Electrophysiological Differences between Neurogliaform Cells from Monkey and Rat Prefrontal Cortex

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Running head: Neurogliaform cells in monkey and rat prefrontal cortex

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Abstract

Current dogma holds that a canonical cortical circuit is formed by cellular elements that are basically identical across species. However, detailed and direct comparisons between species of specific elements of this circuit are limited in number. In this study, we compared the morphological and physiological properties of neurogliaform (NGF) inhibitory neurons in the prefrontal cortex (PFC) of macaque monkeys and rats. In both species, NGF cells were readily identified based on their distinctive morphological features. Indeed, monkey NGF cells had only a few morphological features that differed from rat, including a larger soma, a greater number of dendrites, and a more compact axonal field. In contrast, whole-cell recordings of the responses to injected current steps revealed important differences between monkey and rat NGF cells. Monkey NGF cells consistently generated a short-latency first spike riding on an initial depolarizing hump, whereas in rat NGF cells, the first spike appeared after a substantial delay riding on a depolarizing ramp not seen in monkey NGF cells. Thus, although rat NGF cells are traditionally classified as late-spiking cells, monkey NGF cells did not meet this physiological criterion. In addition, NGF cells in monkey appeared to be more excitable than those in rat, because they displayed a higher input resistance, a lower spike threshold and a higher firing frequency. Finally, NGF cells in monkey showed a more prominent spike frequency adaptation as compared to rat. Our findings indicate that the canonical cortical circuit differs in at least some aspects of its constituent elements across species.
Introduction

It is widely accepted that a canonical cortical circuit is conserved across mammalian species (see for review, Nelson 2002; Douglas & Martin 2004). As a consequence, interspecies differences in cortical function are thought to reflect solely differences in the number of these circuits and in their extrinsic connections. However, some existing data suggest that the interneuron component of the circuit also differs across species. For example, rodents and primates differ considerably in the proportions of neocortical interneurons immunoreactive for specific calcium-binding proteins. Parvalbumin-positive interneurons are the most prevalent interneuron subpopulation in rat PFC (Gabbott et al. 1997; Kawaguchi and Kubota 1997), whereas calretinin-positive interneurons outnumber other interneuronal subtypes in monkey PFC (Conde et al. 1994; Gabbott and Bacon 1996). Moreover, subtypes of cortical interneurons also appear to differ in their developmental origin between rodents and humans (Xu et al. 2004; Butt et al. 2005; Letinic et al. 2002).

The morphological and functional diversity of cortical interneurons (Kawaguchi 1995; Markram et al. 2004; Nelson et al. 2006) makes cross-species comparisons challenging because they require identification of the same interneuron subtype. NGF cells are a very suitable population of interneurons for interspecies comparisons because they possess distinctive morphological properties that are readily recognized across cortical regions in many species (Jones 1984; Lund and Lewis 1993; Kawaguchi 1995). NGF cells have a spider-like appearance with a small round soma, short dendrites and densely arborizing axons that synapse primarily on the distal dendritic spines of pyramidal cells (Kisvarday et al. 1990; Kawaguchi and Kubota 1997; Tamas et al. 2003). In rat neocortex, NGF cells provide long-lasting inhibition onto pyramidal cells via connections that contain both GABA$_A$ and metabotropic GABA$_B$ postsynaptic receptors (Tamas et al. 2003), enabling NGF cells to modulate the firing rates of pyramidal cells during sustained activity (Pouille and Scanziani 2001; Mittmann et al. 2004). In addition, NGF cells in rat neocortex connect inhibitory interneurons of different classes via gap junctions, and thus could contribute to neocortical interneuron synchrony (Simon et al. 2005).

Previous studies have established that rat cortical NGF cells are characterized by the late-spiking electrophysiological phenotype \textit{in vitro} (Kawaguchi 1995; Chu et al. 2003; Tamas et al. 2003) and \textit{in vivo} (Zhu and Zhu 2004). Our recent physiological study of interneurons in monkey dorsolateral PFC (Krimer et al. 2005) suggested that NGF cells in primates might differ from those
in rats. However, comparison of our data with the published data in rat was not possible at that time because of the significant differences in age of the studied animals. Here, we directly compared morphological and physiological properties of NGF cells between adult monkeys and adult rats. The hallmark physiological feature of NGF cells in rat, a prominently delayed first spike latency [“late spiking” cells], did not hold for monkey NGF neurons. Furthermore, NGF cells in monkey appeared to be markedly more excitable than in rat: they had a lower spike threshold, higher input resistance and higher firing frequency.

