# Altered GluA1 Function and Accumbal Synaptic Plasticity in the *Clock*Δ19 Model of Bipolar Mania

## *Supplemental Information*

### Supplementary Methods

### Animal Behavior

*Locomotor Response to Novelty.* Mice were placed inside automated locomotor activity chambers equipped with infrared photobeams (San Diego Instruments, San Diego, CA) to measure activity. Data were collected continuously for 90 minutes and analyzed in 5-minute time bins.

*Elevated Plus Maze*. During the light phase, mice were tested in a dimly lit room and light was measured at each open arm to ensure consistent exposure. During the dark phase, overhead red lights were used. Mice were placed in the center of the elevated plus maze and both the number of entries into the open and closed arms and the total time spent in the open arms (in seconds) were recorded over a 10-minute period.

*Cocaine Conditioned Place Preference (CPP).* A biased protocol was used to assess place preference. On the pre-test day, mice were allowed to explore all chambers of the place-conditioning apparatus for 20 minutes to determine inherent bias. On conditioning days 1 and 3, mice were given a saline injection (i.p.) paired with the preferred chamber of the apparatus, and on days 2 and 4, they received a cocaine injection (5mg/kg; i.p.) paired with

the non-preferred chamber. Conditioning sessions lasted 20 minutes. Following conditioning, on day 6, mice were tested again for time spent on either side of the apparatus and the CPP score was calculated by subtracting the pre-conditioning time spent in the cocaine-paired side from the time spent in the cocaine-paired side on the test day. Data from mice that spent a majority of time in the center of the apparatus were eliminated from analysis.

**Whole-cell Patch-clamp Recordings**

Borosilicate glass pipettes (3-5MΩ) were filled with (in mM): 117 Cs-$MeSO_3$, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 2.5 Mg-ATP, 0.25 Na-GTP, 5 QX-314; pH 7.3 adjusted with CsOH. For miniature EPSC (mEPSC) and current clamp recordings, a solution containing (in mM): 119 K-$MeSO_4$, 2 KCl, 1 $MgCl_2$, 1 EGTA, 0.1 $CaCl_2$, 10 HEPES, 2 Mg-ATP, 0.4 Na-GTP; pH 7.3 adjusted with KOH, was used interchangeably with no measured differences. A constant-current isolated stimulator (DS3; Digitimer) was used to stimulate excitatory afferents with a monopolar electrode to record evoked currents (eEPSCs). Single or paired pulses were generated using Clampex software (Molecular Devices). Stimulus intensity was adjusted to generate currents with amplitude between 50 and 500pA. Cells with run-up or run-down of more than 15% were excluded from analysis, as were electrophysiologically identified interneurons. Picrotoxin (50μM, Sigma Aldrich) was included in the external perfusion aCSF to block $GABA_A$ receptors. TTX (1μM, Tocris, Bristol, UK) was used for mEPSC recordings. For EPSC experiments, D-APV (50μM, R&D Systems, Minneapolis, MN) was bath applied to block NMDARs at +40mV. In some experiments, D-APV was not applied and the peak amplitude of AMPAR current was measured at -70mV and the NMDAR peak amplitude taken

at 40mV, 35ms from the AMPAR peak. Series resistance for all recordings was monitored continuously. Cells with a change in series resistance beyond 20% were excluded from data analysis. Synaptic currents were recorded with a MultiClamp 700B amplifier (Molecular Devices). Signals were filtered at 2.6-3 kHz and amplified 10 times, then digitized at 20 kHz with a Digidata 1322A analog-to-digital converter (Molecular Devices).

