Intrinsic voltage dynamics govern the diversity of spontaneous firing profiles in basal forebrain noncholinergic neurons

Saak V. Ovsepian,1,2 J. Oliver Dolly,2 and Laszlo Zaborszky1
1Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, New Jersey; and 2International Center for Neurotherapeutics, Research and Engineering Building, Glasnevin, Dublin City University, Dublin, Ireland

Submitted 8 July 2011; accepted in final form 7 April 2012

Ovsepian SV, Dolly JO, Zaborszky L. Intrinsic voltage dynamics govern the diversity of spontaneous firing profiles in basal forebrain noncholinergic neurons. J Neurophysiol 108: 406 – 418, 2012. First published April 11, 2012; doi:10.1152/jn.00642.2011.—Spontaneous firing and behavior-related changes in discharge profiles of basal forebrain (BF) neurons are well documented, albeit the mechanisms underlying the variety of activity modes and intermodal transitions remain elusive. With the use of cell-attached recordings, this study identifies a range of spiking patterns in diagonal band Broca (DBB) noncholinergic cells of rats and tentatively categorizes them into low-rate random, tonic, and cluster firing activities. It demonstrates further that the multiplicity of discharge profiles is sustained intrinsically and persists after blockade of glutamate-, glycine/GABA-, and cholinergic synaptic inputs. Stimulation of muscarinic receptors, blockade of voltage-gated Ca2+-, and small conductance (SK) Ca2+-activated K+ currents as well as chelating of intracellular Ca2+ concentration accelerate low-rate random and tonic firing and favor transition of neurons into cluster firing mode. A similar trend towards higher discharge rates with switch of neurons into cluster firing has been revealed by activation of neuropeptide Y (NPY) receptors with the NPY or NPY, receptor agonist [Leu31 Pro34]-NPY. Whole cell current-clamp analysis demonstrates that the variety of spiking modes and intermodal transitions could be induced within the same neuronal population by injection of bias depolarizing or hyperpolarizing currents. Taken together, these data demonstrate the intrinsic and highly variable characteristic of regenerative firing in BF noncholinergic cells, subject to powerful modulation by classical neurotransmitters, NPY, and small membrane currents.

neuropeptide Y; modulation; pacemaker; spontaneous activity


The present study analyses the spontaneous firing of noncholinergic cells in the diagonal band Broca (DBB) in acute brain slices. It shows that the variety of outputs in these putative GABA- or possibly also glutamatergic) neurons is generated intrinsically, subject to modulation by synaptic inputs, intracellular Ca2+ concentration ([Ca2+]i), apamin-sensitive Ca2+ activated K+ currents (I\textsubscript{K,Ca}), and neuropeptide Y.

Address for reprint requests and other correspondence: S. V. Ovsepian, International Center for Neurotherapeutics, Research and Engineering Bldg., Glasnevin, Dublin City Univ., Dublin 9, Ireland (e-mail: saak.ovsepian@gmail.com).
(NPY). It also demonstrates that various discharge patterns with intermodal transitions can be induced within the same neuronal population through biasing their membrane potential with small depolarizing or hyperpolarizing currents.

METHODS

Prelabeling of DBB cholinergic neurons with Cy3-IgG192. Experiments were performed in accordance with the U.S. Public Health Service Policy on Human Care and Use of Laboratory Animals and the National Institute of Health and approved by the Rutgers University Institutional Board. Under deep anesthesia (ketamine: 90 mg/kg; xylazine: 10 mg/kg ip), Cy3-192IgG (2–4 μl; 0.4 mg/ml; 0.5 μl/min) was stereotaxically injected into the lateral ventricles of juvenile Sprague-Dawley rats (postnatal 12–14 days old) with a Hamilton syringe (22-gauge needle). Because BF cholinergic cells are the only forebrain neurons expressing low-affinity nerve growth factor receptor p75, Cy3-192IgG-injected animals were killed and used for electrophysiological recordings.

Slice preparation with identification and recordings from noncholinergic DBB cells. Under deep anesthesia (with ketamine: 120 mg/kg; xylazine: 10 mg/kg), the Cy3-192IgG-preinjected brain was decapitated and the brain was removed and placed in low Na⁺, low Ca²⁺, high Mg²⁺ containing ice-cold artificial cerebrospinal fluid (slicing aCSF) of the following composition (in mM): 75 sucrose, 85 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, 4 MgCl₂, and 25 glucose, with pH 7.4, continuously bubbled with 95% O₂-5% CO₂.