The finding that similar anatomical units of PFC circuitry demonstrate functional differences between monkey and rat suggests that species differences are present in the canonical cortical circuit.
Methods

Slice preparation

Brain slices were obtained from adult (P56-P135, 350-550 g) and juvenile (P19-P28) male Wistar rats and adult (4-5 yrs old, 3.5-6.0 kg) male long-tailed macaque monkeys (Macaca fascicularis). All animals were treated in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Rats were deeply anesthetized with halothane and decapitated. The brain was quickly removed and immersed in ice-cold pre-oxygenated artificial cerebrospinal fluid (ACSF). Tissue blocks containing the prelimbic cortex in rats (Paxinos and Watson, 1998) were excised for further slicing. The protocol employed to obtain tissue blocks from monkey PFC has previously been described (Gonzalez-Burgos et al. 2000). Briefly, animals were treated with ketamine hydrochloride (25 mg/kg, intramuscular), dexamethasone phosphate (0.5 mg/kg, intramuscular), and atropine sulfate (0.05 mg/kg, subcutaneous). Endotracheal anesthesia was maintained with 1% halothane in 28% O₂/air. Monkeys were placed in a stereotaxic apparatus and a craniotomy was performed over the dorsolateral PFC. The dura was removed in a location determined by stereotaxic coordinates and by the position of relevant sulcal landmarks. Small blocks of tissue containing a portion of dorsal area 9 and both the medial and lateral banks of the principal sulcus (area 46) were excised. After the surgery all animals recovered quickly with no impairments in eating or drinking, and no overt behavioral deficits were observed. Animals were allowed to recover for 2-4 weeks, after which a second craniotomy under terminal anesthesia was performed. Animal were given an overdose of pentobarbital (30 mg/kg) and were perfused through the heart with ice-cold modified ACSF. A tissue block containing portions of areas 9 and 46 from a non-homotopic portion of the intact brain hemisphere was quickly excised and placed in ice-cold ACSF. Subsequent treatment of the tissue was the same for both procedures.

Coronal slices (350 µm thick) were cut with a vibratome (Leica VT1000S, Leica, Germany). Slices were incubated at 37°C for 0.5-1 h and further stored at room temperature. Slices were transferred to a recording chamber perfused with ACSF at 31-32°C. Through all steps of the experiments, ACSF of the following composition was used (in mM): 126 NaCl, 2.5 KCl, 1.25
NaH₂PO₄, 1 MgSO₄, 2 CaCl₂, 24 NaHCO₃, 10 dextrose. ACSF was perfused with 95% O₂/5% CO₂ gas mixture. Tissue slices from the same monkeys were also used in other experiments.

**Electrophysiological recordings**

Whole-cell voltage recordings were made from layer 2-3 neurons visualized using IR-DIC videomicroscopy equipped with Zeiss Axioskop 2 FS microscope, 40x water immersion objective and a Dage-MTI NC-70 video camera (Dage-MTI Television, Michigan City, IN). Interneurons were selected based on their small and round cell body and lack of apical dendrite. Patch electrodes were filled with an internal solution containing (in mM): 114 K-gluconate, 6 KCl, 10 HEPES, 4 ATP-Mg and 0.3 GTP. pH was adjusted to 7.25 with KOH. Biocytin (0.5%) and Alexa 488 or 568 (0.075%) were added to the solution for later morphological identification of the recorded neurons. Electrodes had 5-12 MΩ open-tip resistance. Voltages were amplified with an IE-210 electrometer (Warner Instruments, Hamden, CT) or MultiClamp amplifier (Axon Instruments, Union City, CA) operating in a bridge-balance mode. Signals were filtered at 5, or 4 kHz in the IE-210 and the MultiClamp, respectively, and acquired at a sampling rate of 20 kHz using a 16 bit-resolution Power 1401 interface and Signal 2.03 software (CED, Cambridge, UK). Access resistance and capacitance were compensated on-line. Access resistance typically was 15–30 MΩ and remained relatively stable during experiments (≤ 30% increase) for the cells included in the analysis. Membrane potential was not corrected for the liquid junction potential.

**Electrophysiological Data Analysis**

To characterize the membrane properties of neurons, hyper- and depolarizing current steps were applied for 500 ms in 5-10 pA increments at 0.5 Hz. Input resistance was measured from the slope of a linear regression fit to the voltage-current relation in a hyperpolarizing range. The membrane time constant was determined by single-exponential fitting to the on-phase of the average voltage responses to the small (5-15 pA) hyperpolarizing current steps, which presumably are less affected by voltage-dependent membrane conductances. The ramp coefficient was estimated at the RMP as the voltage difference between the two points: at 75 ms from the beginning of the current pulse and at the end. All action potential measures were taken from the first evoked action potential. Peak amplitude of the action potential and the afterhyperpolarization (AHP) was measured from the action potential threshold (level of voltage deflection exceeding 10 mV/1ms). The amplitude of the
fast component of AHP was measured from the spike threshold downward the fast voltage drop until the point of a marked slowing in the voltage drop to < 10 mV/1ms. The amplitude of the medium component of AHP was measured from the point of the voltage deflection to the most negative voltage peak of the AHP. The slow AHP was measured as the AHP amplitude at 300 ms after the end of a train of spikes. Duration of the action potential was measured at its half amplitude. The spike rise time was measured as 10 to 90% of the total rise time. 10 to 90% spike decay time was measured between the spike peak and its threshold.