**Surface GLUA1 Detection**

Single NAc tissue punches were rapidly micro-dissected using a stainless-steel stylet (1mm diameter) from 1 mm coronal sections obtained from a mouse brain matrix. Punched tissue was consistently chopped with a surgical scalpel. NAc tissue was not pooled between mice. One hemisphere of tissue was immersed in ice-cold artificial CSF (aCSF) containing 2 mM Bis(sulfosuccinimidyl) suberate ($BS^3$) (Pierce) and incubated for 30 min at 4°C on a rotator. Tissue from the other hemisphere was incubated in aCSF alone. The cross-linking reaction was quenched with 100 mM glycine in aCSF for 10 min at 4°C on a rotator. Samples were centrifuged for 2 min at 4°C. Supernatants were discarded and pellets washed once with aCSF. Samples were re-centrifuged, supernatants were discarded, and pellets were sonicated in ice-cold lysis buffer [0.1% NP-40 buffer in Tris-EDTA, pH 7.4, containing 1× protease inhibitor mixture (Sigma-Aldrich, St. Louis, MO), 5 mm NaF, and 1× phosphatase inhibitor mixture (Sigma-Aldrich)]. Protein concentration was determined by DC assay (Bio-Rad, Hercules, CA) and 20 μg of protein was loaded on a 4–15% gradient Tris-HCl gel (Bio-Rad) and run at 100 V in 1X TGS buffer (Bio-Rad). Proteins were transferred onto PVDF membranes for 1.5 hrs at 500mA constant current in cold 1X TG buffer (Bio-Rad). Membranes were re-wet and blocked for 4hrs in Odyssey Blocking Buffer (LI-COR

Biosciences, Lincoln, NE). They were further processed for GLUA1 immunoblot analysis and probed with mouse anti-GAPDH (37kDa; 1:10000, Santa Cruz Biotechnology, Dallas, TX) and rabbit anti-GluA1 (106kDa; 1:500, Pierce). Following overnight primary incubation at 4°C, blots were incubated in fluorescent secondary antibodies including goat anti-rabbit 800 and goat anti-mouse 680 (1:400, LI-COR Biosciences). Blots were imaged using the LI-COR Odyssey system. Intensity of protein bands was normalized to GAPDH and surface protein levels were determined as the subtraction of the intracellular band intensity from the total band intensity (in arbitrary units). The surface-intracellular ratio was calculated from the obtained values. Rhythms and acrophase measures of GLUA1 expression were determined by multiple harmonic regression using CircWave v1.4 software available from circwave.org. Curves were fit to 2 sine waves and the center of gravity of each fitted waveform was used to determine acrophase.

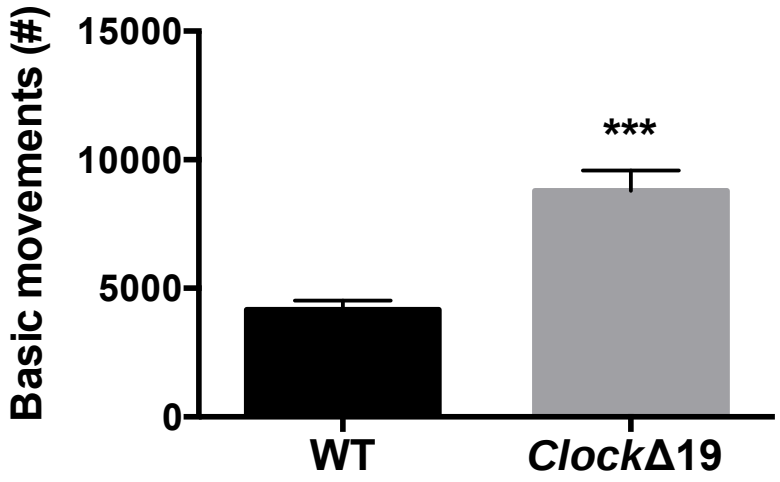
**Immunohistochemistry**

Mice were perfused with 1x phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS (pH 7.4). Brains were post-fixed then transferred to 30% sucrose solution. Sections (30μm) were taken and processed for GFP, NeuN and DAPI. Briefly, floating sections were rinsed 3 x with PBS to remove fixative then blocked for one hour in PBS containing 0.2% Triton-X and 5% Normal Donkey Serum (Jackson ImmunoResearch, West Grove, PA). Sections were incubated overnight at room temperature with the primary antibodies: rabbit anti-GFP (1:20,000; Abcam, Cambridge, UK) and mouse anti-NeuN (1:500; Millipore, Temecula, CA) on a rotary shaker. The following day, sections were rinsed 3 x with in PBS and incubated with donkey anti-rabbit conjugated to a 488nm fluorophore and

