Supplementary Material

Ishikawa et al., Homeostatic Synapse-driven Membrane Plasticity in Nucleus Accumbens Neurons

1) Active and passive membrane properties following hSMP.

Both active and passive membrane properties were measured in NAc MSNs following the expression of hSMP. As shown in the table below, both the resting membrane potential (RMP, measured right after the formation of whole-cell mode; the stabilized RMPs after the dialysis of internal solution were more hyperpolarized, usually at ~80 mV) and input resistance (R_in, measured after stabilization of the RMP) were not significantly different between hSMP-expressing MSNs and control MSNs. In active membrane properties, the MSNs expressing hSMP and control MSNs exhibited similar duration of action potential (AP, measured by the duration at half peak), AP threshold (measured at the edge of the uprising of AP), fAHP (see main manuscript for definition), and rheobase (measured by a 300-ms pulse). However, the amplitudes of AP (from AP threshold to the peak of AP, p < 0.05) and mAHP (p < 0.01) were significantly increased. The change in mAHP was extensively discussed in the main manuscript. It remains to be determined the ionic basis underlying the change in AP amplitude.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control n = 21</th>
<th>DCS 5 hrs n = 18</th>
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</thead>
<tbody>
<tr>
<td>Passive</td>
<td></td>
<td></td>
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<tr>
<td>RMP (mV)</td>
<td>-73.33 ± 0.97</td>
<td>-73.94 ± 1.21</td>
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<tr>
<td>R_in (MΩ)</td>
<td>142.76 ± 5.22</td>
<td>131.46 ± 5.51</td>
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<tr>
<td>Active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Amplitude (mV)</td>
<td>65.10 ± 1.57</td>
<td>70.02 ± 1.66*</td>
</tr>
<tr>
<td>AP Halfwidth (ms)</td>
<td>0.77 ± 0.03</td>
<td>0.80 ± 0.03</td>
</tr>
<tr>
<td>AP Threshold (mV)</td>
<td>-38.81 ± 0.83</td>
<td>-40.22 ± 1.00</td>
</tr>
<tr>
<td>fAHP (mV)</td>
<td>14.38 ± 0.51</td>
<td>15.27 ± 0.57</td>
</tr>
<tr>
<td>mAHP (mV)</td>
<td>6.30 ± 0.55</td>
<td>8.84 ± 0.74**</td>
</tr>
<tr>
<td>Rheobase (pA)</td>
<td>125 ± 9 (n = 8)</td>
<td>155 ± 22 (n = 11)</td>
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</table>

2) In vivo manipulation of the excitatory synaptic strength by PSD95 and PSD95-RNAi

To focus on the synaptic activity, we adopted two independent approaches. One approach involved depleting the synaptic transmission and detecting the induction of hSMP by the same pharmacological treatments (Fig. 6A-C). In another approach, we attempted to detect whether
hSMP can be induced in the in vivo NAc MSNs upon experimental manipulations of excitatory synaptic strengths. The main finding of this section is presented in the main manuscript. Here, we demonstrate that with our molecular manipulations, the strength of excitatory synapses was regulated in the intended directions. Briefly, we used viral-mediated gene transfer technique to express PSD95 or PSD95-RNAi (see Experimental Procedures in the main manuscript), which, in NAc MSNs in vivo, bi-directionally regulated the synaptic scaffolding protein PSD95. It has been shown that over-expression of PSD95 increases the excitatory synaptic strength, whereas interference (RNAi)-mediated knocking down of this protein causes the opposite effect (Schluter et al., 2006). We stereotaxically injected the lentiviruses expressing PSD95 or PSD95-RNAi in the NAc of anesthetized rats and 7-11 days later prepared the NAc slices. Using pairwise recordings (see Experimental Procedures), we verified that NAc MSNs expressing PSD95 exhibited larger AMPAR EPSCs, whereas MSNs expressing PSD95-RNAi exhibited smaller AMPAR EPSCs (relative to EPSC amplitude of uninfected neighbors: GFP, 1.3 ± 0.22, n = 8, p = 0.21; PSD95, 2.96 ± 0.47, n = 8, p < 0.01; RNAi, 0.58 ± 0.1, n = 8, p < 0.01; paired t-test, Fig. S1A,B). On the other hand, NMDAR EPSCs were not significantly affected by these molecular manipulations (relative to EPSC amplitude of uninfected (uninf) neighbors: GFP, 1.28 ± 0.22, n = 8, p = 0.24; PSD95, 1.44 ± 0.28, n = 8, p = 0.16; PSD95-RNAi, 0.83 ± 0.12, n = 8, p = 0.20; paired t-test, Fig. S1C,D). It is important to note that the in vivo activity state of NMDARs relies on AMPAR-mediated depolarization of postsynaptic membrane. Thus, although lacking a direct effect on synaptic NMDARs, expression of PSD95 or PSD95-RNAi are expected to up- or down-regulate the activation level of synaptic NMDARs in vivo due to the elevated activity of synaptic AMPARs. With these manipulations focusing on synaptic activity in vivo, we performed a set of double-blind experiments (see Experimental Procedures) and observed that alterations in excitatory synaptic strength in NAc MSNs induced compensatory changes in membrane excitability in NAc MSNs (see Fig. 6F, G in main manuscript).