We used an adaptation ratio (AR) to describe spike frequency adaptation in evoked trains. The AR was calculated as a ratio between the first and the last interspike interval for different current intensities. Since the AR changes with the increase of the stimulation current we employed the AR coefficient for the interspecies comparison. The AR coefficient was estimated from the linear regression of AR versus current at 2x threshold level of current.

The onset frequency was calculated at the first interspike interval, the steady-state frequency was calculated as average instantaneous frequency within the last 200 ms of the response to depolarizing current pulses where firing frequency remained relatively stable.

**Morphological Analysis**

To identify cell morphology during the experiments, neurons were filled with the fluorescent dyes Alexa 488 or 568 (0.075% solution, Molecular Probes, Eugene, OR) and with biocytin (0.5%, Molecular Probes) added to the internal solution. Cells were filled for at least 30 minutes to ensure complete cell labeling by the dyes. Slices were fixed in cold 4% paraformaldehyde for at least 72 hr, then transferred into an anti-freeze solution (ethylene glycol and glycerol in 0.1 M phosphate buffer), and stored at -80°C. Some neurons filled with a fluorescent dye were confocally reconstructed using an Olympus Fluoview 500 confocal microscope (Olympus America Inc, Melville, NY) with FITC and CY3 filters. Images were acquired with Fluoview software (Olympus America Inc, Melville, NY) and further processed using Imaris (Bitplane INC, Saint Paul, MN) and Photoshop (Adobe System Incorporated, San Jose, CA) programs. To process for biocytin, the slices were rinsed in 0.1 M Na-phosphate buffer, and then serially resectioned at 50 µm. After the sections were treated with 1% H₂O₂ for 2-3 h at room temperature, they were incubated for 12 h at 4°C in blocking serum containing 2% goat serum, 1% bovine serum albumin and 0.4% Triton X-100. After rinsing, the sections were incubated with the avidin-biotin-peroxidase complex
(Vectastain Elite ABC kit; 1:100; Vector Laboratories, Burlingame, CA) in 1% bovine serum albumin for 4 h. Sections were rinsed, stained with Ni-3,3’-diaminobenzidine (DAB), mounted on gelatin-coated glass slides, dehydrated and coverslipped.

Of 30 monkey NGF cells, 14 cells were identified based on the confocal reconstructions and consecutive biocytin processing, 3 cells were identified solely based on confocal reconstructions, and 13 cells were identified only by the DAB reaction. Of 28 rat NGF cells (adult and juvenile), 18 cells were identified based on confocal reconstructions and DAB processing, 3 cells were identified based on confocal reconstructions, 2 cells were identified solely by visualizing in the UV light, and 5 cells were identified only by DAB processing.

For a quantitative morphological analysis 22 monkey (9 fully reconstructed, 13 - dendritic trees only) and 9 rat (7 fully reconstructed, 2 - dendritic trees only) biocytin-processed NGF cells were reconstructed with the Neurolucida neuron tracing system (Microbrightfield Inc., Colchester, VT) using Axiophot 2 Zeiss microscope equipped with a 100x planapochromate objective (NA 1.4) and 2.5x optovar. The intermediate image was further captured on a video camera and projected on a computer LCD screen. These reconstructions were used for a quantitative morphological analysis, which was made in NeuroExplorer (Microbrightfield Inc., Colchester, VT). Horizontal and vertical spans of NGF cells were measured as the distances between the two extreme axonal endings in horizontal and vertical dimensions, respectively.

**Statistical analysis**

Two-tailed t-tests were used for group comparisons in most of the cases. Fisher exact test was used to compare percentage of cells with different numbers of dendritic branches. Unless otherwise noted values are presented as mean ± SD. Statistical tests were performed using Microsoft Excel or Statistica 6.1 (Statsoft Inc., Tulsa, OK). 14 NGF cells from monkey dorsolateral PFC were reported in our previous publication (Krimer et al., 2005).
Results

Morphology of monkey and rat NGF cells

Interneurons from monkey and rat PFC were identified as NGF cells based on the morphological features described earlier (Jones 1984; Lund and Lewis 1993; Kawaguchi 1995). In these cells, a large number of primary dendrites (typically more than 6) arose radially from a small round soma located in layers 2-3 (Fig. 1,2). Dendrites were short, smooth or slightly beaded, and gave rise to only a few secondary branches which formed a relatively small sphere centered on the soma. The axon of NGF cells originated from the cell body or one of the primary dendrites, branched and established a dense axonal mesh with thin shafts. Axons occupied a volume several times larger than that of the dendrites, but still were confined mainly within layer 2-3 where the somata were located. Axons rarely extended into layers 1 or 5. Both dendrites and axons sometimes curved back towards the soma.