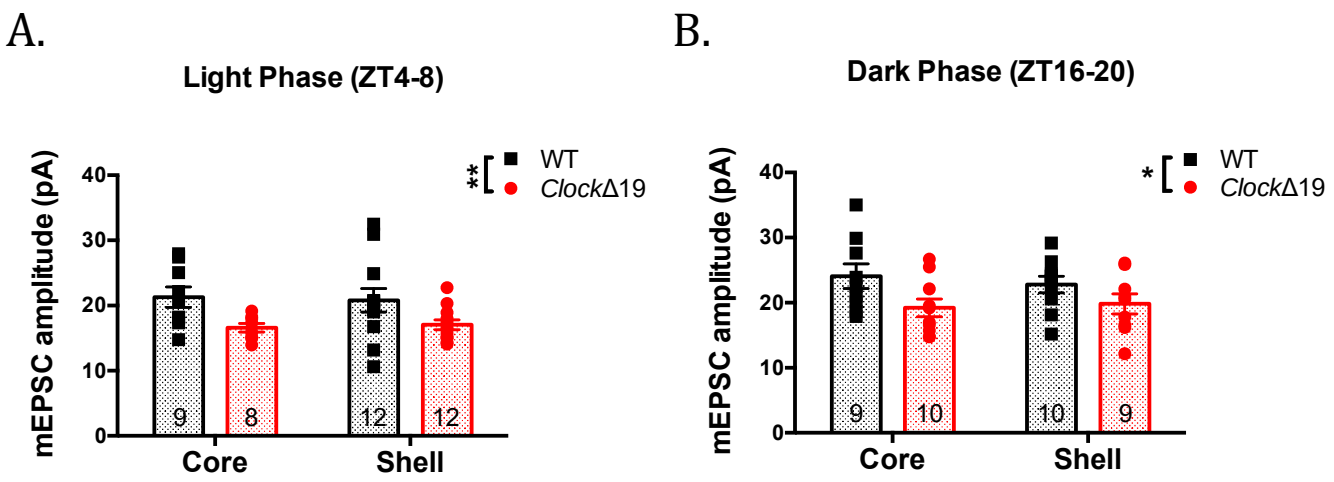
donkey anti-mouse 546 (1:400; Invitrogen, Carlsbad, CA) for two hours at room temperature on a rotary shaker. Following a final wash in PBS, sections were mounted and coverslipped with DAPI mounting medium (Vector Labs, Burlingame, CA) and imaged at 4x magnification on a fluorescent microscope (Olympus, Center Valley, PA).

