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Homeostatic Recovery of Downstate-Upstate Cycling in Nucleus Accumbens Neurons

Brian R. Lee, Ping Mu, Daniel B. Saal, Catherine Ulibarri, and Yan Dong

Program in Neuroscience, Department of VCAPP, Washington State University, Pullman, WA 99164

Abstract

Homeostatic plasticity is a powerful cellular mechanism through which neurons adjust intracellular and intercellular resources to stabilize their functional output through the ever-changing environment. Here, we report a novel form of homeostatic plasticity that nucleus accumbens (NAc) neurons use to regain their functionally active state once it is lost. *In vivo*, NAc neurons periodically alternate between a functionally active upstate and a functionally quiescent downstate. The upstate of NAc neurons is immediately lost following severe environmental changes, such as deep anesthesia and truncation of excitatory synaptic inputs. Using short-term slice cultures, our current study demonstrates that NAc neurons initially lose but gradually recover their upstate-downstate cycling after shifting to the *in vitro* condition. Furthermore, we show that this homeostatic recovery of the upstate is mediated by increased synchronization of presynaptic activity. Given that being in the upstate is required for *in vivo* NAc neurons to fire action potentials, the homeostatic recovery of upstate may underlie an important cellular mechanism for NAc neurons to maintain their functional output against severe environmental fluctuations.

INTRODUCTION

Homeostatic neuronal plasticity is an important self-correcting mechanism through which neurons adjust cellular resources to restore their functional output after a disturbance [24]. Homeostatic plasticity occurs at the synaptic and membrane levels. For example, an extracellular interference that tends to reduce the intrinsic membrane excitability triggers a compensatory increase in the intrinsic membrane excitability [3,4], whereas a decrease in presynaptic input induces a homeostatic increase in the postsynaptic responsiveness [27,28]. Here, we report a novel form of homeostatic plasticity that occurs between presynaptic terminals. This form of homeostatic plasticity acts to maintain the overall functional output of NAc neurons.

NAc neurons *in vivo* are often called bimodal neurons because they cycle between two functional states, the downstate and the upstate [12]. During downstates the NAc neurons dwell at a hyperpolarized resting membrane potential (~ -80 mV) and remain largely quiescent. Upon synchronous excitatory synaptic inputs, NAc neurons climb to a depolarized potential (~ -50 mV), which is commonly referred to as the upstate potential [12]. During upstates, the NAc neurons are prone to fire action potentials. Extensive evidence suggests that the upstate potential is initiated and maintained by synchronous activities of glutamatergic synapses [13,

Corresponding author: Yan Dong Phone: 509-335-5960 Fax: 509-335-4650 Email: yandong@vetmed.wsu.edu Address: PO Box 646520, 205 Wegner, Washington State University, Pullman, WA 99164.

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30]. Thus, to carry out their function, the NAc neurons must first receive synchronous glutamatergic synaptic input to climb onto the upstate membrane potential.

It is thought that synchronization of excitatory synaptic inputs to the NAc neurons is mediated by simultaneous activation of the projecting glutamatergic neurons located in the cortical and subcortical regions. Either silencing the somas of these projecting neurons or truncating the projecting fibers from these neurons immediately prevents the generation of upstate in NAc neurons [12,16,18]. Accordingly, in the thin coronal brain slice in which the glutamatergic projecting neurons are absent, NAc neurons do not normally exhibit the downstate-upstate cycling. Our current results show that the bimodal property of NAc neurons gradually recovered in the thin brain slices when these slices were maintained for a sufficiently long period of time. Furthermore, we demonstrate that the homeostatic recovery of bimodal property is mediated by gradually-increased synchronization of presynaptic release, presumably from the glutamatergic fibers that were truncated from their cell bodies. These observations suggest that homeostatic neuroplasticity occurs at intracellular and intercellular levels and play an important role in maintaining/recovering neuronal function.