For a quantitative inter-species comparison NGF cells from adult monkey and rat PFC were reconstructed using transmitted light microscope (monkey: dendritic trees were reconstructed in 22 cells, full reconstructions were made in 9 cells; rat: dendritic trees - in 9 cells, full reconstructions - in 7 cells). Soma size was significantly (40%) larger in monkey NGF cells than in rat (Table 1). The number of primary dendrites was also significantly (43%) greater in monkey than in rat, although the total dendritic length did not differ between the species (Table 1). Dendrites of rat NGF cells tended to branch more often than in monkey; half of the rat NGF cells possessed 6th order dendrites, whereas most of the monkey NGF cells had dendrites that branched only to the 3rd or 4th order (Fig. 2C); however this difference between the two species was not statistically significant (Fisher exact p>0.1). Sholl analysis demonstrated that the dendrites in monkey NGF cells had a more compact proximal distribution as compared to rat. First Sholl’s circle of 10 µm had more dendritic intersections in monkey than in rat (Fig. 2D).

Axons in monkey NGF cells branched more frequently than in rat; however, the total axonal length did not differ between the two species (Table 1). Axons in monkey NGF cells, similar to their dendrites, had a tendency for a denser perisomatic distribution than those in rat (Fig. 2E). However, more distally from the soma (200-400 µm) rat NGF cells had more intersections than those in monkey. Therefore, even though the total dendritic and axonal lengths were comparable in
the two species, both axonal and dendritic distributions of NGF cells were more compact in monkey than in rat.

Thus, although all the cells included in this study belonged to the ‘neurogliaform phenotype’, quantitative analyses revealed some morphological differences between the species.

**Subthreshold membrane properties of NGF cells in adult rat and monkey**

Whereas the resting membrane potential of NGF cells was comparable in the two species (Table 2), some of the other subthreshold membrane properties appeared to be different. Thus, input resistance was significantly larger in NGF cells from monkey than from rat (Table 2, Fig. 3A, B), indicating their greater excitability. Hyperpolarizing current steps of the same strength generated larger voltage responses in NGF cells from monkey than from rat. Accordingly, I-V curves obtained from the subthreshold responses to hyperpolarizing current pulses in monkey cells were much steeper than in rat (Fig. 3B). The current-voltage plot revealed time-independent inward rectification in both monkey and rat NGF cells (Fig. 3B). In addition, 56% of monkey NGF cells showed a time-dependent depolarizing sag in their hyperpolarizing voltage responses, which was never seen in rat neurons.

In response to subthreshold depolarizing current steps, rat NGF cells generated depolarizing ramps especially noticeable at membrane potentials close to spike threshold (Fig. 3C). Such responses may be attributed to low-threshold potassium currents (Connor & Stevens 1971). A ramp coefficient was estimated at the RMP and was found to be larger in rat NGF cells than in monkey (Fig. 3D). This depolarizing ramp was previously described as a distinctive feature of NGF cells in immature rats (Kawaguchi, 1995).

In striking contrast, monkey NGF cells did not generate a ramp in response to the depolarizing current steps. Moreover, when the cells were held at a more hyperpolarized membrane potential (close to -80 mV), they showed a hump-like upward voltage deflection at the beginning of the responses to current injection (Fig. 3C).

**Firing properties of NGF cells in adult monkey and rat**

**Single spike properties.** Single spikes of monkey and rat NGF cells were similar in a number of parameters, including spike amplitude, spike duration, spike rise and decay time, and the shape of the AHP (Table 2, Fig. 4A). However, differences in AHP amplitude, spike threshold and first spike
latency were observed. In monkey NGF cells AHPs following single spikes had larger amplitudes than those in rat, but similar form (Fig. 4A). In both species AHP had a complex shape and could be divided into two components - an early, fast AHP and a delayed, medium duration AHP (Fig. 4B). The differences in AHP amplitude were due to the differences in the fast component (monkey 12±3 mV and rat 17±3 mV, respectively, p<0.05), but not in the medium duration component (monkey 7.4±4.1 mV and rat 7.6±3.2 mV, respectively; p>0.1). Action potential threshold was considerably lower in NGF cells from monkey than from rat PFC (Fig. 4C, Table 2), which could be associated with the higher input resistance in monkey NGF cells (Segev and London, 1999; Prescott et al., 2006), as well as with the other mechanisms, and, again, could be indicative of greater excitability of monkey NGF cells as compared to rat.

