 1993; Koos *et al*, 2004). The presynaptic patch pipette was filled with potassium-based internal solution (in mM: 130 K-methanesulfate, 10 KCl, 10 Hepes, 0.4 EGTA, 2 $MgCl_2$, 3 Mg-ATP, 0.25 Na-GTP; pH 7.3, 290mM mOsm), while postsynaptic pipettes were filled with high chloride, cesium-based internal solution (in mM: 15 $CsMeSO^4$, 140 CsCl, 4 TEA-Cl, 0.4 EGTA (Cs), 20 Hepes, 3 Mg-ATP, 0.25 Na-GTP, 5 QX-314(Br), pH 7.3, 290mM mOsm) to enhance IPSCs. This arrangement allowed for connectivity to be tested in one direction.

To evoke uIPSCs, presynaptic cells were held in current-clamp mode with membrane potential adjusted to -60mV, to ensure reliable action potential generation. The postsynaptic cell

was held at -70mV in voltage-clamp mode. Brief current injections (1ms, 1300pA) into the presynaptic cell were used to evoke single action potentials. When paired pulses were used, the inter-pulse interval was 50ms. For non-stationary multiple probability fluctuation analysis (MPFA) recordings, a train of 6 action potentials were evoked in the presynaptic cell at 20Hz. Since the rate of connectivity is heavily dependent on whether connections are preserved, we always 1) recorded from pairs deep within the slice where local fibers are better preserved; and 2) recorded pairs with similar distance, to avoid bias and normalize recording conditions. NBQX (5μM) was included in the bath to isolate uIPSCs, except during LTD experiments.

Simultaneous dual voltage-clamp recordings were used to compare excitatory postsynaptic currents (EPSCs) in $CB1^+$ FSIs and MSNs. Cells were held at -70 mV unless otherwise stated. For all EPSC recordings, electrodes were filled with cesium-based internal solution (in mM: 135 $CsMeSO_4$, 5 CsCl, 5 TEA-Cl, 0.4 EGTA (Cs), 20 Hepes, 3 Mg-ATP, 0.25 Na-GTP, 1 QX-314(Br), pH7.3, 290mM mOsm). Picrotoxin (100 μM) was included in aCSF to inhibit $GABA_A$-mediated currents. Presynaptic afferents were electrically stimulated with a constant isolated stimulator (Digitimer, UK), using a monopolar electrode (glass pipette filled with aCSF). When paired pulses were used, the inter-pulse interval was 50 ms. To assess depolarization-induced suppression of excitation (DSE) in $CB1^+$ FSIs, voltage-clamp recordings were used to record EPSCs. These recordings were made in the same manner as dual recordings. All chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Tocris (UK).

Data acquisition, analysis, and statistics

Connection probability was calculated as the percentage of functionally connected pairs from the total number of pairs sampled. Pairs were determined to be functionally connected if presynaptically evoked action potentials elicited uIPSCs in the postsynaptic cell (**Fig. 1 A,B**). uIPSC amplitudes were determined by averaging the current amplitudes over a 1-ms segment around the uIPSC peak relative to its baseline. Synaptic delay of uIPSC was determined by

measuring the time from the peak of the evoked action potential to the deflection of the postsynaptic uIPSC. The decay kinetics of uIPSCs was assessed by fitting the decaying segment of uIPSCs with a single exponential function to obtain the decay time constant, τ. The activation kinetics of uIPSCs was assessed by the time to peak, which was measured as the time interval between the downward deflection of the uIPSC to the peak amplitude. Paired pulse ratio (PPR) was measured by dividing the amplitude of the second uIPSC by that of the first. The coefficient of variance (CV) analysis was done as previously described (Kullmann, 1994). Briefly, CV was estimated from 30-50 consecutive trials. Sample variances (SVs) were calculated for uIPSC amplitudes and sweep noises. CV was calculated as the square root of the difference for the sample variances ($SV_{uIPSC} - SV_{noise}$), divided by the mean uIPSC amplitude.

For MPFA, ~50 uIPSCs were used from each cell at 6 different release probability conditions achieved by a 6-pulse train of presynaptic action potentials at 20Hz (Clements and Silver, 2000; Neumann *et al*, 2016; Scheuss and Neher, 2001; Scheuss *et al*, 2002; Silver, 2003). The mean amplitude of uIPSCs at each time point of the train (I) was calculate by averaging the peak amplitudes of all uIPSCs at this time point. Variance of uIPSCs at each time point in the train was calculated and plotted against their mean. With the assumptions that presynaptic release sites operate independently and that the release probability of synapses is uniform, the amplitudes of uIPSCs can be expressed as:

$$I = NPrQ \qquad \text{[Eq 1]}$$

where N is the number of release sites, Pr is the presynaptic release probability, and Q is the quantal size. For a binomial model, the variance (σ^2) of uIPSC amplitudes can be expressed as:

$$\sigma^2 = NQ^2Pr(1 - Pr) \qquad \text{[Eq 2]}$$

Based on the above equations, the following equation can be derived:

$$\sigma^2 = IQ - (I/N)^2 \qquad \text{[Eq 3]}$$

This equation predicts the parabolic relationship between σ^2 and I. As such, the variance-mean relationship was fit with Eq 3 to estimate N and Q. Pr was then calculated with Eq 1.