3) Age-dependent modulation of membrane excitability of NAc MSNs.

In our experiments, rats received a 5-day cocaine regimen starting at ~30 days postnatal. These rats then experienced a withdrawal period of either 2 days or 3 weeks. Thus, the rats with different withdrawal paradigms were examined at two different ages, ~37 days or ~52 days. As such, to determine the potential effects of different withdrawal periods on membrane excitability, it is important to understand whether the age of the animals plays a role in regulating the membrane excitability. Indeed, it has been shown that the intrinsic membrane excitability (measured by evoked
action potential firing) exhibits age-dependent changes in central neurons (Frick et al., 2007; Tombaugh et al., 2005; Yan et al., 2009). When examining the membrane excitability of NAc MSNs from animals of different ages, we observed a similar age-dependent regulation; the frequency of evoked action potential firing was gradually decreased when animals became older (F(3, 320) = 65.59, p = 0.00, two-factor ANOVA; p = 1.00, p23-25 vs. p30-31; p = 0.00, p30-31 vs. p41-42; p = 0.00, P41-42 vs. P49-52; Bonferroni posttest; n/m = 11/10, 11/6, 12/7, and 10/5 for P23-25, 30-31, 41-42, and 49-52, respectively; Fig. S2). These results indicate that there is a age-dependent shift in the basal membrane excitability of NAc MSNs. Thus, the membrane excitability of NAc MSNs does not remain at the same level at the two time points of short- (2-day) and long-term (3-week) withdrawal. Accordingly, we performed each set of experiments using a group of age-matched rats (Fig. 8).

4) Detailed statistical results:

Figure 1: 100 pA: Kyn, 11.5 ± 2.1; control, 5.2 ± 1.8; 200 pA: Kyn, 25.7 ± 2.1; control, 18.1 ± 1.9; number of cells/number of rats, or “n/m”, = 8/4 and 7/4 for Kyn and control, respectively; F(1, 26) = 13.1, p < 0.01, two-factor ANOVA.

Figure 2: E, F: 100 pA: control, 5.2 ± 0.9, DCS, 2.3 ± 0.7, APV, 9.7 ± 1.4; 200 pA: control, 16.5 ± 2.2, DCS, 12.9 ± 1.6, APV, 22.4 ± 2.1; F(2, 48) = 12.8, p < 0.01, two-factor ANOVA; p < 0.01 for DCS vs. control; p < 0.01, APV vs. control; Bonferroni posttest; n/m = 6/4, 10/5, and 11/6 for control, DCS, and APV, respectively. G, I: 100 pA: control, 5.1 ± 0.7; APV, 4.8 ± 0.9; 200 pA: control, 18.0 ± 1.8; APV, 17.8 ± 1.6; F(1, 20) = 0.03, p = 0.86, two-factor ANOVA, n/m = 5/3 and 7/4 for control and DCS, respectively. H: 100 pA: control, 5.1 ± 0.8; APV, 5.0 ± 0.7; 200 pA: control, 18.2 ± 2.1; APV, 18.4 ± 1.4; F(1, 42) = 0.22, p = 0.91, two-factor ANOVA; n/m = 12/6 and 11/6 for control and APV, respectively.

Figure 3: F(3, 249) = 14.8, p < 0.01, two-factor ANOVA; p < 0.01 between 1 hr and other time points, Bonferroni posttest; the effect size of time was estimated by the ‘mean difference’ between 1 hr and other time points: 3hr, 1.90; 5 hr, 1.92; 8 hr, 2.73.