All NGF cells from rat PFC demonstrated the characteristic delayed firing pattern (Table 2, Fig. 4D,E). This delay of the first spike progressively shortened with subsequently stronger current stimulation. Based on this observation, rat NGF cells have been previously defined as late-spiking interneurons (Kawaguchi 1995; Chu et al. 2003). In contrast, at a near-threshold level of stimulation current, monkey NGF cells fired with a significantly shorter first spike latency than those in rat (Table 2, Fig. 4D,E). In addition, the first spike latency in rat NGF cells was dependent on the membrane potential, as demonstrated by manipulations of the resting membrane potential. The first spike latency was longer at more hyperpolarized membrane potentials and progressively shortened at more depolarized membrane potentials (286±139 ms and 194±131 ms, p<0.05, t-test paired comparison, n=9). Similar observations were made in NGF cells from immature rats (Kawaguchi 1995). In contrast, monkey NGF cells failed to increase delay of their 1st action potential when they were current-clamped at membrane potentials close to -80 mV (Fig. 4D). The estimated latency of the first spikes evoked at the more hyperpolarized level was 57±23 ms which was basically indistinguishable from the 1st spike latency at the RMP (72±33 ms, p>0.10, t-test paired comparison, n=5).

**Firing pattern properties.** Representative examples of the firing pattern in monkey and rat NGF cells are shown on the Figure 5A. In both species, the temporal pattern of firing was characterized by an early adaptation of spike frequency - a considerable increase in duration from the first to the second interspike interval. Although the increase in duration between the first and the second interspike interval was small with near threshold stimulation currents, it progressively increased with stronger currents (Fig. 5A,B). In addition, in both monkey and rat NGF cells the AR
progressively decreased with the increase in stimulation current (Fig. 5C). When average AR was plotted against stimulation current intensity, a significant negative relationship was revealed (slope of -4.7 for rat and -4.6 for monkey).

Although the temporal structure of firing pattern was similar in monkey and rat NGF cells, monkey cells showed more prominent spike frequency adaptation. The AR was smaller in monkey than in rat for all current intensities (Fig. 5C). In addition, in rat NGF cells the first interspike interval was considerably shorter than the remaining interspike intervals, which in turn were of relatively constant duration in the same sweep. While rat NGF cells showed almost no adaptation from the second to the last interspike interval, monkey NGF cells continued to adapt: the ratio of the second interspike intervals to the last interspike intervals (at the level 2× threshold) was close to 1 in rat and considerably smaller in monkey (0.92±0.17 (n=19) and 0.68±0.18 (n=21), respectively, p<0.001). It was shown before that Ca2+-dependent slow K+ currents may be responsible for spike frequency adaptation in different types of neurons (Sah 1996; Lopez de Armentia et al. 2004), and the AHP that follows a train of action potentials, slow AHP, could be the best indicator of their activity. Indeed, the slow AHP (measured at 300 ms after the end of the voltage step) was larger in monkey than in rat NGF cells (1.92±0.76 (n=12) and 0.64±0.39 (n=10), p<0.001) (Fig. 5D).

To further compare the excitability of monkey and rat NGF cells, we measured evoked firing frequency in these cells. Since NGF cells showed spike frequency adaptation, both onset and steady-state frequencies were compared between monkey and rat NGF cells. The onset and steady-state frequency at 2x threshold was higher in monkey NGF cells than in rat (Table 2). We also found a significantly steeper slope in the frequency/current plot from monkey NGF cells than from rat (onset frequency: 1.3±0.12 Hz/pA (n=17) and 0.3±0.03 Hz/pA (n=19), p<0.001; steady-state: 0.24±0.16 Hz/pA and 0.1±0.04 Hz/pA, respectively, p<0.001, Fig. 5E,F). The difference in onset frequency was more prominent than the difference in steady-state frequency between the two species. Accordingly, NGF cells in monkey generated more spikes than rat at firing threshold (3.1±1.5 (n=30) and 2.0±1.0 (n=19), p<0.01).

**Firing properties of NGF cells in juvenile rats**

Matching different mammalian species by a certain phase of their brain development is a challenging task (Clancy et al. 2001). Membrane properties of neurons have been shown to undergo changes through the time span of postnatal development in rat neocortex and seem to achieve a
mature state by the 3rd-4th postnatal week in rat (Zhou and Hablitz 1996; Zhu 2000). To address the stability in membrane properties of NGF cells from the maturation point until post-adolescence we also analyzed NGF cells from younger rats (P19-P28, n=9). We found that almost all subthreshold and suprathreshold properties of NGF cells in younger animals were similar to those found in adults. Importantly, NGF cells from juvenile rats displayed the late-firing pattern with just suprathreshold stimulation currents (Fig. 6A), and had firing threshold indistinguishable from the adults.