METHODS

NAc slice preparation and culture

Detailed procedure for obtaining and culturing NAc slices can be found in our previous publications [5,8]. Briefly, young (p19-21) Sprague-Dawley rats were deeply anaesthetized with isoflurane and decapitated. Coronal NAc slices (200- μ m thick) were obtained in ice-cold sterile low Ca^{2+} solution containing (in mM): 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4 , 1.2 MgCl_2 , 0.625 CaCl_2 , 18 NaHCO_3 , and 11 glucose, and then placed on Millicell Millipore culture plate inserts in wells containing Neurobasal-A Media with 4% B-27 and 1% Glutamax-I Supplements (Invitrogen) for 1-24 hrs until it is transferred to the recording media for electrophysiological recordings. In a subset of preparations, cortical regions were completely removed and the bimodal NAc neurons were still readily observed.

Electrophysiology

Standard whole-cell current- or voltage-clamp recordings were used in all experiments (with MultiClamp 700B amplifier). For current-clamp, bridge balance and the input resistance were monitored continuously by applying a 100 msec hyperpolarizing current pulse following each synaptic stimulus. In voltage-clamp recordings, a small (5-10 mV) hyperpolarizing step was applied following each stimulus to monitor input resistance throughout the experiment. If access resistance changed by more than 10%, data subsequent to this change were excluded. The intracellular and extracellular solutions used can be found in our previous publications [6,7]. The recording bath contained (in mM): 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4 , 1.2 MgCl_2 , 2.5 CaCl_2 , 18 NaHCO_3 , and 11 glucose, and was equilibrated at 31–34°C with 95% O_2 /5% CO_2 . To examine EPSCs, the extracellular solution contained picrotoxin (0.1 mM), which blocked GABA_A -mediated currents. Stimuli were applied through bipolar stainless steel electrodes, which were placed in the dorsolateral region of the recorded neurons (~100 μ m). Stimulation parameters included: frequency, 0.1 Hz; intensity, 50-200 μ A; duration, 0.1 msec. The membrane potential was held at -70 mV. Recordings were made primarily from the medium spiny neurons within NAc shell, which were identified by their anatomical landmarks [2].

Definition of bimodal neurons

We operationally define that during the 5-min whole-cell current-clamp recordings, any depolarizing membrane oscillations with amplitude >7 mV, duration >0.1 sec, and >1 action

potential firing during the period represent the upstates [15-17]. We also define that any NAc neurons that exhibited >10 upstates during the 5-min recording are bimodal neurons.

Statistics

All values were presented as mean±standard error. Statistical significance was assessed using a *t*-test unless otherwise specifically indicated. The *p* values were presented as <0.05, and <0.01. If >0.05, results were indicated as “not significant”.

RESULTS

Whereas in acute slice, the NAc neurons seldom exhibit the upstate-downstate cycling, our recording shows that ~50% of NAc neurons (n=9/20) exhibited this bimodal membrane property in one-day old slice cultures (Fig1. A,B). Two-Gaussian analysis reveals that the membrane potential of these bimodal NAc neurons primarily distributed at two different voltages, the relatively hyperpolarized voltage (-76 ± 7 mV, n=6) and the relatively depolarized voltage (-55 ± 6 mV, Fig. 1C). This pair of membrane potentials is similar to the upstate and downstate membrane potentials observed *in vivo* [10, 13]. Because NAc neurons in acute brain slice do not exhibit the bimodal property, the bimodal property of NAc neurons in 1-day slice culture must be ‘regained’ within the *in vitro* environment. To monitor the recovery process, we recorded NAc neurons at three time points (1, 6, and 24 hrs) in culture. At the time point 1 hr, none of the recorded neurons (n=21) exhibited upstate activity. At the time point 6 hrs, one out of twenty recorded neurons exhibited clear downstate-upstate oscillations. In addition, four recorded neurons (n=4/20) exhibited bursting, upstate-like membrane depolarization (Fig. 1D,E). During these bursting activities no action potentials were observed. We operationally defined these bursting, upstate-like neurons as bursting neurons. At the time point 24 hrs, half of the recorded NAc neurons (n=12/24) exhibited clear downstate-upstate oscillations and seven neurons exhibited the bursting membrane property (Fig. 1A, B). These results suggest: 1) the bimodal membrane property of NAc neurons is gradually regained after being lost through the slice preparation procedure; 2) the bursting neurons appear to be the neurons that are transforming to the bimodal neurons; and 3) given that no other interferences are involved, the recovery of bimodal property is likely due to a homeostatic process that occurs within the intracellular or extracellular microenvironment of NAc neurons.

