

# 四种皮质间神经元亚型树突状树的守恒特性

## Conserved properties of dendritic trees in four cortical interneuron subtypes

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树突树影响突触整合和神经元的兴奋性，但似乎以相当任意的模式发展。利用电子显微镜和序列重建技术，我们分析了四种形态不同的新皮质中间神经元亚型的树突树，揭示了两种共同的基本组织原理。首先，树突内任何给定点的横截面积与该点远端所有树枝段的总长度成正比。与这一观察结果一致，总横截面积在分叉点几乎完全守恒。第二，在更近、直径更大的树突位置，树突横截面逐渐变得更椭圆。最后，计算机模拟显示这些守恒的形态学特征限制了细胞体 EPSPs 的距离依赖性滤波，并促进了细胞体去极化分布到所有的树突房室。因为所有研究的中间神经元都具有这些特征，所以它们可能代表了树突树不同形态的共同组织原理。

树突的形态直接影响突触整合和神经元的兴奋性。树突的被动特性会阻碍电压信号从远端突触位置有效地传播到胞体和轴突，在那里动作电位通常是启动的。树突膜固有的电导和电容以距离依赖的方式衰减和扩大突触电位，而变细树突的不对称性加剧了突触电位。然而，逐渐变细的树枝晶的直径和表面积也逐渐减小，这将增加局部输入阻抗，并在较远的枝晶位置降低局部电容。通过在远端产生快速和大的局部突触反应，逐渐变细的树突限制了树突中电压衰减的功能影响，这个过程有时被称为被动正常化，并优化树突内突触电流的传导。树突树的结构也会影响神经元的兴奋性和动作电位输出模式，即使树突的树突总长度和表面积相同。树突分支点的几何结构通过影响胞体和树突间电流的传递，调节树突拓扑结构对突触整合和兴奋性的影响。树突树有许多形状和大小。尽管树突形态的相似性经常被用来将神经元分为离散的亚型，但没有两个树突树是完全相同的：单个树突片段在分支点的位置上显得曲折和随意。我们询问皮层 GABA 能中间神经元树突状树状结构是否有共同的原理。由于光学显微镜在亚毫米水平上缺乏精确测量树突尺寸的分辨率，我们结合光学显微镜和系列电子显微镜的重建来比较四种新皮质非锥细胞亚型 12-16 的树突尺寸和形状。我们的数据揭示了所有亚型共有的树突状树的一些保守的形态学特征，这些特征限制了细胞体 EPSPs 的距离依赖性过滤，并促进了细胞体去极化分布到树突隔间中。这些特征可能代表了树突状树多样形态的共同组织原则。

### 结果

利用大鼠额叶皮质切片标本中充满生物素的非锥体神经元的神经系统重建，分析了马丁诺蒂 (Martinotti, MA) 细胞、快速尖峰篮状细胞 (fast-spiking basket cell, FS)、双花束 (double-bouquet, DB) 细胞和大篮状 (large basket, LB) 细胞的树突状树状结构 (图 1a)。这些神经元的生理特性见补充表 1。树突树状结构由 NeuroSida 进行三维重建神经形态组织 (<http://neuromorpho.org/neuromorpho/index.jsp>)。之所以选择这些细胞，是因为它们在 300 $\mu\text{m}$  厚的组织切片中基本上是完整的树突树 (图 1b)。沿着没有切断末端的树枝状分支，以 50 $\mu\text{m}$  的间隔选择了 85 个树突片段 (图 1b 中树状图上的红色圆圈，平均长度 16.8-6.8 $\mu\text{m}$ )。一些树枝状片段包含分叉点。电子显微镜下观察到的树突状结构 (见图 1)。

为了探索树突如何随着距离逐渐变细，我们首先研究了树突横截面积和与胞体的距离之间的关系 (图 2a, 上图)。通过将树枝状柱体的体积除以其长度 (0.18-2.7  $\mu\text{m}$ ) 来计算横截面积，其中没有附着棘，从而得出圆柱体的平均值。用枝晶柱的表面积除以其长度，计算出枝晶的平均周长。当选择二维横截面时，这些方法降低了潜在的变异性。近端树突截面的横截面积 (0.1-2.2 $\mu\text{m}^2$ ) 不同，而所有远端树突节段的横截面积都很小 (0.5 $\mu\text{m}^2$ )。树突厚度在树突的前 50  $\mu\text{m}$  处急剧变细，在那里经常发现分叉 (图 1b, 2a)。在树突状分支的远端，分枝很少，逐渐变细的程度变小，但仍然显著。在连续的树状段之间绘制的轨迹线 (图 2a, 下面板) 显示母树突分叉成两个大小不同的较小子枝。在近端位置的初始分

叉后出现的较小分支完全可以解释位于胞体附近的所有小截面树突。这些较薄的近端树突只延伸了很短的距离，通常没有额外的分叉，而较厚的近端子树突延伸了很长的距离，并且有频繁的额外分叉。尽管树突横截面积在细胞内和单个细胞内变化，但值得注意的是，对于任何树突位置，横截面积与从参考点向外扩展的总树突长度有很好的相关性（图 2b，也可参见在线补充图 2）。在这项分析中，我们只使用具有自然、非切断末端的重建片段。