With the Vibrotome Series 1000 (St. Louis, MO), forebrain segment containing DBB was sliced (300 μm, coronal plane) and tissue was transferred for a 30-min incubation at 32°C (95% O₂-5% CO₂, 50 M KCl, 0.5% sodium azide). After the slicing procedure, the tissues were placed in low Na⁺, low Ca²⁺, high Mg²⁺ containing aCSF containing the following (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, 2 MgCl₂, and 25 glucose, with pH 7.4, continuously bubbled with 95% O₂-5% CO₂. ChAT- and Cy3-positive neurons of the DBB nucleus, and hyperpolarizing current pulses (30-pA increments; 1s) from hyperpolarized holding potentials, which was maintained closed to −65 mV by steady current passed through the patch pipette. For measurements of the action potential voltage peak and after-hyperpolarizing potentials (AHPs), a reference voltage was defined during the upstroke where dV/dt exceeds 5 V/s (see Fig. 2, A2 and B2). This voltage was also taken as a voltage threshold for generation of action potentials in response to depolarizing current injection (Osepeian and Friel 2008) (IgorPro, 6.01; WaveMetrics, Lake Oswego, OR). Action potential width was evaluated as the width of the spike waveform at half-amplitude, measured from the same reference voltage. Stretches of spike trains of ≥5-min duration were used for assessments of spontaneous firing parameters with threshold crossing macro (Clampfit 10.0; Molecular Devices). Action potential discharge rate and regularity [interspike interval variation coefficient (ISI CV)] were measured for each individual cell before and after treatment. Firing was defined as tonic if two or more action potentials (range 2–31) were generated per second with maximum ISI variability (ISI standard deviation) not exceeding 50% of the mean ISI. Cluster firing was defined empirically as spike sequences (>3 action potentials; typically 5–9) with individual action potentials interspersed by periods of quiescence, which exceeded at least three times the mean ISIs within the spike packs (clusters). Profiles not meeting these criteria were considered as low-rate random firing.

Double labeling of DBB cholinergic neurons and fluorescence microscopy. Experimental procedures conformed to the guidelines approved by the Dublin City University Ethics Committee under the license of the Department of Children and Health, Ireland. The surgical procedure (n = 3) and lateral ventricular injections of Cy3-IgG192 (n = 2) or saline (n = 1) were performed as described above; animals were allowed to recover for 2 days. On the third day, they were deeply anesthetized with sodium pentobarbital (Euthatal, Pfizer; 200 mg/kg ip) and perfused transcardially with 150 ml 0.1 M PBS, pH 7.4, followed by a 100 ml 4% paraformaldehyde, pH 7.4. The brains were removed from the skull and postfixed in 4% paraformaldehyde, pH 7.4, at 4°C overnight, cryoprotected with 30% sucrose solution in 0.1 M PBS, and sectioned (coronal, 35-μm thick) on freezing microtome (Leica, CM3050 S). The free-floating sections from genu of corpus callosum to the crossing of anterior commissure were collected in PBS, washed three times, and incubated in 0.4% Triton X-100 for an hour in the dark box at room temperature. After three thorough washes, the tissue was incubated in blocking solution containing 5% BSA and 2% rabbit serum for 1 h followed by incubation in anti-choline acetyltransferase (ChAT) polyclonal antibody (Millipore; AB144P) in 1:100 dilutions in the same blocking solution with 0.4% Triton X-100 in 0.1 PBS, overnight in the dark box (room temperature). Following five rounds of wash with 0.1 PBS (5 min each), sections were incubated in secondary rabbit anti-goat polyclonal antibody (1:1000 dilutions) labeled with FITC for 2 h. This was followed by thorough rinsing of the tissue in 0.1 PBS and mounting on charged glass slides. After being air-dried, slices were covered with Vectashield hardest medium. The internal control for the staining was carried out with omission of primary antibody. Field micrographs were obtained (×40 objective) using laser scanning microscope in epifluorescence mode (pinhole wide open; AxioObserver, Carl Zeiss). Argon and helium/neon lasers provided the 488 and 568 nm lines for excitation. The emitted signals were sampled in 407 intrinsically firing of noncholinergic BF neurons