Figure 4: B: relative to control: DCS, 1.08 ± 0.14; DCS + APV, 0.97 ± 0.11; F(2, 12) = 0.78, p = 0.49, n/m = 5/4, one-factor ANOVA; D: relative to control: DCS, 1.63 ± 0.30; DCS + APV, 0.72 ± 0.13; n/m = 6/4; F(2, 15) = 6.27, p < 0.01, one-factor ANOVA; p < 0.05 control vs. DCS; p = 0.31 control vs. DCS + APV, Bonferroni posttest. E, F: F(1, 105) = 5.83, p < 0.05, two-factor ANOVA, n/m = 9/5 and
8/5 for control and DCS, respectively. 4G, H: \( F_{(1,119)} = 6.77, p < 0.05 \), two-factor ANOVA, \( n/m = 10/5 \) and \( 9/5 \) for control and D-serine, respectively.

Figure 5: A, B: \( F_{(1,105)} = 2.1, p = 0.047 \), 3-hr DCS vs. control; \( F_{(1,259)} = 10.77, p < 0.01 \), 5-hr DCS vs. control; \( F_{(1,91)} = 12.94, p < 0.01 \), 5-hr DCS + 3-hr wash vs. control. C, D: \( F_{(2,119)} = 0.11, p = 0.90 \), two-factor ANOVA, \( n/m = 7/4, 6/4, \) and \( 7/5 \) for control, 5 µM APV, and 50 µM APV, respectively. E, F: \( F_{(3,255)} = 19.85, p < 0.01 \), two-factor ANOVA; \( p < 0.01 \), control vs. DCS; \( p < 0.01 \), control vs. DCS + 5 µM APV; \( p = 1.0 \), control vs. DCS + 50 µM APV; Bonferroni posttest.

Figure 6: B: \( F_{(1,126)} = 0.05, p = 0.82 \), two-factor ANOVA, \( n/m = 9/5 \) and \( 11/6 \) for control and Baf, respectively. C: \( F_{(2,192)} = 21.4, p < 0.01 \), \( n/m = 9/5, 10/5, \) and \( 8/5 \) for control, DCS, and Bal + DCS, two-factor ANOVA; \( p < 0.01 \), control vs. DCS; \( p = 0.30 \), control vs. Bal + DCS; \( p < 0.01 \), DCS vs. Bal + DCS, Bonferroni posttest. F, G: \( F_{(3,320)} = 28.3, p < 0.01, n/m = 22/14, 7/6, 8/6, 7/5 \) for uninf, GFP, PSD95, and RNAi, respectively, two-factor ANOVA; \( p = 1.00 \), uninf vs. GFP; \( p < 0.01 \), GFP vs. PSD-95; Bonferroni posttest.

Figure 7: B: in mV: control, 6.30 ± 0.55, \( n = 21 \); DCS, 8.84 ± 0.74, \( n = 18 \); \( t_{(37)} = -2.78, p < 0.01 \), t-test. C: in mV: control, 14.38 ± 0.51, \( n = 21 \); DCS, 15.27 ± 0.57, \( n = 18 \); \( t_{(37)} = -1.17, p = 0.25 \), t-test. D-F: relative amplitude in Ibx: fAHP, 0.68 ± 0.07, \( n = 5, t_{(4)} = 3.53, p < 0.05 \), paired t-test; mAHP, 0.89 ± 0.13, \( n = 5, t_{(4)} = 1.08, p = 0.34 \), paired t-test. G: \( F_{(1,56)} = 0.99, p = 0.35 \), two-factor ANOVA, \( n/m = 5/4 \) and \( 5/4 \) for control and Ibx, respectively. H-J: relative amplitude in apamin: fAHP, 0.95 ± 0.03, \( n = 5, t_{(4)} = 1.43, p = 0.23 \); mAHP, 0.92 ± 0.10, \( n = 5, t_{(4)} = 0.54, p = 0.62 \), paired t-test. K: \( F_{(1,56)} = 0.07, p = 0.79, n/m = 9/5 \) and 7/5 for control and apamin, respectively, two-factor ANOVA. L: \( F_{(2,352)} = 34.6, p < 0.01, n/m = 21/12, 18/11, \) and \( 8/7 \) for control, DCS, and DCS + apamin, respectively, two-factor ANOVA; \( p < 0.01 \), control vs. DCS; \( p < 0.01 \), DCS vs. DCS + apamin, \( p = 1.00 \), control vs. DCS + apamin; Bonferroni posttest. M, N: mAHP in mV: control, 6.20 ± 0.66, \( n/m = 16/9 \); DCS, 8.79 ± 0.93, \( n/m = 14/9 \); DCS + apamin, 4.84 ± 1.11, \( n/m = 8/7 \); \( F_{(2,33)} = 5.37, p < 0.01 \), one-factor ANOVA; \( p < 0.05 \), control vs. DCS; \( p < 0.01 \), DCS vs. DCS + apamin; \( p = 0.66 \), control vs. DCS + apamin, Bonferroni posttest.