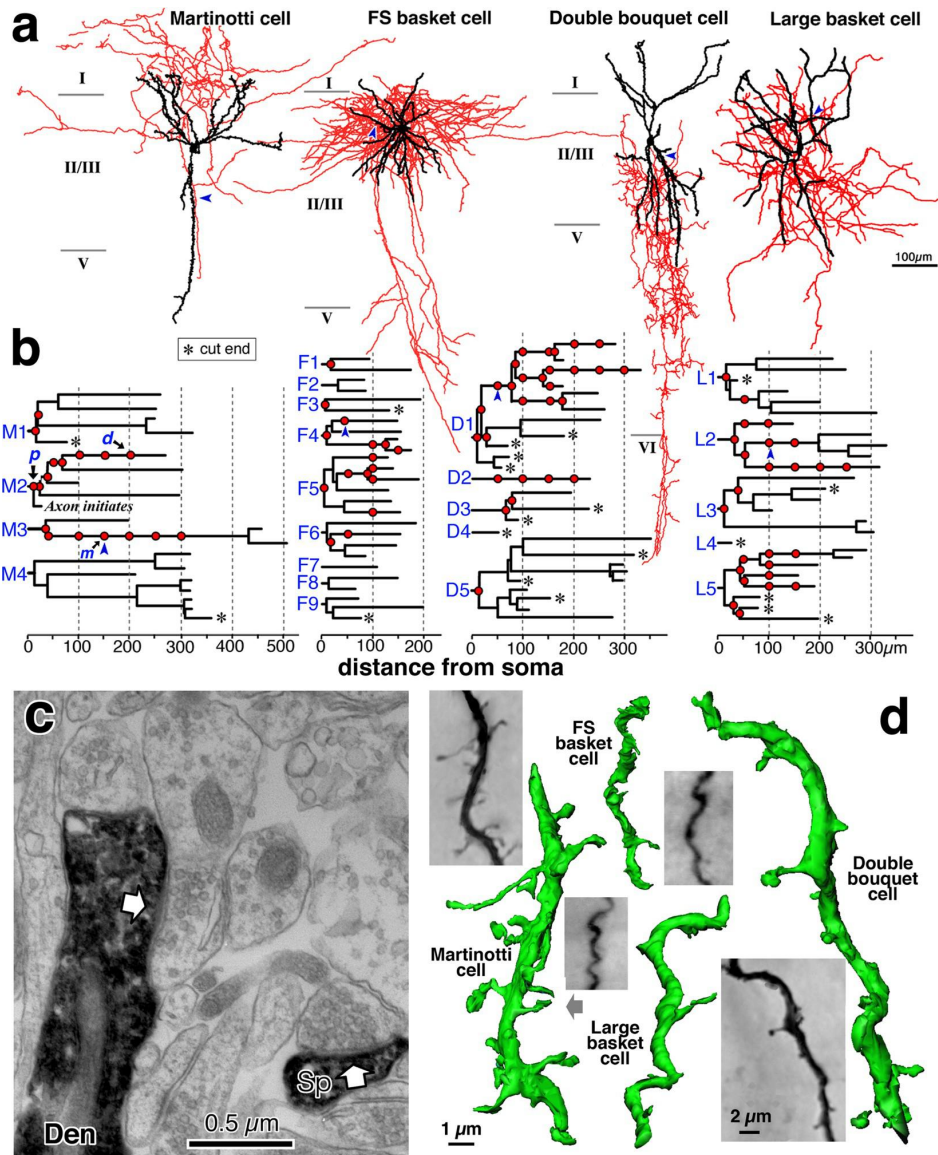


图 1 代表 4 种非锥体神经元亚型的新皮层细胞的树突结构。(a) 神经元形态。轴突用红色表示，树突和细胞体用黑色表示。MA 细胞的轴突分支延伸到第 I 层，而 FS 细胞的轴突乔木呈水平伸长。DB 细胞有一个下行狭窄的轴突分枝，LB 细胞有一个大的胞体和篮状终端。(b) 相同神经元的树状图。红色圆圈表示经过连续 EM 切片进行三维重建的段。星号表示树突的末端被切断。对树突状树进行标记(M1-M4, F1-F9, D1-D5, L1-L=)，并在后面的图中引用。Martinotti 细胞 p、m、d 段超微结构图像分别如图 3 g-i 所示。(c) 超微结构显示 MA 细胞的树突(dendrite, Den)和棘头(spine head, Sp)，与(a)和(b)箭头所示的树突段相对应，白色箭头表示这些结构上发生突触。(d) (a)和(b)中箭头表示的树突的多聚焦照片，以及同一树突段连续 EM 图像重建的树突。灰色箭头表示(c)所示的脊椎和树突。

我们的重建显示，树枝状的横截面不是近似的圆形，而是不规则的椭圆。我们使用椭圆度指数来量化这一特征，该指数通过将重构树枝晶的横截面积除以具有相同周长的真圆的面积确定，其中真圆的指数为 1，直线的指数为 0。在所有神经元中，树突横截面的平均椭圆度指数为  $0.64 \pm 0.15$  (范围: 0.33-0.95,  $n=163$ ) (图 3)。FS 细胞的树突比双花束细胞的树突稍平坦，但明显平坦 ( $p=0.029$ , 图 3c, 表 1)。在所有神经元的树突中，较小和较远的树突往往比较近、较大的树突更圆 (图 3a)。有趣的是，我们发现所有被研究神经元的横截面积和周长之间存在线性关系 (图 3e)，表明树突体积和表面积随着离胞体的距离以恒定的速率减少，而对于具有圆形横截面的树突则不是这样。如图 3f 所示，在距胞体 30-40 μm 远的树突位置，表面积体积比相对均匀。在更近的位置，表面积体积比变小。然而，近端树突节段往往充满大量细胞内膜结合的小室 (图 3g-i; 例如，线粒体、内质网、高尔基体等)，这表明我们的测量可能会不成比例地低估这些近端位置的表面积与细胞质体积比。