The brains were removed from the skulls and postfixed in 4% paraformaldehyde, pH 7.4, followed by a 100 ml 4% paraformaldehyde, pH 7.4. The brains were removed from the skulls and postfixed in 4% paraformaldehyde, pH 7.4, at 4°C overnight, cryoprotected with 30% sucrose solution in 0.1 M PBS, and sectioned (coronal, 35-μm thick) on freezing microtome (Leica, CM3050 S). The free-floating sections from genu of corpus callosum to the crossing of anterior commissure were collected in PBS, washed three times, and incubated in 0.4% Triton X-100 for an hour in the dark box at room temperature. After three thorough washes, the tissue was incubated in blocking solution containing 5% BSA and 2% rabbit serum for 1 h followed by incubation in anti-choline acetyltransferase (ChAT) polyclonal antibody (Millipore; AB144P) in 1:100 dilutions in the same blocking solution with 0.4% Triton X-100 in 0.1 PBS, overnight in the dark box (room temperature). Following five rounds of wash with 0.1 PBS (5 min each), sections were incubated in secondary rabbit anti-goat polyclonal antibody (1:1000 dilutions) labeled with FITC for 2 h. This was followed by thorough rinsing of the tissue in 0.1 PBS and mounting on charged glass slides. After being air-dried, slices were covered with Vectashield hardest medium. The internal control for the staining was carried out with omission of primary antibody. Field micrographs were obtained (×40 objective) using laser scanning microscope in epifluorescence mode (pinhole wide open; AxioObserver, Carl Zeiss). Argon and helium/neon lasers provided the 488 and 568 nm lines for excitation. The emitted signals were sampled in a frame mode at spatial resolution of 30-nm per pixel with 1.5-μs dwell time. ChAT- and Cy3-positive neurons of the DBB nucleus, which in rat anatomically extends between septum and caudal exten-
sion of the horizontal limb of the DBB bordering with magnicellular preoptic area (Zaborszky et al. 1999), were sampled for current analysis. Neuronal cell bodies were counted within defined subregions of the DBB nucleus and tabulated for analysis. Colocalization of Cy3 and FITC is estimated based on the presence of two labels within the same pixel of digitally acquired images, using colocalization macro (Zen 2008).

Data analysis and statistical significance. Data are reported as means ± SE with statistical significance estimated using paired or unpaired Student’s t-test. In experiments involving multiple-repeated comparisons, one-way ANOVA was applied for significance assessment. The difference between samples was defined as significant if P value was <0.05.

Drugs and chemicals. All chemicals and drugs were obtained from Sigma (St. Louis, MO) except Cy3-IgG192, which was purchased from Advanced Targeting Systems, and NPY, NPY1, and NPY5 receptor agonists [Leu31,Pro34] NPY and [D-TRP34]-NPY, which were obtained from Tocris (Tocris Biosciences). Secondary rabbit anti-goat polyclonal antibody labeled with FITC was purchase from Abcam.

RESULTS

Intraventricular injection of Cy3-IgG192 labels exclusively DBB cholinergic neurons. Numerous Cy3-labeled neurons were visible in medial septum, in DBB, in ventral pallidum, and in more caudal BF nuclei in fixed brain slices of Cy3-IgG192 injected animals. At higher magnification, distinctly punctuate intracellular presence of Cy3 was evident, consistent with its uptake and concentration in endosomal compartments (Hartig et al. 1998; Kacza et al. 2000). Immunostaining of MS and DBB containing slices for ChAT revealed vast majority of DBB ChAT-positive profiles being also labeled with Cy3-IgG192 (97.4 ± 1.9%; Fig. 1, A–C). Similarly, almost all Cy3-IgG192-positive profiles in the DBB were also labeled positive for ChAT (98.7 ± 1.1%; Fig. 1C). These data are consistent with an high specificity of Cy3-IgG192 as a marker for labeling cholinergic neurons in DBB nuclei and agree with earlier studies, showing ~100% in situ colabeling of ChAT-positive cells with Cy3-IgG192 in rostral BF nuclei (Hartig et al. 1998). Congruently, whole cell recordings from DBB Cy3-IgG192-positive and -negative neurons in 300-μm slices (n = 6 and n = 11, respectively) revealed characteristics electrophysiological profiles (Fig. 2, A and B, and Fig. 3, A–C) with hyperpolarization-activated inward rectifier current seen in all but only Cy3-IgG192-positive neurons (6/6, 100%; Fig. 2A). In contrast, the majority of Cy3-unlabeled (8/11, ~72%) cells responded to hyperpolarizing stimuli by delayed depolarizing sag potential, attributed to slow activating Ih current (Fig. 2B), with the rest exhibiting relatively linear I/V relation within negative potential ranges. Differences were also revealed in responses of these cells to suprathreshold depolarizing stimuli, with all Cy3-IgG192-positive cells exhibiting a characteristic shoulder (outward rectification) before the onset of action potentials firing from more depolarized voltages (voltage threshold: −32.4 ± 2.1 mV vs. −37.3 ± 1.8 mV; P = 0.021) with action potentials being slower (half width: 0.54 ± 0.03 ms vs. 0.31 ± 0.02 ms; P < 0.001) and followed by more prominent AHPs (19.5 ± 3.1 mV vs. 4.2 ± 1.2; P < 0.001; Fig. 2, A and B). These observations verify the adequacy of Cy3-IgG192 in vivo prelabeling as a reliable method for identification of noncholinergic neurons in the DBB. It should be emphasized, however, that Cy3-IgG192 cannot be applied with the same certainty for differentiating cholinergic from noncholinergic cells in more caudal nuclei of the BF, due to the fact that ChAT-positive profiles therein do not always express p75 nerve growth factor receptor (Ferreira et al. 2001; Nickerson Poulin et al. 2006).