NGF cells from juvenile rats tended to have less adaptation of firing than those in adult rats (Fig. 6A-C), although the difference did not reach the level of significance. 2 of 9 juvenile NGF cells demonstrated acceleration of firing frequency from onset to the end of the spike train in response to near-threshold stimulation currents. Notably, as in adult rats, the AR in juvenile rats decreased with increase of stimulation current (mean slope of -3.1±2.2), which seems to be a characteristic feature of NGF cells from different species.
Discussion

The results of this study revealed important differences between monkey and rat PFC in the intrinsic physiological characteristics of NGF cells. In contrast to rat, the first spike in monkey NGF cells was not delayed, challenging the physiological definition of NGF cells as ‘late-spiking’ cells. Furthermore, NGF cells in monkey were more excitable than in rat since they demonstrated lower spike threshold, higher input resistance and firing frequency. Finally, monkey NGF cells showed more prominent spike frequency adaptation than those in rat. In contrast, morphological characteristics of NGF cells were qualitatively similar between the species and the differences were only appreciated using quantitative measures. Our findings suggest that interspecies differences can be detected at the level of intrinsic cellular properties.

Morphological analysis of monkey and rat NGF cells

All cells included in the analysis satisfied the morphological criteria defined for the ‘NGF’ phenotype. In particular, NGF cells had a relatively large number of dendrites – on average 8, which corresponds to the data reviewed by Jones (1984), but is greater than reported previously in layer 1 of immature rat neocortex (Hestrin and Armstrong 1996). Although the morphology of NGF cells was qualitatively similar between monkey and rat, morphometric analysis revealed several differences including a more compact distribution of processes, less prominent branching, larger soma and larger number of primary dendrites in monkey than in rat NGF cells. Average horizontal span of axons was smaller in monkey than in rat NGF cells, although in our study the values in monkey were somewhat larger than those previously reported in Golgi-based data from monkey PFC (Lund and Lewis 1993). The width of the axonal field is critical for circuitry organization since it defines a potential spread of inhibition in the cortex provided by an interneuron.

Membrane properties of NGF cells

Several physiological membrane properties of NGF cells, including spike amplitude, spike duration, and low steady-state firing rate, were preserved across the species. However, many of the important physiological properties differed between monkey and rat NGF cells. First, in striking contrast to rat, monkey NGF cells did not show late-spiking firing with the near-threshold stimulation currents. Voltage dependence of the 1st spike latency in rat NGF cells demonstrated in this study suggests the
contribution of the low-threshold outward K⁺ currents to their electrical activity (Connor & Stevens 1971; Banks et al. 1996; Yang et al. 1996). Delayed spike firing can be critical to the role of NGF cells in microcircuit function. It is shown that the cells that respond with a delayed 1st spike to depolarizing current steps may respond with a similar delay to synaptic stimulation (Beggs et al. 2000). Thus, in rat PFC, sustained inputs might be significantly more efficient than transient inputs to elicit NGF cell firing, while, sustained and transient synaptic inputs might be similarly effective to recruit NGF cells from monkey PFC. Delay in firing can as well affect transition from the ‘down’ states to the ‘up’ states (Sanchez-Vives & McCormick 2000) since currents that underlie this delay can limit the subthreshold depolarization associated with the onset of the ‘up’ state (Gabel & Nissenbaum 1998).

Also, in this study monkey NGF cells appeared to be more excitable than those in rat because they displayed higher input resistance, lower spike threshold, and higher firing frequency. Cells that show a higher responsiveness to excitatory stimuli were shown to be more effective in mediating synaptic plasticity, because higher excitability can facilitate activation of neurons by fewer glutamate inputs (Schmidt-Hieber et al. 2004).

Finally, we found that rat NGF cells show less pronounced spike frequency adaptation than those in monkey. According to the previous studies from young rats, NGF cells demonstrate a non-adapting firing pattern with low current intensities and slightly adapting with the increase of stimulation current (Kawaguchi 1995; Simon et al. 2005). This difference in adaptation could be a developmental phenomenon since we found that NGF cells from juvenile rats tended to have less adaptation than those from adult animals. Some juvenile NGF cells demonstrated acceleration of firing frequency, the phenomenon shown previously by Simon et al. (2005).