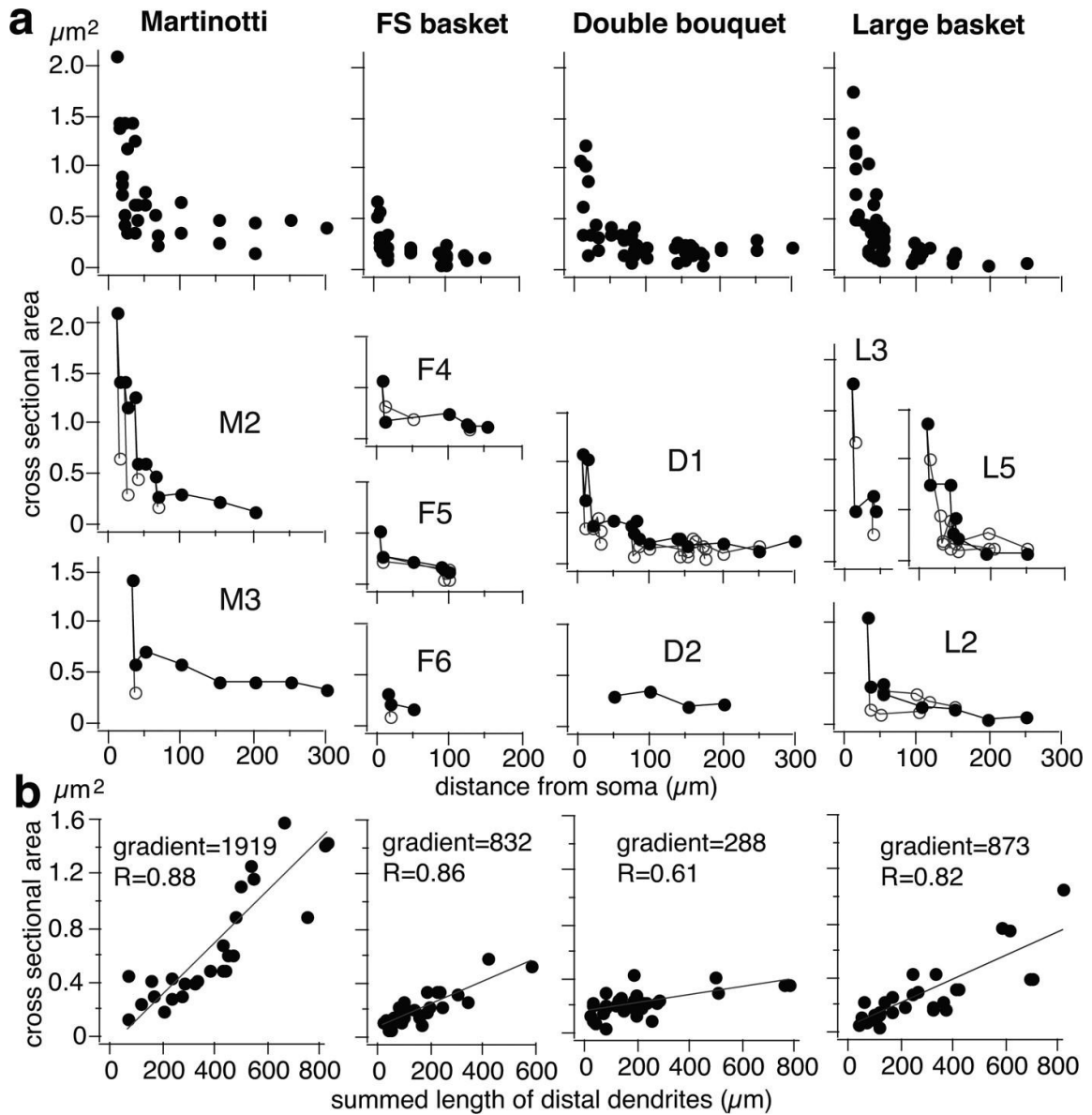


图2 非锥细胞体亚型的树突尺寸。(a)上面板:树突段的横截面积与其到索体的距离。大树突经常发现附近的细胞体,而只有小树突观察到远端位置。在近端也发现了一些小的树突。下面板:部分树木(M2、M3、F4 F6、D1、D2、L2 L4)轨迹线所示的树突线段截面积。在枝状分叉处,子枝分裂成较大的(黑色圆)和较小的(白色圆)的裂片。(b)所有非锥细胞体的树突横截面积与参考点远端树突总长度有良好的相关性。

我们的重建还显示,当树突向外延伸时,树突会发生扭曲,这一特征可以解释在光学显微镜下观察到的树突直径波动(图3d和在线补充视频1)。因此,用光学显微镜测量枝晶直径可能不可靠。

为了便于对枝晶中电压信号的计算建模,Rall描述了一种特殊情况,即枝晶支路在母枝晶和子枝晶之间具有均匀的输入电导,即所谓的三次半幂律,这使得在终端分支处具有均匀电音速距离的树枝状树可以被建模为简单的电缆。虽然已经有人尝试用光学显微镜来测量父分支和子分支的直径,但是对于真实神经元的分支点是否遵循精确的规则(如Rall的 $3/2$ 幂律)仍然存在争议。我们分析了枝晶分支点的EM3D重建(图4a),以直接解决这个问题,并发现母枝晶的横截面积与两个子枝的总横截面积匹配得很好(图4b)。为了量化这种关系,我们通过将两个子枝的总枝晶横截面积除以母枝晶的横截面积来计算横截面积匹配指数(表2)。我们发现,所有细胞类型中的大多数分支都具有近乎完美的亲子横截面积匹配。匹配的横截面积可能允许恒定数量的微管通过分叉点到达远端树突(图4c),这可以优化树突树中的传输机制。事实上,使用具有7或10 nm z步进的扫描电镜图像,我们发现树突片段中微管的数量与其横截面积呈线性相关(图5a-f)。我们还计算了镍-DAB处理的钙视网膜蛋白阳性(GABA能双束细胞的特征)两个分叉点的母支和子枝中的微管数目。母枝中微管的数量(60和62微管)与子枝中的微管总数几乎相同(分别为62和63微管)。我们还通过未标记的树突状分叉( $n=4$ ;可能是锥细胞体)追踪微管(图5g-i,补充视频2)。发现母树突中的微管连续进入两个子树突中的一个(图5k,1,补充视频3)。因此,母枝和子枝的微管总数几乎相同。这些结果表明,保持分支点的横截面积,可能有助于维持母枝和子枝中微管数量的一致性,从而促进微管依赖性向树突状树的运输。