Spontaneous firing activity of DBB noncholinergic neurons in acute slices. Cell-attached recordings were used to examine the spontaneous firing activity of medium and large size Cy3-IgG192-negative neurons within vertical and horizontal limbs of DBB (Fig. 3, A–C). The vast majority of tested cells (108 from 116, 93.1%) showed continuous firing during entire recording session. Consistent with single unit in vivo data (see Discussion), noncholinergic BF neurons in slice revealed a variety of discharge profiles, which were tentatively classified in three categories. The bulk (74/108; 68.5%) exhibited tonic firing (discharge range: 2–31 Hz; means ± SE: 9.18 ± 1.2 Hz), while the rest generated action potentials randomly (12/108; 11.1%) at relatively lower rates (discharge range: 0.1–5.2 Hz; means ± SE: 3.1 ± 0.7 Hz) or fired spikes in clusters (22/108; 20.3%; range: 15–45 Hz; means ± SE: 20.2 ± 2.4; Fig. 3, D–G). Comparison of the discharge rates revealed significant differences between three profiles (P < 0.0001, one-way ANOVA). The heterogeneity of firing patterns was reflected also in ISI distribution histograms, with a relatively regular firing tonic profiles yielding Gaussian shaped ISI distribution histograms, contrasting to skewed ISI distribution of random or cluster firing cells (Fig. 3, E–H). Accordingly, the ISI CV was the lowest in tonic (means ± SE: CV = 0.22 ± 0.1) followed by random (means ± SE: CV = 0.58 ± 0.13) and being the
highest in clusters firing profiles (means ± SE: CV = 0.86 ± 0.14; P = 0.00032, one-way ANOVA; Fig. 3H). Thus, in the absence of long-range synaptic inputs in acute brain slices, DBB neurons discharge spontaneously, similar to single units of BF shown in vivo (Zaborszky and Duque 2003).

GABAergic synaptic inputs modulate the output of DBB noncholinergic neurons. To establish if main synaptic inputs contribute to ongoing firing of DBB neurons, the effects of glutamate, GABA/glycine, and acetylcholine receptor blockers on spiking of these cells were examined. Application of broad spectrum glutamate and GABA/A/glycine receptor blockers (5 mM kynurinate and 200 μM picrotoxin, respectively) (Altman et al. 1976; Stone 1993) caused notable discharge acceleration in tonic firing profiles (n = 9; rate increase: 32.5 ± 6.2%, P = 0.00052) with reduction in ISI CV (P < 0.0001; Fig. 4, A1 and A2). These changes were visible in ISI distribution histograms, which become narrower and shifted towards lower ISIs (Fig. 4A2). Similar treatment induced discharge acceleration and ISI CV reduction in random and cluster firing neurons, albeit these changes reached statistical significance only in the first group (n = 4, rate increase: 41.2 ± 12.1%, P = 0.032; n = 5, rate increase: 17.3 ± 9.1%, P = 0.25; not shown). Next, relative contribution of inhibitory inputs to modulation of spontaneous firing was assessed, given that neurons in rostral BF receive intense GABA/glycinergic synaptic inputs (Segal 1986; Bengtson and Osborne 2000). Markedly, picrotoxin, a potent blocker of GABA/glycine-activated Cl⁻ channels fully replicated the acceleration of spontaneous firing with ISI CV reduction produced by its coapplication with kynurinate (n = 5; firing rate increase: 38.5 ± 5.2%, P = 0.00039; 3.2-fold reduction in ISI CV, P < 0.0001; Fig. 4, B1 and B2). Similar experiments with only kynurinate revealed no alterations in the rate and regularity of spontaneous firing (rate: P = 0.48; CV: P = 0.51; n = 4). These data are consistent with substantial inhibitory but not excitatory synaptic activity in DBB noncholinergic cells in acute brain slices. As noncholin-
ergic cells receive profuse innervations from local cholinergic neurons (Zaborszky et al. 1986, 1999; Brauer et al. 1998), next the effects of blockade of muscarinic-receptor mediated endogenous cholinergic drive with atropine (n/H11005) and activation of muscarinic cholinergic receptors with muscarine (n/H1100510) were assessed in the presence of kynurinate and picrotoxin. No changes in discharge rate or regularity of tonic (n/H110055, P/H110050.78) and cluster firing (n/H110054, P/H110050.31) cells were revealed upon application of atropine (10 nM/H9262) (Morton and Davies 1997). In contrast, both tonic (discharge rate increase: 32.4 ± 5.1%; n = 7, P = 0.029) and cluster firing (discharge rate increase: 24.4 ± 4.6%, n = 3, P = 0.008) were accelerated after exposure of slices to muscarine (20 μM) (Peinado 2000).