**Real-time Polymerase Chain Reaction**

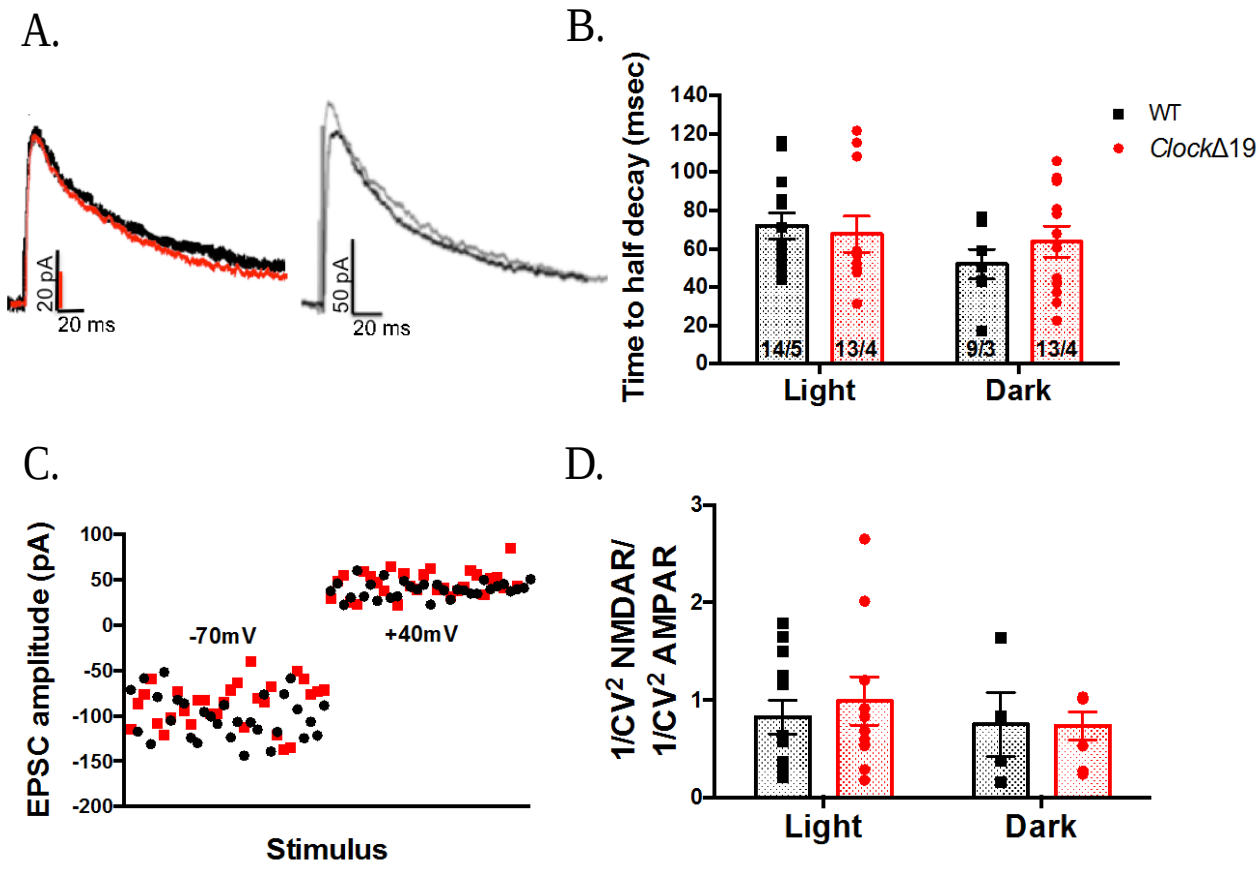
Primers used for qPCR: *GluA1* Fwd: 5' – ACCCTCCATGTGATCGAAATG-3'; *GluA1* Rev: 5'-GGTTCTATTCTGGACGCTTGAG-3'; *Gapdh* Fwd: 5'-CTTTGTCAAGCTCATTTCCTGG-3'; *Gapdh* Rev: 5'-TCTTGCTCAGTGTCCTTGC-3'. Micro-dissected NAc tissue punches were homogenized both mechanically and by QIAshredder homogenization spin-column (Qiagen, Hilden, Germany). Total RNA was isolated using the RNeasy Plus Micro Kit (Qiagen) as per manufacturer guidelines. gDNA was eliminated with the provided gDNA Eliminator column. RNA concentration and quality was determined using NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized from 100ng total RNA using SuperScript VILO Master Mix (Invitrogen). Relative gene expression was measured by qPCR with 1ng of cDNA mixed with Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) and primers listed above. Reactions were run in duplicate in an Applied Biosystems 7900HT Fast Real-time PCR System (Applied Biosystems, Foster City, CA). Relative gene expression was calculated using the comparative Ct (2^ΔΔCt) method and normalized to each sample's corresponding *Gapdh* mRNA levels.