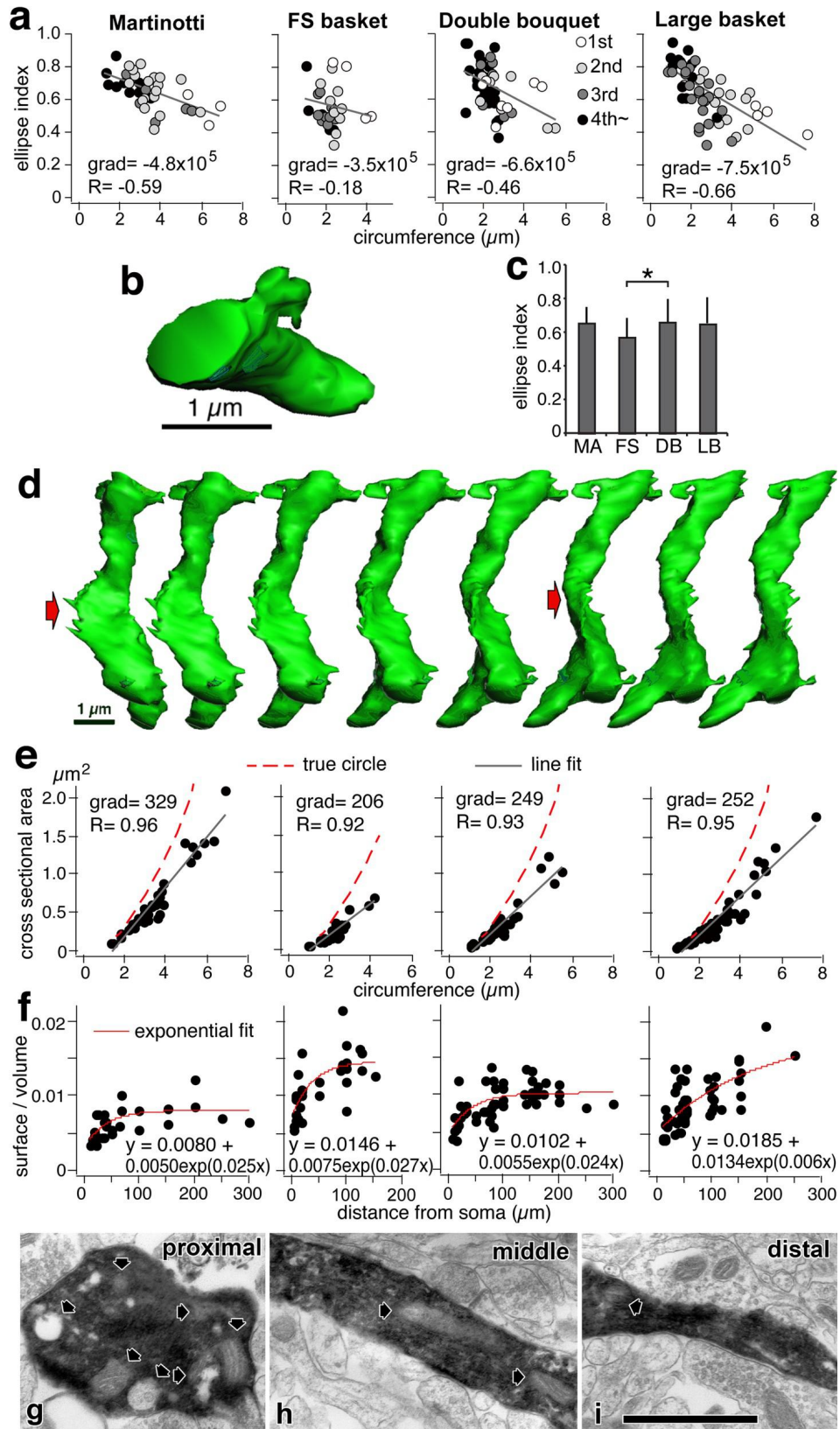


图3 非锥细胞体树突的横截面为不规则椭圆。(a) 在所研究的四个神经元中，每个神经元的树突节段的椭圆度指数与横截面积的关系。大多数枝晶的椭圆度指数一般在0.4到0.9之间。近端（较厚）树突比远端（较薄）树突更平坦。圆的索引是1，直线的索引是0。(b) Martinotti细胞树突节段的重建图像。横截面是一个椭圆。(c) FS细胞的平均椭圆度指数小于双花束细胞(\*Tukey, p50.029)。MA, 马丁诺蒂细胞; FS, FS篮状细胞; DB, 双花束细胞; LB, 大篮状细胞。(d) 旋转三维图像椭圆树突节段的大篮状细胞。从不同角度(箭头)观察时,表现厚度变化很大,从单一角度观察时,沿节段长度的明显直径波动是明显的,就像在光学显微镜下一样。(e) 根据横截面周长绘制的横截面面积显示了两个变量之间强烈的线性相关性。显示真圆线(红点曲线)和线性拟合线。较大的枝晶位于真圆曲线的下方,而较小的枝晶倾向于在曲线上或接近曲线。它们的枝晶在横截面上更倾向于椭圆形,而在横截面上则更倾向于较小的圆形。(f) 表面积与体积比与体节的距离呈常数,尤其是距体节30~40 μm或更远。(g-i) 马丁诺蒂细胞树突的超微结构在图1b的树状图中显示为p、m、d。注意,近端树突(g)包含许多细胞器,而中部(h)和远端树突(i)仅包含少量细胞器。比例尺,1 μm。

Rall 对分歧点的计算简化被称为 3/2 幂律(3/2 power law)。我们用这个公式评估了非锥体神经元树突分支处的电导匹配

$$\sqrt{c_p \times a_p} = \sqrt{c_{d1} \times a_{d1}} + \sqrt{c_{d2} \times a_{d2}} \quad (1)$$

其中  $c$  为树突的周长,  $a$  为树突的横截面积,  $p$  为亲本树突,  $d1$  和  $d2$  为子代树突(见方法)。我们发现, 大多数分支的父子  $\sqrt{c \times a}$  具有近乎完美的匹配, 至少对于皮质间神经元来说, 这证实了 Rall 在其计算模型中用于简化的原始假设(图 4d)。为了量化这种关系, 我们通过将两个子树突的分支  $\sqrt{c \times a}$  除以母树突的  $\sqrt{c \times a}$  来计算电导匹配指数 (表 2)。匹配父子分支的电导可以补偿树突拓扑结构变化对突触整合和尖峰生成的影响。

我们惊讶地发现, 皮质非锥体神经元的分支点显示出近乎完美的亲子横截面积匹配以及电导匹配。为了比较这两种关系, 我们绘制了两个公式:

$$a_p = a_{d1} + a_{d2} \quad (2)$$

$$r_p^{3/2} = r_{d1}^{3/2} + r_{d2}^{3/2} \quad (3)$$

在单个图上标识同时满足两个公式的值。为了绘制这两种关系, 我们使用  $E$  (椭圆度指数) 和  $a$  (横截面积) 将方程 (3) 转换为一种形式 (见方法)

$$\left(\frac{a_p}{E_p}\right)^{\frac{3}{4}} = \left(\frac{a_{d1}}{E_{d1}}\right)^{\frac{3}{4}} + \left(\frac{a_{d2}}{E_{d2}}\right)^{\frac{3}{4}} \quad (4)$$