Voltage-gated ICa 2+ and apamin-sensitive IK Ca stabilize the intrinsic firing activity of noncholinergic DBB neurons. Next, the role of voltage-activated Ca 2+ (ICa 2+) and IK Ca in regulating the spontaneous firing of noncholinergic DBB cells was examined after pharmacological blockade of ionotropic glutamatergic and GABA/glycinergic synaptic inputs. Inhibition of high- and low-voltage activated ICa 2+ by cobalt (CoCl 2, 100 μM) caused a rapid discharge rate increase and switched tonic firing cells into cluster firing mode (10 min after treatment: firing rate increase: 134.6 ± 11.6%; CV increase: 4.3-fold; P < 0.0001; n = 9; Fig. 5, A1 and A2). In seven neurons, high rate irregular firing induced by cobalt persisted during the entire (15 min) recording session, while in two cells, discharge acceleration was followed by cessation of firing (not shown). The effects of cobalt were mimicked by another inorganic voltage-activated ICa 2+ blocker cadmium (CdCl 2, 200 μM; n = 5, 10 min after treatment: firing rate increase: 126.7 ± 16.2%; CV increase: 3.6-fold; P < 0.0001), as well as by a selective small-conductance IK Ca (SK) channel inhibitor apamin (50 nM; n = 6; 10 min after treatment: firing rate increase: 89%; CV
increase: 3.2-fold; \( P < 0.0001 \); Fig. 5B), suggesting a stabilizing role of high-voltage activated \( I_{Ca^{2+}} \) and SK \( I_{KCa} \) on firing activity of BF noncholinergic neurons. The stabilizing role of \( Ca^{2+} \) on intrinsic pacemaking was further confirmed by whole cell current clamp recordings through dialysis of neurons with a rapid \( Ca^{2+} \) chelator BAPTA (20 mM). Introduction of BAPTA via patch pipette rapidly accelerated the spontaneous spiking of noncholinergic neurons \(( n = 7 \); firing rate increase: 180.6 ± 19.4% at 25–35 s after breaking the membrane; \( P < 0.0001 \)) and switched tonic firing cells into high-frequency cluster firing activity mode (Fig. 5, C–E). Similar to some of cobalt-treated cells, acceleration of firing caused by BAPTA was followed by cessation of spiking, with membrane potential settling at depolarized voltages \((-42.5 \pm 2.1 \text{ mV}, n = 7 \) ). Intriguingly, in five cells silenced by BAPTA, firing could be restored by injection of hyperpolarizing current (Fig. 5E). Collectively, these findings demonstrate that both the rate and pattern of spontaneous firing in DBB noncholinergic neurons are under control of inhibitory synaptic inputs, voltage-activated \( I_{Ca^{2+}} \), SK \( I_{KCa} \), and intracellular [Ca\(^{2+}\)] (Fig. 5F).