One potential mechanism through which NAc neurons restore their bimodal membrane property is the change of intrinsic membrane properties, which may lead to periodic oscillation of membrane potential. Indeed, it has been shown that the striatal neurons, a type of neurons geographically adjacent and electrophysiologically similar to NAc neurons, express a set of voltage-gated ion channels that may generate rhythmic membrane oscillation [25]. It is possible that the ion channel-based rhythmic mechanism is homeostatically activated/potentiated in NAc neurons during the course of slice culture. To test this possibility, we used whole-cell current-clamp technique to examine the intrinsic membrane property of NAc neurons at two time points (1 and 24 hrs) in slice culture. In the test of passive membrane properties, we ran a step protocol to measure the current-voltage relationship (I-V curve) of NAc neurons and found that the I-V curve, and thus the input resistance, was not significantly affected during the course of slice culture ($p>0.05$, ANOVA, Fig. 2A, B). We then measured the positive membrane property by running a set of depolarizing current steps. These current steps evoked action potential firing in NAc neurons, which is often used to represent the general intrinsic membrane excitability. The number of evoked action potentials of NAc neurons was not significantly different between the two measuring time points ($p>0.05$ ANOVA, Fig. 2C, D). These results suggest that intrinsic membrane property is not the primary expression site for the homeostatic mechanism to restore the bimodal property of NAc neurons.

Another potential mechanism that can restore the bimodal property of NAc neurons is the homeostatic change in synapses; if excitatory synaptic input is gradually increased and synchronized, it will result in a summated depolarization plateau of the membrane potential (i.e., the upstate potential). To examine this possibility, we used whole-cell current-clamp technique to monitor spontaneous excitatory postsynaptic potentials (sEPSPs) at three time points, 1, 6, and 24 hrs. Because the sEPSPs in NAc slice culture were so frequent and heavily overlapped, measuring the frequency and amplitude of individual sEPSPs was technically prohibitive. Instead, we measured the integrated area, defined as the net change of membrane potential (active membrane potential - baseline potential) times the unit period of time (10-sec). The integrated area gradually increased when measured at the time points 1, 6, and 24 hr in culture, indicating that the spontaneous synaptic activities (generated by spontaneous presynaptic release of glutamate) increased progressively over the course of slice culture (Fig. 3A,B). These results suggest that the gradual increase in the intensity and synchronicity of spontaneous excitatory synaptic activities may mediate the homeostatic restoration of the upstate of NAc neurons.

Is the potentiated synaptic activity in cultured NAc neurons mediated by increased efficacy of individual synapses, or by increased spontaneity and synchronicity of total synapses without changes in the efficacy of individual synapses? To address this question, we examined the efficacy of basic excitatory synaptic transmission. We first used whole-cell current-clamp technique to measure miniature (m) EPSPs (in TTX) in the NAc neurons at the three time points and found that neither the amplitude (in mV: 1 hr, 2.31 ± 0.58 , $n=6$; 6 hr, 2.45 ± 0.95 , $n=7$; 24 hr, 2.02 ± 0.27 , $n=6$) nor the frequency (in Hz: 1 hr, 27.4 ± 5.1 , $n=6$; 6 hr, 24.2 ± 7.8 , $n=7$; 24 hr, 20.7 ± 9.2 , $n=6$) of mEPSPs was different (Fig. 1A,C,D). We then used whole-cell voltage-clamp technique to examine the paired pulse ratio (PPR) of EPSCs. The PPR (interpulse interval: 50-ms) of EPSC in NAc neurons also remained unchanged along the course of slice culture (1 hr, 1.05 ± 0.32 , $n=9$; 24 hr, 1.02 ± 0.19 , $n=9$, Fig. 3E,F). Finally, we measure the ratio of AMPAR EPSC:NMDAR EPSC (AMPA:NMDAR ratio), a parameter that reflects the postsynaptic composition of glutamatergic receptors. The peak AMPAR EPSCs was measured at the holding potential of -70 mV and the peak NMDAR EPSCs was measured at the holding potential of +40 mV in the presence of NBQX (5 μ M), an AMPAR-selective antagonist. No change was observed in AMPAR:NMDAR ratio in NAc neurons between the time points 1 and 24 hrs (1 hr, 2.15 ± 0.34 , $n=9$; 24 hr, 2.21 ± 0.32 , $n=9$, Fig. 3G,H). These results suggest that the basic properties of individual excitatory synapses (presynaptic quantal release and postsynaptic responsiveness) are not altered along the course of culture. Thus, the recovery of upstate in NAc neurons in slice cultures is likely mediated by homeostatic increases of the spontaneity and synchronicity of total presynaptic terminals.