利用我们对母树突横截面积的测量, 我们绘制了一系列可能的解决方案, 以匹配子树突的横截面积和电导。我们发现, 对于典型的树突尺寸, 这两种关系非常相似, 并且子枝晶的值始终接近两种关系的预测范围 (图 4e, 表 1)。我们测量了从三维重建子枝获得的数据之间的最小距离, 并将其与预测的横截面积和电导匹配值进行了比较。我们发现大篮状细胞中的树突状分支更接近横截面积匹配的预期值, 但其他细胞类型的数据对这两个概念都没有明显的偏好 ( $p > 0.05$ , 图 4e)。因此, 皮质非锥细胞体的分支点倾向于保留横截面积和电导匹配。

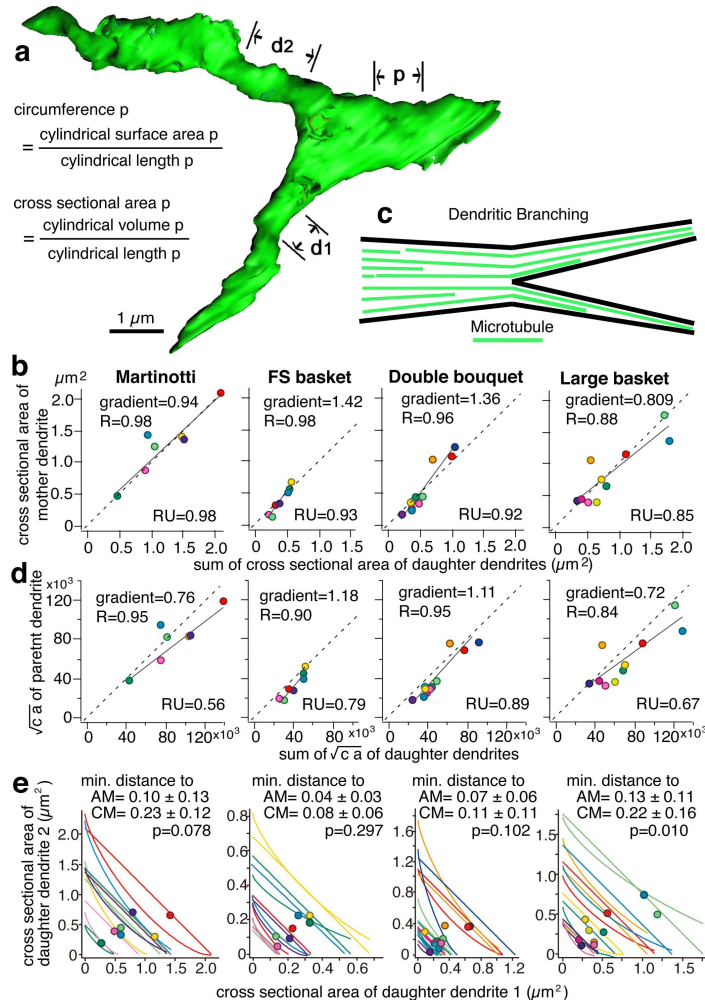


图4 树枝状分叉的横截面积保持不变，并遵循 Rall's  $s^{3/2}$  幂律。(a) 在大篮状细胞中重建树突状分支点。周长和横截面积以单位枝晶长度的平均值计算。(b) 母枝晶的横截面积与子枝晶的总截面积非常相似。线表示线性拟合，点线表示统一线。R: 拟合线的相关系数，RU: 与统一线的相关系数。(c) 图示微管在分支点的分布。在母树突中发现的微管与其中一个子树突相连。(d) 绝大多数枝条的亲电导 ( $A_p$ ) 几乎完全匹配。线表示线性拟合，点线表示统一线。R: 拟合线的相关系数，RU: 与统一线的相关系数。(e) 直线表示两个子枝晶的可能值范围，其横截面积总和与母枝晶的测量值相匹配 (面积匹配线: AM)。与直线颜色相同的弧表示两个子枝晶横截面积的值范围，其遵循 Rall's 电导匹配  $3/2$  幂律 (电导匹配曲线: CM)。对于典型的枝晶尺寸，这两条线是相似的。每个子枝晶的测量横截面积用颜色编码为直线和圆弧的圆圈表示，大多数都在这两种关系的预期值范围内。显示到 AM 和 CM 的最小距离。

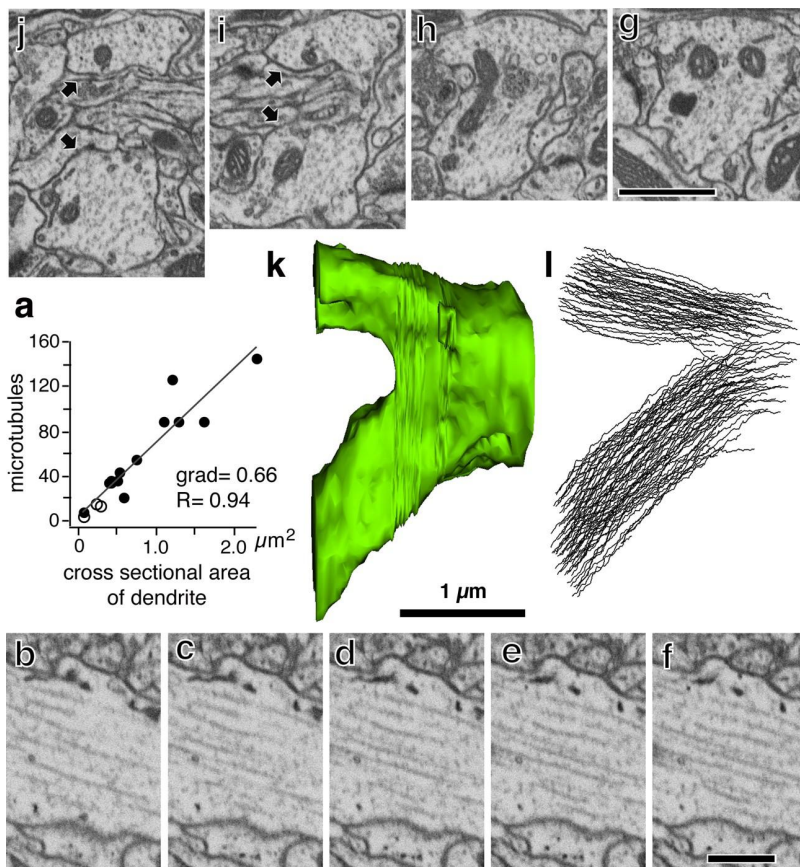


图5 树突分支点微管分布。(a) 微管数量与树突横截面积呈线性相关。黑色树突和圆形树突。(b-f) 树突树 (10 nm, 5 nm 分辨率) 的系列 FIB/SEM 图像。同样的微管可以很容易地从一片追踪到另一片。比例尺, 0.5 毫米。(g-j) 分叉枝晶 (300nm 间距, 5nm 横向分辨率) 的系列 FIB/SEM 图像。母分支 (g) 逐渐改变形状 (h, i) 并分叉为两个子分支 (箭头, (j))。比例尺, 1 $\mu$ m。k、分叉分支的三维重建 (g-j)。(l) 在 (k) 所示的分叉分支中观察到的微管痕迹。母树突中的微管与两个子树突中的一个相连