NPY and [Leu\(^{31}\),Pro\(^{34}\)] NPY accelerate spontaneous firing of noncholinergic cells. The presence of NPY-positive neurons in the BF and modulation of cortical activity by this peptide when injected into the BF (Zaborszky and Duque 2003; Duque et al. 2007; Toth et al. 2007) encouraged us to investigate its potential role in governing the discharge characteristics of BF cholinergic (Zaborszky et al. 2009) and noncholinergic cells. Of the 6 NPY receptor subtypes (NPY1–6), NPY1 and NPY5 are shown to be residing at postsynaptic elements, where they are negatively coupled to voltage-gated Ca\(^{2+}\) currents (Gehlert 1994; McQuiston et al. 1996; Lin et al. 2003). In the presence of kynurinate and picrotoxin, application of NPY (0.5 \( \mu \text{M} \)) caused gradual discharge acceleration in noncholinergic neurons (in 8 of 9 tested cells, firing rate increase: 67.7%; \( P = 0.0016 \)), an effect accompanied with an increase in ISI CV \(( P = 0.0003 \) ) and emergence of spike clusters (Fig. 6A). In a similar way, the effects of NPY\(_1\)-
receptor-selective agonist [Leu<sup>31</sup>,Pro<sup>34</sup>]-NPY (0.5 μM) (Klapstein and Colmers 1997) and NPY<sub>5</sub>-receptor-selective agonist [D-TRP<sup>34</sup>]-NPY, NPY<sub>5</sub> (0.5 μM) (Pronchuk et al. 2002) were tested (Fig. 6, B and C). Like NPY, application of [Leu<sup>31</sup>,Pro<sup>34</sup>]-NPY caused firing acceleration (in 7 of 8 tested cells, firing rate increase: 67.7%; P < 0.0019) with transition of neurons into cluster spiking mode. These changes were associated with enhancement of ISI CV (P < 0.00012), reflected in leftward shift of the ISI distribution histogram (Fig. 6B). Alterations of spiking caused by NPY and [Leu<sup>31</sup>,Pro<sup>34</sup>] contrasted to the lack of visible effects on spontaneous firing rate and regularity in slices exposed to NPY<sub>5</sub>-receptor agonist [D-TRP<sup>34</sup>]-NPY (n = 8; rate change: P = 0.54; ISI CV change: P = 0.32, respectively; Fig. 6C). To establish membrane potential changes associated with NPY-induced acceleration of spiking, the effects of this peptide were examined using whole cell current clamp recordings. From four spontaneously firing Cy3-IgG192-negative cells tested, in three NPY (0.5 μM) caused increase in both discharge rate (72.3 ± 11%; P = 0.0021) and ISI CV (3.4 ± 0.3-fold) associated with a shift of the interspike voltage towards more depolarized potentials and reduction of AHPs (Fig. 7, A and B). These effects reversed only partially after a 15- to 20-min washout (34.1 ± 9%; 2.2 ± 0.2-fold) of the peptide. Overall, these findings suggest that the majority of noncholinergic DBB cells are responsive to NPY, which via stimulation of NPY<sub>1</sub> receptors, accelerates and switches their spiking into cluster firing mode.

Distinct discharge modes of noncholinergic DBB neurons are interconvertible and can be mimicked by injection of bias currents. A key question in BF research is if the variety of single unit discharge profiles documented in vivo reflects
heterogeneity of neuron types rigidly tuned for defined firing patterns or it is an indicative of an array of dynamic states of the same cell population capable of supporting multiple outputs. Our findings show that DBB noncholinergic cells can switch among random, tonic regular, and cluster firing modes. Because intermodal transitions associate with changes in discharge rate and membrane potential, we proposed that the variety of patterns could be induced by biasing the membrane potential of these cells towards more depolarizing or hyperpolarizing voltages. To test this, effects of steady positive or negative currents on firing modes of DBB noncholinergic cells were examined under the blockade of both excitatory and inhibitory synaptic inputs. Figure 8, A–C, illustrates examples of intermodal switch in noncholinergic neuron caused by bias current injection. As shown, tonic regular activity is switched to cluster firing mode by depolarization while constant hyperpolarizing current turns a cluster firing neuron into tonic (Fig. 8, A and B). With such manipulations, a low-rate random firing neurons could be switched into tonic, which in turn converted into cluster firing by injection additional depolarizing current or vice versa (Fig. 8, B and C, and Fig. 9). Similar experiments with low rate random ($n = 5$), tonic ($n = 11$), and cluster firing ($n = 6$) cells demonstrated that variety of functional states are highly dynamic and interconvertible regardless their initial firing profile. Notably, a strong depolarizing current could also mimic effects of BAPTA or cobalt, occasionally causing complete blockade of spiking with emergence of sub-threshold membrane potential oscillations ($n = 11$; Fig. 8D), a feature attributed to BF GABAergic neurons stimulated by prolonged depolarizing currents (Alonso et al. 1996). Taken together, these findings are consistent with dynamic nature of intrinsic pacemaker mechanisms of BF noncholinergic cells, which are likely to contribute to the variety of firing modes described by in vivo studies.
in several brain structures, including hippocampus (Alger and Nicoll 1980; Collingridge et al. 1984; Toth et al. 1997), cerebral cortex (Salin and Prince 1996), BF cholinergic neurons (Khateb et al. 1998), and cerebellum (Ovsepian and Friel 2010), described herein, spontaneously firing noncholinergic cells (108 from 116 cells examined with cell attached recordings exhibited intrinsic firing, 93.1%) could represent the GABAergic neuronal population of the BF. Such conjecture is consistent with a failure of kynurenate to induce notable changes in the firing activity of noncholinergic cells, implying a relatively quiescent state of DBB local glutamatergic cells. It also provides an important clue that might explain the somewhat lower discharge rates of tonic firing noncholinergic cells in slices, compared with those found in vivo (Pang et al. 1998; Duque et al. 2000; Hassani et al. 2010), where long-range excitatory inputs can activate BF noncholinergic neurons. Although it is tempting to speculate that disproportionate effects of picrotoxin on tonic, random, and cluster firing (discharge rate increase: ~32, ~41, and ~17%, respectively) could reflect disproportionate levels of inhibitory drives in these cells, other mechanisms that might also contribute to regulating the efferent outposts of intrinsic activity of noncholinergic neurons should be considered (Jaeger et al. 1997; Gauck and Jaeger 2000). The hyperpolarization-activated cation I, voltage-gated [Ca2+], KCa currents (Griffith 1988; Alonso et al. 1996; Easaw et al. 1997; Bengston and Osborne 2000; Sotty et al. 2003) and persistent Na+ current (Ovsepian SV and Zaborszky L, unpublished data) identified in BF noncholinergic cells are likely to be critical in driving intrinsic activity. The flexible nature with capability to produce a range of activity patterns influenced by neurotransmitters and modulators suggests that both endogenous firing mechanisms and prevailing synaptic inputs would contribute to the ascending noncholinergic drive with important implications for population activity of neurons in recipient cortical fields. Indeed, as demonstrated by us and others, blockade of GABA/glycinergic synaptic transmission or activation of cholinergic or NPY receptors strongly alter the main parameters of spike trains in BF noncholinergic cells.