DISCUSSION

Homeostatic recovery of neuronal function has been widely observed in both human and animal models following a variety of brain damages, but the underlying cellular mechanisms have not been well understood. Our current study demonstrates that NAc neurons can regain an important *in vivo* electrophysiological property within 24 hours *in vitro*. Furthermore, our results suggest that this recovery process is mediated by homeostatic changes in presynaptic activities. Below, we discuss five specific points related to the slice culture preparation, which help better understand this novel form of homeostatic plasticity.

First, is the upstate of NAc neurons in slice culture driven by synchronized glutamatergic synaptic inputs? Results from our recent work [8] demonstrate that similar to that observed *in vivo* [11-14,18], application of glutamate receptor antagonists abolishes the upstate of NAc neurons in slice cultures, suggesting that glutamatergic input is the key driving force for downstate-upstate transition in cultured NAc neurons. Furthermore, in a subset of slice cultures

in which cortical regions were completely eliminated, upstates were still present (data not shown). Thus, synchronization of synaptic activity should happen at the synaptic terminals that were truncated from their cell bodies. It is, however, possible that cortical neurons, when present, contribute to the generation of upstate in NAc neurons; it has been reported that in the slice preparations, cortical neurons often automatically generate rhythmic activations [20-22], and thus may rhythmically activate NAc neurons.

Second, are truncated presynaptic terminals functional after 24 hrs *in vitro*? Indeed, the truncated axons from projecting glutamatergic neurons are well preserved and well functional, evidenced as that both evoked and spontaneous glutamatergic synaptic transmission can be recorded from long-held NAc slices; these synaptic transmissions are not only stable, but also subject to experience-dependent plastic changes, indicating that the truncated axons can still maintain sufficient nutritional and functional activities [1,5,8]. Furthermore, because the glutamatergic inputs from cortical and subcortical regions are highly convergent at *proximal* dendrites of NAc neurons [31,32], the glutamatergic terminals preserved in the thin NAc slices should originate from most projection regions.

Third, how can the truncated glutamatergic terminals survive in the 1-day NAc slice culture? Local protein synthesis and protein translocation occur within axons and dendrites after being truncated from their cell bodies [9,29], suggesting that some basic biochemical and biophysical properties of neural processes are maintained locally. Moreover, the truncated terminals reseal their membrane at the broken site such that the cell contents are preserved. These so-called pseudo-cells have been thoroughly examined in retina slices, because such pseudo-cells from bipolar cells are big enough for ‘whole-cell’ recordings. A number of studies show that the bipolar pseudo-cells have resting membrane potentials, can be hyperpolarized and depolarized, and maintain their local networks and basic functions [19,26]. Thus, the glutamatergic synaptic transmission observed in our NAc slice cultures is likely to be generated from the preserved glutamatergic terminals that were truncated from their cortical and subcortical origins.

Fourth, Can action potentials occur spontaneously in the truncated glutamatergic terminals? sEPSPs are generated by both action potential-dependent and independent presynaptic activities. When action potentials were prevented (in TTX), sEPSPs (or more accurately, mEPSPs, under this condition) became much less active (Fig. 3). These observations indicate that action potentials can be spontaneously generated in the presynaptic terminals in NAc slice culture.