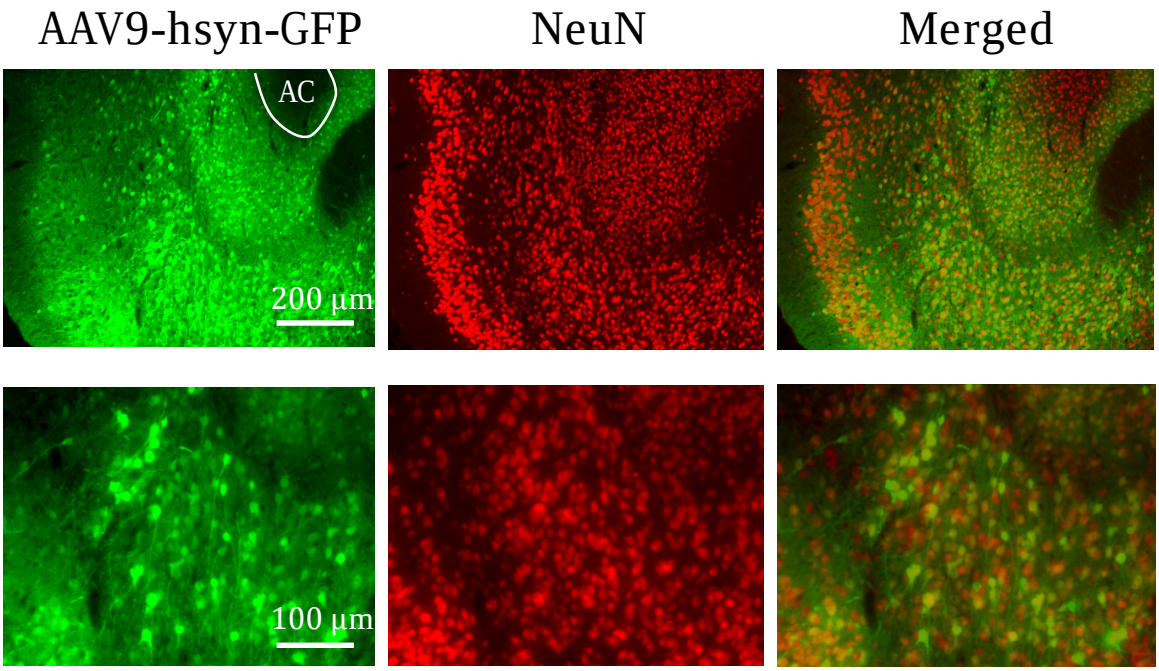

**Supplementary Figure S1.** Locomotor activity of 6-9wk old *Clock*Δ19 mice in a novel environment, as measured by basic movements during a 60 min session is significantly increased compared with age-matched WT littermates.