为了测试树突状分叉是否具有功能意义，我们在重构神经元形态的计算模型中模拟突触输入 (真实形态模型; 可在 ModelDB: <https://senselab.med.yale.edu/modeldb>, 型号 140299) 和形态修改消除支电导匹配 (电导失配模型) (补充图 3 和见方法)。我们用突触电流 ( $\alpha$  函数, 2nS) 在树突树的不同位置 (间隔 10 $\mu$ m) 刺激树突。在形态学上真实的模型神经元中, 兴奋性突触后电位 (EPSPs) 引起的细胞体去极化峰值 (图 6a) 显示出有限的突触位置依赖性可变性 (图 6c 中靠近底部的黑线和图 6d 中的彩色线条), 而突触输入部位的局部 EPSPs (图 6b) 具有高度的易变性和位置依赖性 (图 6c 中的彩色线条)。在电导失配模型中, 分支点不显示电导匹配, 峰值细胞体 EPSPs 振幅 (图 6e 中靠近底部的黑线和图 6f 中的彩色线) 存在较大的可变性, 如细胞体 EPSPs 振幅的变化系数所量化 (图 6g)。接下来我们研究了稳态细胞体去极化对树突的被动侵袭。体电流注入 (40pA) 使具有形态真实分支点的神经元的树突状分支去极化更加均匀, 树突状树状树中各个位置的峰值去极化变化较小 (图 7a)。相比之下, 体电流注入在电导失配模型中产生了树突的可变去极化 (图 7b), 变异性的的大小随着离胞体的距离而增加 (图 7c)。

最后, 我们研究了细胞体大小与树突状树之间的关系。我们发现在四种神经元亚型中, 细胞体和树突体积以及细胞体和树突表面积之间存在线性关系 (图 7d, e, 表 3)。细胞体体积约为树突总体积的 1.4 倍, 细胞体表面积约占树突总表面积的 7%。这些数据与假设相一致, 即较大的躯体, 包含更多的细胞内细胞器, 如核糖体, 可能需要支持更大和更精细的树突树。

讨论我们的结果揭示了树突状结构的一些特征, 这些特征是树突状树多样性和多样性的基础。首先, 树突横截面积与所有远端树突节段的总长度成正比, 而与远端分支点的数目无关。第二, 非锥体神经元树突呈椭圆形而非圆形, 椭圆

度随树突大小而减小，随距胞体距离的增加而增大。这一特性倾向于限制沿枝晶的表面与体积比的变化。最后，我们发现分支点保留了母枝晶的总横截面积。母子树突横截面积的密切匹配可以优化微管依赖性的树突内运输，促进细胞体去极化向树突状分支的有效电分布，这一过程可以减少树突拓扑结构对神经元兴奋性的影响。这种形态特性也优化了EPSPs传播对胞体的保真度。此外，我们发现，在所有的神经元亚型中，细胞体体积和表面积可以预测树突的总体积和表面积。虽然我们的结果是从青少年大鼠的神经元中获得的，但我们在体内固定的成年大鼠皮质中非锥细胞体的免疫组织化学染色树突中发现了类似的结果（22个片段，未显示数据，见免疫组化方法）。