Fifth, do any other processes contribute to the homeostatic recovery of the upstate in NAc neurons in slice cultures? Homeostasis is one of the most complicated physiological properties that often simultaneously involve numerous factors. Thus, in addition to synchronous presynaptic activity, other cellular processes may also be involved in homeostatic recovery of the upstate in NAc neurons. Lobster stomatogastric ganglion neurons (SGN) *in vivo* normally exhibit rhythmic action potential bursting when receiving synaptic input. This synaptically driven membrane oscillation of SGNs is very similar to the bimodal membrane property of the NAc neurons. When these SGNs are dissociated and their synaptic inputs are removed, their rhythmic membrane property is lost. But after 77 hrs of incubation with culture medium, the rhythmic membrane property recovers through homeostatic adaptation of intrinsic membrane property [23]. However, this membrane-mediated homeostatic compensation of synaptic input is not the case in NAc neurons; our results show that the intrinsic membrane property of NAc neurons remained largely unaltered (Fig. 2). Other possible, yet untested, mechanisms include changes in glutamatergic transporter, re-organization of presynaptic terminals, and activation of other neurotransmitter systems (e.g. acetylcholine or dopamine).

Functional implication

The NAc is an essential brain site that mediates reward, emotion and motivation. It is generally believed that the *in vivo* synchronization of excitatory synaptic input to NAc starts from the cell bodies of projecting neurons [12]. Alternatively, our results show a locally occurring, terminal-based synchronizing mechanism. This novel mechanism may potentially play two important roles in maintaining the stable functional output of NAc neurons: 1) Results from *in vivo* studies show that experimental activation of projecting somas produces upstate or upstate-like membrane oscillations in some, but not all, NAc neurons [12]. Thus, the terminal-based synchronizing mechanism may complement the soma-based mechanism to achieve upstate of overall NAc neurons; 2) under certain pathological conditions when soma-based synchronizing mechanism is disabled (e.g., disruption of the projecting regions), terminal-based mechanism can be activated to restore the basic function of NAc neurons.

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FIG. 1.

The NAc neurons gradually restored the bimodal property in vitro. **A** An example NAc neuron showing that the bimodal membrane property was restored in one-day-old slice cultures. **B** The upstate membrane potential and action potential firing during the upstates in a slower time course. **C** Membrane potential of the bimodal neuron in **A** was fit by two-Gaussian distribution (gray area). **D** Examples traces showing progressive increase in the number of both bursting neurons and upstate neurons over the course of slice culture. **E** Summary showing progressive increase in the number of bimodal neurons over the course of slice culture. **F** Summary showing progressive increase in the number of bursting neurons over the course of slice culture.

FIG. 2.

The intrinsic membrane property was not affected during slice culture. **A** Example membrane properties of NAc neurons from 1-hr (left) and 24-hr-old (right) NAc slice cultures. **B** Summary showing that the input resistance of NAc neurons was not significantly affected over the course of slice culture. **C** Example traces of evoked action potential firing recorded from NAc neurons from 24-hr-old slice cultures. **D** Summary showing that the evoked action potential firing of NAc neurons was not affected over the course of slice culture.

FIG. 3.

Spontaneous presynaptic release, but not the efficacy of individual excitatory synapses, was upregulated during the course of slice culture. **A** Example spontaneous (s) and miniature (m) EPSPs in NAc neurons over the course of slice culture. Dashed lines represent the base lines that were used to calculate the integrated area. **B** Summary showing that the integrated area of sEPSPs in NAc neurons was progressively increased over the course of slice culture. **C** Summary showing that the amplitude of mEPSPs in NAc neurons was not altered over the course of slice culture. **D** Summary showing that the frequency of mEPSPs in NAc neurons was not altered over the course of slice culture. **E** Example paired-pulse (interpulse interval: 50 ms) induced AMPAR EPSCs in NAc neurons from 1-hr (left) and 24-hr-old (right) slice

cultures. **F** Summary showing that paired-pulse ratio (PPR) was not altered over the course of slice culture. **G** Example AMPAR and NMDAR EPSCs in NAc neurons from 1-hr (left) and 24-hr-old (right) slice cultures. Dashed lines represent baselines used for measuring the peak of EPSCs. **H** Summary showing that the ratio of AMPAR EPSC:NMDA EPSC in NAc neurons was not altered over the course of slice culture.