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Homeostatic recovery of downstate–upstate cycling in nucleus accumbens neurons

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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.

Keywords: Nucleus accumbens; Upstate; Homeostatic plasticity

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 nucleus accumbens (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 [13,30]. 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

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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.

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-μm 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 h 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.

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 ms 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 $GABA_A$R-mediated currents. Stimuli were applied through bipolar stainless steel electrodes, which were placed in the dorsolateral region of the recorded neurons (~100 μm). Stimulation parameters included: frequency, 0.1 Hz; intensity, 50–200 μA; duration, 0.1 ms. 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].

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

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".

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 1-day-old slice cultures (Fig. 1A and 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 h) in culture. At the time point 1 h, none of the recorded neurons ($n = 21$) exhibited upstate activity. At the time point 6 h, 1 out of 20 recorded neurons exhibited clear downstate–upstate oscillations. In addition, four recorded neurons ($n = 4/20$) exhibited bursting, upstate-like membrane depolarization (Fig. 1D and 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 h, half of the recorded NAc neurons ($n = 12/24$) exhibited clear downstate–upstate oscillations and seven neurons exhibited the bursting membrane property (Fig. 1A and 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 h) 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 and 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 and 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