Although heterogeneity of NGF cells was beyond the scope of this study, more than one class of NGF cells might exist in the cortex. Thus, the cluster analysis of monkey interneurons demonstrated that the group of NGF cells included two physiological clusters (Krimer et al. 2005). Neurochemical and associated topographic heterogeneity of NGF cells have been reported in human motor cortex (Kalinichenko et al. 2006). According to Hestrin and Armstrong (1996), NGF cells from neocortical layer I possess physiological features not found in NGF cells from the other cortical layers described elsewhere. In addition, different subpopulations of NGF cells in terms of intrinsic properties and short-term dynamics were found by Price et al. (2005) and Zsiros and Maccaferri (2005) in the hippocampus of immature rats.
NGF cells in the PFC circuitry

NGF cells in rat were described to have delayed first spike and, thus, were defined physiologically as ‘late-spiking’ interneurons (Kawaguchi 1995; Chu et al. 2003). Since monkey NGF cells do not demonstrate delayed first spike, the existing physiological definition cannot be applied to them. Several properties of the NGF cells, however, are shared between monkey and rat PFC, among them are a relatively slow steady-state firing rate and an increasing spike frequency adaptation with increase in stimulation current (progressively increasing adaptation) (Kawaguchi 1995; Krimer et al. 2005). Thus, one of the physiological definitions of NGF cells could be ‘slow-firing interneurons’, or ‘progressively adapting’. However, application of these characteristics as defining features of NGF cells is also difficult. For example, an increase in adaptation, while is clearly present in adult monkey NGF cells, is more difficult to appreciate in NGF cells from juvenile rats, which basically lack adaptation. Thus, perhaps only a combination of properties can accurately describe specific physiological type of interneurons (Markram et al. 2004; Yuste 2005).

Distinct cellular properties of one of the interneuronal classes between monkey and rat might further elucidate the nature of the functional differences between primate and rodent PFC (Preuss 1995; Brown & Bowman 2002; Uylings et al. 2003). Our findings indicating that the canonical cortical circuit differs in at least some aspects of its constituent elements across species, suggest the need for caution in extrapolating data from rodents to primates, and, ultimately, to humans. This approach is critical for the studies aiming to understand pathophysiological mechanisms of mental illnesses, such as schizophrenia, associated with dysfunction of interneurons in the PFC (Lewis et al. 2005).

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Figure 1. Sites of recording and morphology of monkey and rat NGF cells. A. Sites of interest (arrow): dorsolateral PFC (areas 9 and 46) in monkey and prelimbic cortex in rat. B. Examples of NGF cells from monkey (B) and rat (C) PFC. Dendrites and axons are shown separately.
Figure 2. Morphological analysis of monkey and rat NGF cells. Morphological variety of NGF cells form monkey (A) and rat (B) PFC. C. Percentage of cells with the dendrites of different orders in monkey (n=22) and rat (n=9). Note that the dendrites of monkey cells tended to branch less as compared to rat. D. Sholl analysis of dendritic arbor from monkey and rat NGF cells. Average dendritic intersections are plotted against the distance from the soma. Primary dendrites of monkey NGF cells had more intersections than those from rat. Error bars represent SE. E. Sholl analysis of the axonal arbor from 9 monkey and 7 rat NGF cells. Average axonal intersections are plotted against the distance from the soma. Asterisk: p<0.05. Error bars represent SE.
Figure 3. Subthreshold membrane properties of NGF cells in monkey and rat. A. Voltage responses to the hyperpolarizing current steps. Both monkey and rat NGF cells showed time-independent inward rectification (also in B). B. Current-voltage plots for traces shown in A. C. Subthreshold voltage responses to depolarizing current pulses. The arrowhead points to the 'hump' in monkey, the arrows point to the 'ramp' in rat. D. The ramp coefficient was larger in rat NGF cells (n=19) than in monkey (n=22). Error bars represent SE.
Figure 4. Properties of the first evoked spike in monkey and rat NGF cells. A. Single action potential in monkey (gray) and rat (black) NGF cells had similar spike amplitude and duration (arrow), but different AHP amplitude (arrowhead). B. Dual component AHP in monkey and rat NGF cells. Note that the fast AHP component was smaller in monkey NGF cells than in rat, while the delayed, medium AHP component was similar in the two species. C. Spike threshold was considerably lower in monkey NGF cells than in rat (action potentials are truncated). D. First suprathreshold response to depolarizing current in rat and monkey NGF cells. Note that the 1st spike in rat NGF cells was delayed (arrow) and the delay is voltage-dependent. E. Graph plots the 1st spike latency against the intensities of the above threshold current in monkey (n=17) and rat (n=19) NGF cells. Error bars represent SE, asterisks indicate p<0.05.
Figure 5. Suprathreshold responses to the depolarizing current steps in NGF cells from monkey and rat. A. Firing pattern produced by NGF cells in response to the current steps of 2 x firing threshold and 3 x firing threshold. B. Temporal frequency structure of spikes in the individual sweeps of monkey and rat NGF cells. In both species the firing pattern was characterized by the early adaptation (arrowhead). C. Plot of averaged AR values against intensity of depolarizing current steps in 21 NGF cells from monkey and 19 NGF cells from rat. Note that monkey NGF cells had smaller ARs than rat for all current intensities. Error bars represent SE. D. Slow AHP in monkey and rat NGF cells. E, F. Plot of firing frequency against depolarizing current pulses of increasing magnitude in monkey (n = 17) and rat (n=19) NGF cells. Onset frequency (E) and steady-state frequency (F) was larger in monkey NGF cells than in rat for all current intensities.
Figure 6. Firing properties of NGF cells from juvenile rats. A. Evoked trains of spikes in response to the current steps of increasing magnitude were reminiscent of those in adult rat. Note that the 1st spike was delayed (arrow). B. Temporal structure of individual spike frequencies in the evoked spike for the two intensities of stimulating current steps was characterized by the early adaptation (arrowhead) similarly to adult rat. C. AR coefficient in NGF cells from juvenile rat (n=9) was comparable to those in adult rat (n=19) (p>0.10). Error bars represent SD.
Table 1. Morphometric analysis of NGF cells from monkey and rat