Intrinsic voltage dynamics can dictate the multiplicity of discharge mode in DBB noncholinergic cells. One of the key findings of this study is that chelation of intracellular [Ca2+] with BAPTA causes membrane depolarization with switch of tonic and low rate irregular firing profiles into cluster firing. A similar trend has also been observed after the blockade of voltage-activated Ca2+ channels, consistent with tight coupling between the endogenous spiking machinery and intracellular [Ca2+] signaling. Our data are consistent with BAPTA (20 mM) (Roussel et al. 2006) being more effective in destabilizing the intrinsic firing machinery of noncholinergic cells, leading to high-frequency cluster firing followed by complete silencing of all tested neurons, compared with inorganic [Ca2+] channel blockers such as cobalt and cadmium, which silenced only a fraction of noncholinergic neurons. A likely explanation for the discrepancy between the effects of BAPTA and [Ca2+] channel blockers could be the additional stabilizing influence of internal-store released [Ca2+] (Velumian and Carlen 1999; Roussel et al. 2006) on regenerative spiking of DBB noncholinergic cells. Indeed, internally applied BAPTA blocks effectively processes relying on cytosolic free [Ca2+] (Velumian and Carlen 1999) while blockers of Ca2+ channels would predominantly suppress functions activated by voltage-gated [Ca2+]...
influx. Comparable depolarizing effects of BAPTA with acceleration of regenerative spiking have been also reported in other central neurons (Williams et al. 2002; Roussel et al. 2006). Intriguingly, through such linkage of intracellular [Ca\(^{2+}\)] with electrogenic machinery of noncholinergic cells, the internal [Ca\(^{2+}\)] stores can play an important role in tuning the basalocortical drive, with direct influence on the dynamics of cortical networks. Because Ca\(^{2+}\) homeostasis in neurons is subject to developmental regulation (Murchison and Griffith 1999, 2007; Toescu and Verkhratsky 2000; Felmy and Schneggenburger 2004; Ovsepian and Friel 2008), noncholinergic ascending drive along with cholinergic inputs (Ovsepian et al. 2004; Hasselmo and Giocomo 2006; Ovsepian 2008) is likely to modulate the state of cortical networks and plasticity mechanisms, with implications for age-related neurodegenerative processes (Harman 2002, 2006; Wojda et al. 2008). In this context, it should be emphasized that several neurotransmitter-activated processes relating Ca\(^{2+}\) signaling and internal store-released [Ca\(^{2+}\)] have been demonstrated in BF neurons (Alreja and Liu 1996; Fort et al. 1998; Wu et al. 2004; Xu et al. 2004). The highly dynamic character of intrinsic firing of these cells was also confirmed through demonstration of the influence of small bias currents on various spiking patterns and their interconvertibility in BF noncholinergic cells, an observation that suggests a continuum rather than multiple neuron types with rigidly defined activity profiles. The latter along with cross-modal flexibility should broaden considerably their tuning curves for a wide range of inputs with the advantage that at any given time broader discharge rates and patterns can be covered by these neurons. Receptive to inputs with more than one variable, greater intrinsic flexibility of noncholinergic cells will therefore also render the population output of these cells less sensitive to the loss of a small number of cells or increase in the level of noise (Lewis and Kristan 1998; Eurich and Wilke 2000; Sanger 2003), improving the reliability of signal transfer from the modulator brain stem and mid-brain nuclei through the BF to higher forebrain structures. Because the best use of population representation dictates that overall entropy (which is related to the variability of the spike patterns) in neuronal network is high (McCulloch 1965; Linas 2001; Sanger 2003), different activity patterns of DBB noncholinergic cells should also improve the capacity of these cells to represent and process simultaneously a range of inputs. Interestingly, the higher proportion of tonic profiles revealed by our experiments.