Figure 8: A: \( F_{(3,352)} = 36.3, p < 0.01 \), two-factor ANOVA; \( n/m = 10/6, 12/6, 13/6, \) and \( 13/6 \) in saline-control, saline-DCS, cocaine-control, and cocaine-DCS, respectively; \( p < 0.01 \), saline-control vs. saline-DCS, cocaine-control, or cocaine-DCS; \( p = 0.58 \), saline-DCS vs. cocaine-control; \( p = 0.60 \), saline-DCS vs. cocaine-DCS; \( p = 1.00 \), cocaine-control vs. cocaine-DCS; Bonferroni posttest. B, C: mAHP in mV: control, 7.05 ± 1.17, \( n/m = 10/6 \); DCS, 11.15 ± 0.44, \( n/m = 12/6 \); cocaine, 10.62 ±
0.68, n/m = 13/6; cocaine-DCS, 10.54 ± 0.61, n/m = 13/6; F(3, 44) = 5.82, p < 0.01, one-factor ANOVA; p < 0.01, control vs. DCS, cocaine, or cocaine + DCS; p = 0.60, DCS vs. cocaine; p = 0.55, DCS vs. cocaine + DCS; p = 0.94, cocaine vs. cocaine + DCS; Bonferroni posttest; fAHP in mV: control, 17.32 ± 0.88, n/m = 10/6; DCS, 15.53 ± 0.82, n/m = 12/6; cocaine, 16.57 ± 0.097, n/m = 13/6; cocaine-DCS, 16.65 ± 0.52, n/m = 13/6; F(3, 44) = 0.78, p = 0.51, one-factor ANOVA. D: F(3, 256) = 17.4, p < 0.01, n/m = 8/5, 10/6, 9/5, and 9/7 for saline, saline + apamin, cocaine, and cocaine + apamin, respectively, two-factor ANOVA; p < 0.01, control vs. DCS; p = 0.30, saline vs. saline + apamin; p < 0.01, saline vs. cocaine; p < 0.01, cocaine vs. cocaine + apamin; p < 0.01, saline vs. cocaine + apamin; p = 0.48, saline + apamin vs. cocaine + apamin; Bonferroni posttest. E: F(3, 320) = 8.52, p < 0.01, n/m = 10/5, 10/5, 12/5, and 13/5 for saline, saline-DCS, cocaine, and cocaine-DCS, respectively, two-factor ANOVA; p < 0.01, saline vs. saline-DCS, cocaine, or cocaine-DCS; p = 1.00, saline-DCS vs. cocaine; p = 1.00, saline-DCS vs. cocaine-DCS; p = 1.00, cocaine vs. cocaine-DCS; Bonferroni posttest.

**Figure Legends**

**Figure S1** In vivo manipulations of the strength of excitatory synapses at NAc MSNs. **A1** A diagram of pairwise recording, in which a virally infected MSN and its adjacent uninfected MSN were sequentially recorded with the same presynaptic stimulation. **B2** AMPAR EPSCs recorded at -70 mV from two example pairs, uninfected MSN vs. PSD95-expressing MSN and uninfected MSN vs. PSD95-RNAi-expressing MSN. **A3** Dual-component EPSCs recorded at +50 mV from two example pairs, uninfected MSN vs. PSD95-expressing MSN and uninfected MSN vs. PSD95-RNAi-expressing MSN. **B** Summaries showing that viral-mediated expressions of PSD95 and PSD95-RNAi in NAc MSNs increased and decreased AMPAR EPSCs, respectively, whereas these two molecular manipulations did not significantly affect NMDAR EPSCs. ***, p < 0.01.**

**Figure S2** Age-dependent changes in evoked action potential firing in NAc MSNs. Summarized results showing that the frequency of evoked action potential firing in NAc MSNs was gradually decreased when the animals became older.

**Reference**