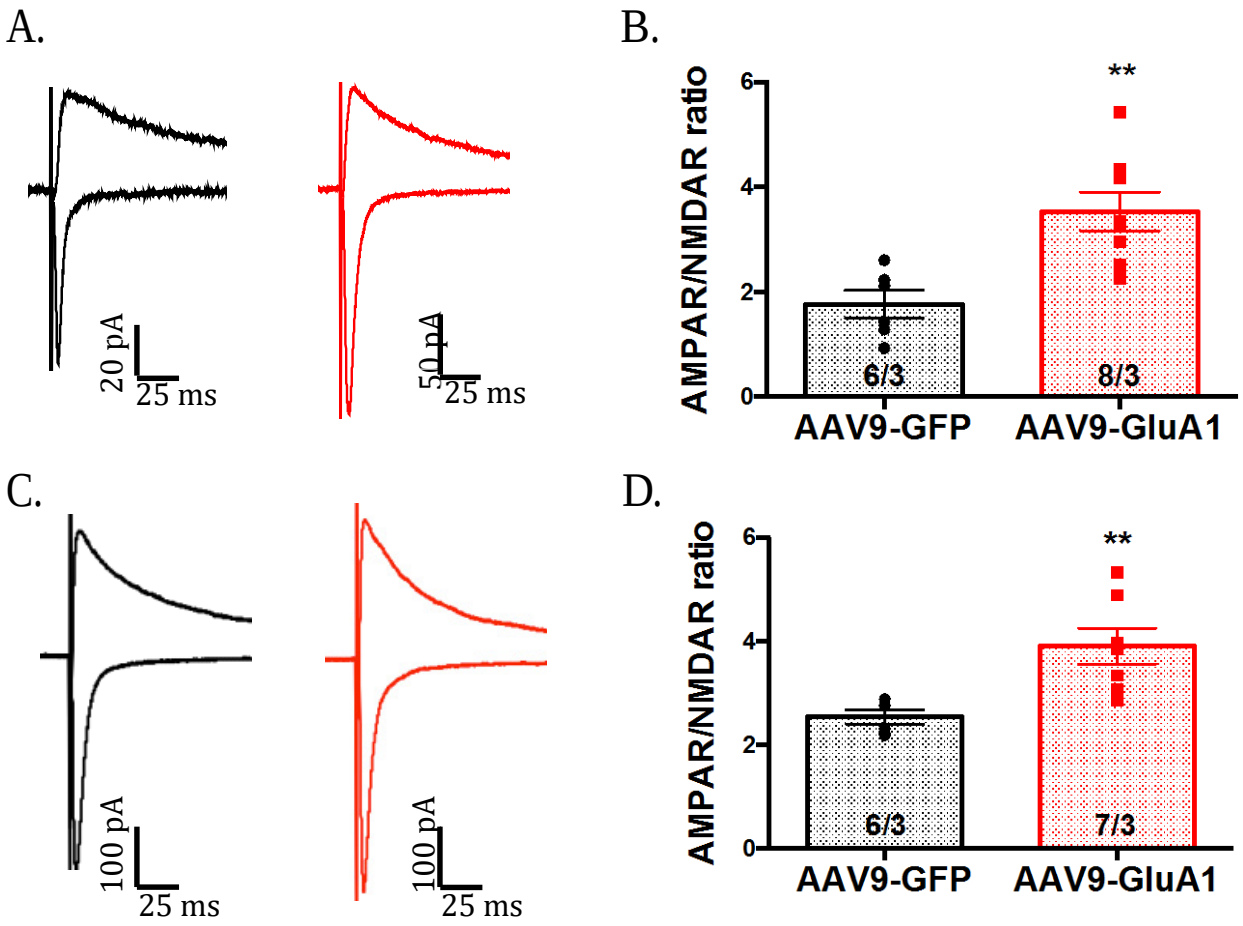

**Supplementary Figure S2.** *Clock*Δ19 MSNs show reduced glutamatergic transmission in both core and shell subregions of the NAc. (A) Summary of mEPSC amplitude of WT and mutant NAc core and shell MSNs during the light phase. (B) Amplitude of mEPSCs in core and shell subregions during the dark phase.