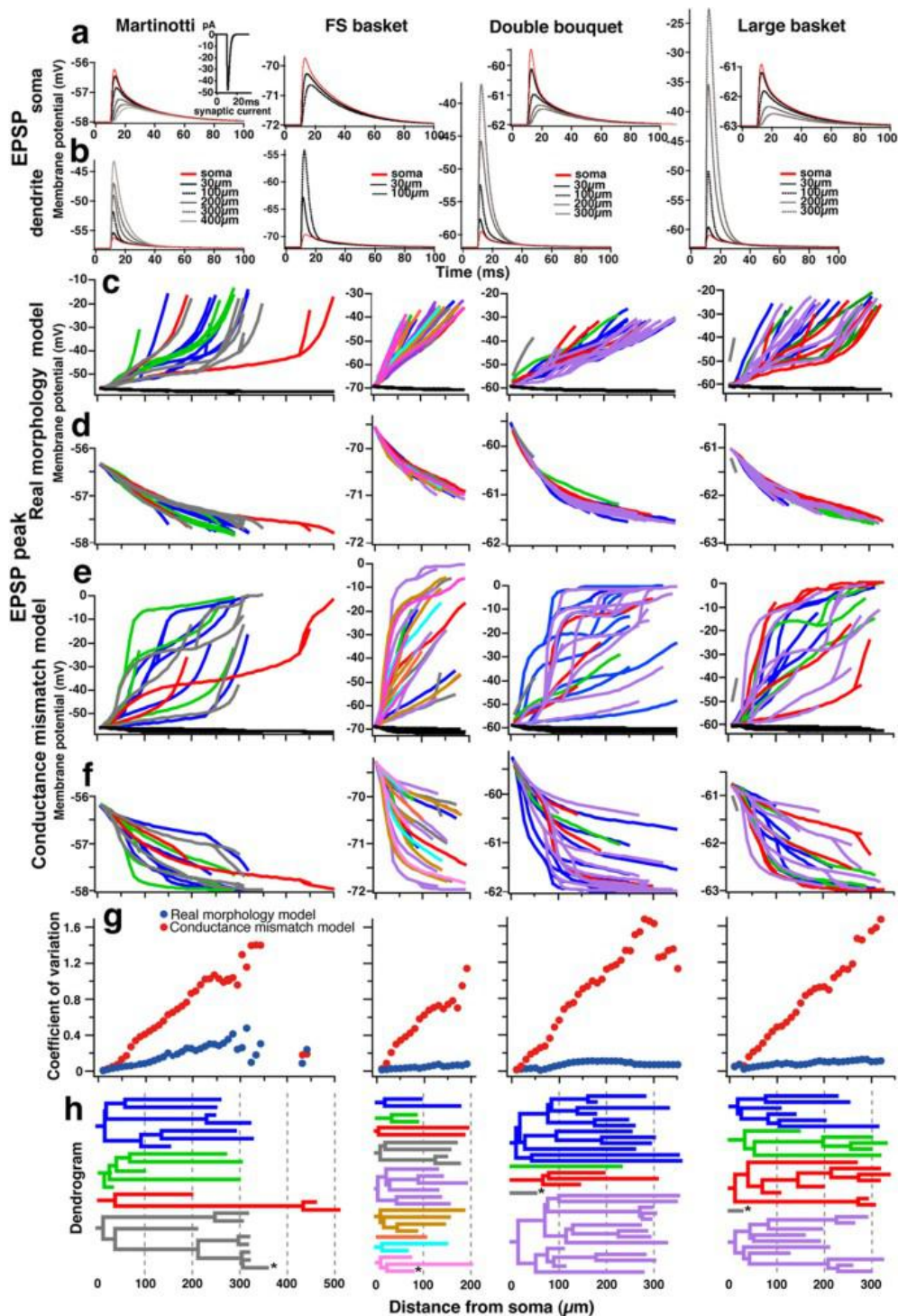


图6 支点几何的功能意义。(a-f) 树突突触反应的衰减。(a, b) 在真实形态模型细胞中树突不同部位产生的突触电流的细胞体 (a) 和局部 (b) 反应。插图显示注入的 $\alpha$ 功能突触电流 (2ns)。(c) 细胞体 (黑色) 和局部突触去极化峰 (颜色编码为树状图, 以 h 表示) 响应真实形态模型细胞树突各部分的突触输入。(d) 峰值细胞体去极化如 (c) 所示, 但规模更大。(e) 电导失配模型树突中突触在胞体 (黑色) 和突触输入处 (颜色编码为 h) 的峰值去极化图。(f) 细胞体去极化峰值, 如 (e) 所示, 但规模较大。不同分支间的偏差比真实模型单元 (d) 大。(g) (d) 和 (f) 所示模型的细胞体去极化峰值随突触输入到离胞体一定距离的树突室的变化系数 (CV) 图。真实形态模型细胞 (蓝圈) 中的 cv 在整个树突长度上非常低, 而电导失配模型细胞 (红圈) 中的 CVs 随着距胞体的距离而增加。(h) 模拟中使用的模型单元的彩色编码树状图。

Table 2 | Morphological feature at dendritic branching point

| AC index              | cross sectional area matching index |          |             |           |             | conductance-matching index |          |             |           |             |             |
|-----------------------|-------------------------------------|----------|-------------|-----------|-------------|----------------------------|----------|-------------|-----------|-------------|-------------|
|                       | EM                                  |          | Model       |           |             | EM                         |          | Model       |           |             |             |
| cell type             | n                                   | 3D image | n           | Real Mor. | C. Mismatch | n                          | 3D image | n           | Real Mor. | C. Mismatch |             |
| <b>Martinotti</b>     | <b>Average</b>                      | 7        | 0.93 ± 0.15 | 18        | 1.00 ± 0.11 | 0.94 ± 0.25                | 7        | 1.12 ± 0.17 | 18        | 1.22 ± 0.15 | 1.14 ± 0.22 |
|                       | <b>range</b>                        |          | 0.64–1.09   |           | 0.64–1.11   | 0.54–1.33                  |          | 0.80–1.29   |           | 0.80–1.45   | 0.71–1.56   |
| <b>FS basket</b>      | <b>Average</b>                      | 7        | 1.08 ± 0.29 | 16        | 1.20 ± 0.23 | 1.27 ± 0.39                | 7        | 1.24 ± 0.21 | 16        | 1.37 ± 0.19 | 1.35 ± 0.26 |
|                       | <b>range</b>                        |          | 0.82–1.69   |           | 0.82–1.89   | 0.84–2.31                  |          | 0.96–1.59   |           | 0.96–1.85   | 0.96–1.84   |
| <b>double bouquet</b> | <b>Average</b>                      | 11       | 1.07 ± 0.21 | 26        | 1.32 ± 0.30 | 1.07 ± 0.21                | 11       | 1.21 ± 0.17 | 26        | 1.45 ± 0.26 | 1.44 ± 0.35 |
|                       | <b>range</b>                        |          | 0.71–1.38   |           | 0.71–1.88   | 0.72–2.32                  |          | 0.84–1.49   |           | 0.84–1.92   | 0.91–2.09   |
| <b>large basket</b>   | <b>Average</b>                      | 10       | 1.03 ± 0.31 | 25        | 1.13 ± 0.23 | 1.12 ± 0.37                | 10       | 1.21 ± 0.29 | 25        | 1.3 ± 0.22  | 1.24 ± 0.31 |
|                       | <b>range</b>                        |          | 0.50–1.56   |           | 0.50–1.56   | 0.40–1.89                  |          | 0.63–1.60   |           | 0.63–1.65   | 0.53–1.76   |

Real Mor.: real morphology model cell, C. Mismatch: conductance-mismatch model cell.

该点远端所有树突的横截面积和总树突长度之间的线性关系表明，树突平滑地变细，与分支点无关，这一形态学特征可通过允许最初大量微管促进细胞内有效转运到树突中靠近树突基部，从胞体到所有远端树突终点连续行进。分支点处总横截面积的守恒与这一假设相一致，即允许被贩运分子均匀分布到树突中。事实上，在检查微管含量的两个神经元间分支点，我们发现子分支中的微管总数几乎与母枝晶中的数量相同。