Results are shown as mean±SEM. Statistical significance was assessed with Fisher's exact test, paired or unpaired two-tail t-tests, one-way ANOVA, or two-way ANOVA with repeated measures, as specified. Cell-based statistics were performed for electrophysiology data. Significance was set at α=0.05

Supplementary Figure legends

Supplemental Figure 1. Tonic eCB signaling suppresses basal CB1-to-MSN inhibitory transmission. **(A)** Example uIPSC traces before (1) and after (2) the application of AM251 (2μM). **(B)** Summary time course showing AM251 (2μM) application increases the amplitude of uIPSCs at CB1-to-MSN synapses. **(C)** Summary showing application of AM251 increased the amplitude of uIPSCs at CB1-to-MSN synapses. **(D)** Summary showing application of AM251 decreased the PPR at CB1-to-MSN synapses. **(E)** Summary showing application of AM251 does not alter the CV at CB1-to-MSN synapses. n/m represents number of cells/number of animals. * $p < 0.05$. Error bars represent SEM.

Supplemental Figure 2. Disynaptic IPSC events in NAc MSNs. **(A1**) Representative traces showing a fast inward current EPSC following by a delayed outward current IPSC when MSN is held at -45mV. Note the end of the EPSC coincides with the action potential firing in the simultaneous recorded $CB1^+$ FSIs. **(A2)** When the $CB1^+$ FSI was held at -70mV in voltage clamp to prevent action potential firing, the amplitude of the EPSC increased while the IPSC disappeared. **(B)** Trace generated by subtracting the MSN responses when the $CB1^+$ FSI was held at -70 (A2) from the MSN responses when the $CB1^+$ FSI was allowed to fire (A1), displaying an isolated outward IPSC. **(C)** Summary showing the amplitude of the inward EPSC

increased while the outward IPSC disappeared after $CB1^+$ FSI was held. n/m represents number of cells/number of animals. ** $p < 0.01$. Error bars represent SEM.

Supplemental Figure 3. Tonic eCB signaling at excitatory synapses in the NAc. **(A)** Example EPSC traces from simultaneously recorded MSN (top) and $CB1^+$ FSI (bottom) before (1) and after (2) the application of AM251 (2μM). **(B)** Summary time course showing AM251 application gradually increases the amplitude of EPSCs evoked in $CB1^+$ FSIs (gray) and MSNs (white). **(C)** Summary showing application of AM251 increased the amplitude of EPSCs in $CB1^+$ FSIs and trended to increase EPSCs in MSNs. **(D)** Summary showing AM251 had no effect on PPR of EPSCs in either $CB1^+$ FSIs or MSNs. **(E)** Summary showing AM251 had no effect on the CV of EPSCs in either $CB1^+$ FSIs or MSNs. **(F)** Summary showing that application of AM251 did not alter the $EPSC_{MSN}/EPSC_{CB1}$ ratio of simultaneously recorded pairs. n/m represents number of cells/number of animals. * $p < 0.05$. Error bars represent SEM.

Supplemental Figure 4. 1hz LFS induces mild LTD at CB1-to-MSN synapses. **(A,B)** uIPSCs traces **(A)** and the time course of uIPSC amplitudes **(B)** from an example CB1-to-MSN pair before (1) and following (2) a single 1hz LFS when MSNs were held at -55mV during delivery of LFS**. (C)** Summary time course showing a single 1hz LFS induces LTD CB1-to-MSN synapses. **(D)** Summary showing the amplitude of uIPSCs are decreased after 1hz LFS (0.75 % baseline; paired two-tail t-test: t_5=7.09, p=0.0009). **(E)** Summary showing the PPR of uIPSCs is decreased after the 1hz LFS (0.90 %baseline; paired two-tail t-test: t_5=4.33, p=0.0075), which is inconsistent with a presynaptic CB1-dependent mechanism. **(F)** Summary showing the CV of uIPSCs trended towards an increase after 1hz LFS (1.26 %baseline; paired two-tail t-test: t_5=2.081, p=0.0919), which is consistent with a presynaptic CB1-dependent mechanism. n/m represents number of cells/number of animals. ** $p < 0.01$. Error bars represent SEM.