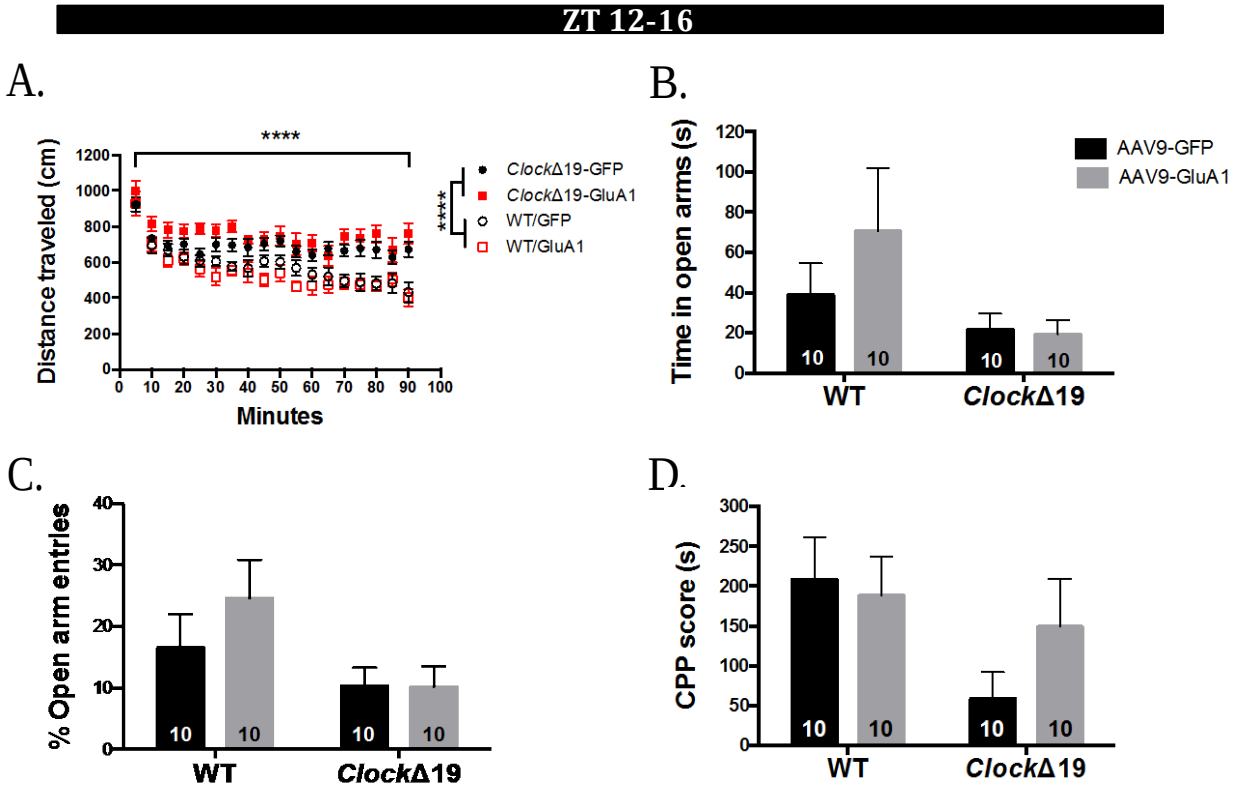

**Supplementary Figure S3.** NMDAR-related properties are not altered by *Clock* mutation. (A) Representative traces of NMDAR-mediated EPSCs in the light phase (WT - black, *Clock*D19 – red) and dark phase (WT - dark grey, *Clock*D19 – light grey) MSNs. (B) Summary of NMDAR EPSC decay kinetics. (C) Sample plot of AMPAR EPSC amplitudes at -70mV and NMDAR EPSCs at 40mV from light phase WT and mutant MSNs. (D) Summary of ratio of $1/CV^2$ NMDAR EPSCs to $1/CV^2$ AMPAR EPSCs from mutant and WT MSNs during both phases.



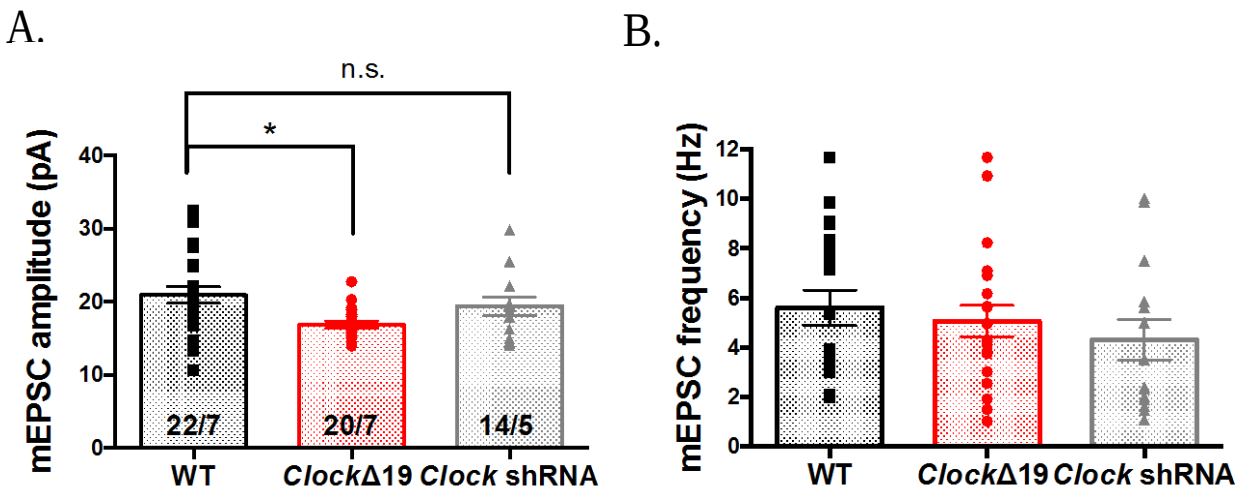

**Supplementary Figure S4.** Neuronal specificity of AAV9 viral constructs in the nucleus accumbens of adult animals following 3 weeks of expression. GFP labeling indicates viral infection (left), labeling of the neuron-specific marker, NeuN (center) and merged overlay image (right).




**Supplementary Figure S5.** Viral overexpression of *GluA1* potentiates MSN excitatory synapses in *Clock*D19 and WT mice. (A) Representative traces of AMPAR EPSCs (at -70mV) and NMDAR EPSCs (at 40mV) in GFP-expressing cells (black) and putative *GluA1*-overexpressing cells (red) in mutants. (B) AMPAR/NMDAR ratio of evoked EPSCs from virally infected cells from mutant slices. (C and D) Representative traces and summary of AMPAR/NMDAR from GFP (black) and *GluA1* expressing cells in WT slices.




**Supplementary Figure S6.** Overexpression of *GluA1* in the NAc does not alter exploratory and reward behavior during the dark phase. (A) Locomotor activity in a novel environment of GFP and GluA1 expressing mutant and WT animals. (B) Time spent in the open arms of the EPM for all groups. (C) Percent open arm entries in the EPM. (D) Cocaine CPP scores for each group in a biased paradigm.




**Supplementary Figure S7.** Knock-down of *Clock* in the NAc of WT mice does not alter excitatory transmission. (A) Summary of mEPSC amplitude in MSNs expressing AAV-*Clock*-shRNA. Compared with WT and mutant MSNs (B) Frequency of mEPSCs from all groups.