Fig. 8. Intrinsic voltage dynamics govern the variety of firing profiles in noncholinergic DBB neurons. A: example of depolarization-induced switch of tonic firing profile into cluster firing (left and middle, respectively) with its return into tonic firing mode after removal of the steady depolarizing current. Intensities of injected currents here and below are indicated above. B: example of cluster firing profile with transition into tonic regular firing mode caused by hyperpolarizing steady current injection. C: low-rate random firing neuron switched into regular tonic firing profile by steady depolarizing current followed by recovery of the initial firing mode after removal of depolarizing stimuli. D: block of firing activity with emergence of the subthreshold membrane oscillations revealed under strong depolarization.
suggests that tonic firing is the most preferred and electrochemically favorable activity state of noncholinergic cells. Given that the discharge modes of these neurons and their dynamic states can be influenced by synaptic inputs and modulator drives, their output at a given time should represent the integral of self-sustaining activity with synaptic drives and modulator inputs (Fig. 10).

Functional implications. Although both cholinergic and noncholinergic components of the ascending basalo-cortical modulator system are well recognized, there is ongoing dispute over the origin of the diversity of various activity profiles in BF projection neurons (Zaborszky and Duque 2003). While some studies, based on discharge characteristics classify three subpopulations of BF neurons (Szymusiak and McGinty 1986), others defined at least five different neuron subtypes (Detari et al. 1987; Detari and Vanderwolf 1987) with neurochemical identity of single units exhibiting various profiles in vivo remaining a matter of controversy. Manns and co-workers, for instance, attributed burst firing activity to cholinergic projection neurons (Manns et al. 2000) while others suggested that bursts are not characteristic to cholinergic cells but can be generated by parvalbumin-positive neurons (Duque et al. 2000; Zaborszky and Duque 2003). Nonetheless, most reports demonstrate a range of firing profiles in BF, extending from low-rate random to high-frequency tonic or burst-cluster firing (Detari et al. 1987; Duque et al. 2000; Zaborszky and Duque 2003; Lin et al. 2006; Lin and Nicolelis 2008). The flexible character of noncholinergic cell outputs in slices revealed here suggests that the various firing patterns found in vivo could be manifestations of different discharge modes of relatively homogeneous cell populations capable of supporting multiple activity profiles. The broad range of activity patterns of noncholinergic neurons described here should enable the use of both rate and temporal codes by these cells for processing inputs and communicating highly dynamic outputs to recipient cortical fields.

ACKNOWLEDGMENTS

We acknowledge the help Inga Antyborzec with immunohistochemistry. We also thank Dr. Tibor Koos, Dr. Valerie O’Leary, and Cargi Unal for insightful comments on this manuscript.

GRANTS

This research was supported by the National Institute of Neurological Disorders and Stroke Grant NS-023945 (to L. Zaborszky) and by a PRTL14 grant from the Irish Higher Education Authority to the Neuroscience Research Stream of Target-Driven Therapeutics and Theranostics Programme (P.I.: O. J. Dolly).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS
Author contributions: S.V.O. and L.Z. conception and design of research; S.V.O. performed experiments; S.V.O. analyzed data; S.V.O., O.J.D., and L.Z. interpreted results of experiments; S.V.O. prepared figures; S.V.O. and L.Z. drafted manuscript; S.V.O., O.J.D., and L.Z. edited and revised manuscript; S.V.O., O.J.D., and L.Z. approved final version of manuscript.

REFERENCES


Ovsepian SV, Friel DD. Enhanced synaptic inhibition disrupts the efferent code of cerebellar Purkinje neurons in leaner Ca\(^{2+}\)/Na\(^{+}\) channel mutant mice. *Cerebellum* 2010 Sep 16. [Epub ahead of print]


Williams SR, Christensen SR, Stuart GJ, Hauser M. Membrane potential bistability is controlled by the hyperpolarization-activated current I\(h\) in rat cerebellar Purkinje neurons in vitro. *J Physiol* 539: 469–483, 2002.