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Monkeys (n=22)</th>
<th>Rats (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma area, µm²*</td>
<td>92±39</td>
<td>66±19</td>
</tr>
<tr>
<td>Number of primary dendrites*</td>
<td>10±2.7</td>
<td>7.0±1.3</td>
</tr>
<tr>
<td>Total dendritic length, µm</td>
<td>1,055±1,036</td>
<td>1,375±649</td>
</tr>
<tr>
<td>Dendritic horizontal span, µm</td>
<td>78±37</td>
<td>107±28</td>
</tr>
<tr>
<td>Dendritic vertical span, µm</td>
<td>104±49</td>
<td>172±111</td>
</tr>
<tr>
<td>Total axonal length, µm</td>
<td>22,951±8,547 (n=9)</td>
<td>29,390±8,845 (n=7)</td>
</tr>
<tr>
<td># of axonal branching points *</td>
<td>180±55 (n=9)</td>
<td>349±158 (n=7)</td>
</tr>
<tr>
<td>Axonal horizontal span, µm*</td>
<td>407±98</td>
<td>540±117</td>
</tr>
<tr>
<td>Axonal vertical span, µm</td>
<td>486±151</td>
<td>547±145</td>
</tr>
</tbody>
</table>

* Significantly different between the two species, p<0.05
Table 2. Intrinsic subthreshold and firing physiological properties of NGF cells in monkey and rat

<table>
<thead>
<tr>
<th></th>
<th>Monkey (n=30)</th>
<th>Rat (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>-67±5</td>
<td>-66±4</td>
</tr>
<tr>
<td>Spike threshold, mV***</td>
<td>-41±4</td>
<td>-35±3</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>58±9</td>
<td>60±7</td>
</tr>
<tr>
<td>Spike half duration, ms</td>
<td>0.63±0.12</td>
<td>0.67±0.07</td>
</tr>
<tr>
<td>Spike 10-90% rise time, ms</td>
<td>0.28±0.05</td>
<td>0.29±0.04</td>
</tr>
<tr>
<td>Spike 10-90% rise rate, mV/ms</td>
<td>184±45</td>
<td>180±25</td>
</tr>
<tr>
<td>Spike 10-90% fall time, ms</td>
<td>0.54±0.09</td>
<td>0.57±0.06</td>
</tr>
<tr>
<td>Spike 10-90% fall rate, mV/ms</td>
<td>95±25</td>
<td>93±13</td>
</tr>
<tr>
<td>1st spike latency, ms***</td>
<td>59±36</td>
<td>209±122</td>
</tr>
<tr>
<td>AHP amplitude, mV***</td>
<td>19±4</td>
<td>25±4</td>
</tr>
<tr>
<td>AR coefficient (2 x threshold)***</td>
<td>0.47±0.15 (n=21)</td>
<td>0.69±0.16</td>
</tr>
<tr>
<td>Onset frequency (2 x threshold), Hz**</td>
<td>50±28 (n=21)</td>
<td>26±11</td>
</tr>
<tr>
<td>Steady-state frequency (2 x threshold), Hz*</td>
<td>24±11 (n=21)</td>
<td>17±4</td>
</tr>
<tr>
<td>Number of spikes at threshold**</td>
<td>3.1±1.5</td>
<td>2.0±1.0</td>
</tr>
<tr>
<td>Input resistance, MΩ**</td>
<td>370±200</td>
<td>238±80</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>11±4</td>
<td>10±4</td>
</tr>
</tbody>
</table>

* Significantly different between the two species, p<0.05; **p<0.01; ***p<0.001