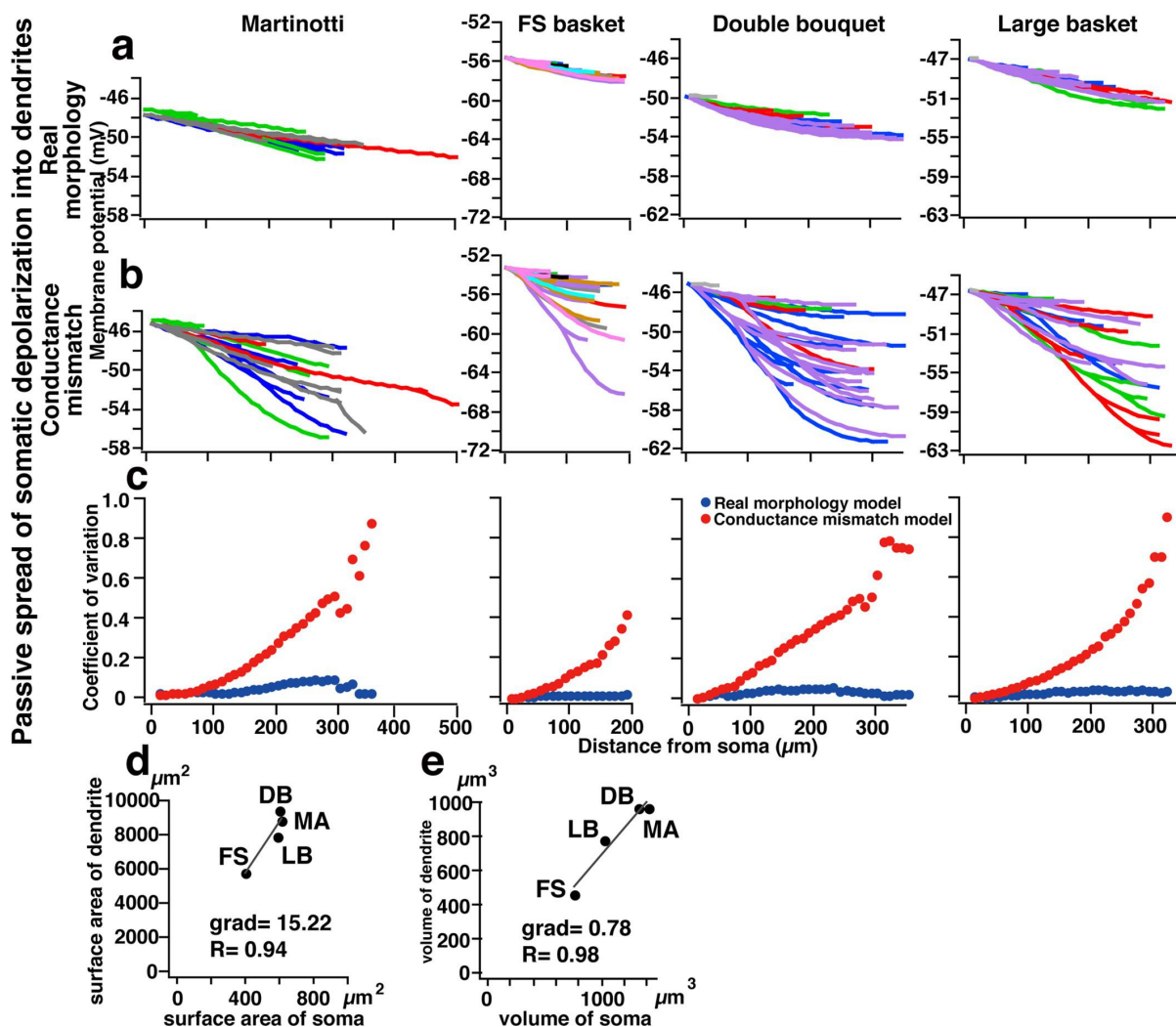


图7 为了使体细胞电压均匀地分布到树突状树中，对树枝状分支点进行了优化。(a)，树突状小室峰值去极化图对40pA体细胞稳态电流脉冲注入真实形态模型细胞的响应。树枝状去极化在树枝状分支中分布均匀。初级树突及其相关树的颜色编码如图6h所示。(b)树突室对注入具有真实形态的神经元胞体的40pA体细胞稳态电流脉冲响应的峰值去极化图。颜色编码如图6h。(c)所示，(a)和(b)中所示模型中，距胞体一定距离的所有树突室峰值去极化的变异系数(CV)图(vs.距胞体的距离)。在整个树突长度范围内，真实形态模型细胞(蓝圈)的树突去极化变异系数很低，而电导失配模型细胞(红圈)中的树突去极化变异系数随离胞体距离的增加而增大。(d)树突的表面积与在真实形态模型细胞中测量的胞体表面积相对应。(e)树突的体积与在真实形态模型细胞中测量的躯体体积相比较。



树突横截面的不规则椭圆形可能在小空间内提供更有效的神经油填充，尤其是考虑到纤维在组织中沿各个方向（垂直、水平和径向）流动。需要进一步研究神经元的堆积效率，以确定椭圆树突片段是否确实有利于神经细胞的组织。或者，近端位置的椭圆横截面将倾向于保持沿枝晶的表面积与体积比。事实上，我们发现树突在离胞体 30-40 毫米远的地方有一个相当稳定的表面积与体积比。这可能有助于使气体交换和分子扩散正常化，进入不同直径的腔室。在树突状细胞膜上均匀分布的离子通道被激活后，它也会趋向于使胞浆离子浓度正常化。给定树突细胞内钙离子的浓度，可以使钙离子的浓度保持在一个独立的位置。

树突形态的改变在神经退行性疾病中有很好的记录。我们的数据表明，在健康神经元中，树突状结构的调控比想象中更为精确，因为树突树状结构的多样性。对病理组织中树突的详细形态进行评估，以检验树突变细和分支点均匀性的改变是否参与了与疾病相关的认知缺陷的产生，这对今后的工作具有重要意义。

我们从四种不同的非锥细胞体亚型中获得的数据揭示了树突状树的形态学特征，这些树突树似乎很适合让分子和电信号均匀地逆向分布到树突树中，同时，增强顺行突触信号传导，限制树突拓扑结构对动作电位输出的影响。由于这些特征在皮质回路中的大小、形状和功能差异很大的皮质非锥细胞体亚型中是保守的，因此树突的这些特征很可能是整个大脑中许多神经元类型的树突所共有的。

**Table 3 | Length, surface area, volume of dendritic trees**

|                |                | length $\mu\text{m}$ | %              | surface area $\mu\text{m}^2$ | %             | % in dendrite | volume $\mu\text{m}^3$ | %             | % in dendrite |
|----------------|----------------|----------------------|----------------|------------------------------|---------------|---------------|------------------------|---------------|---------------|
| MA             | M1             | 1292.0               | 33.5%          | 2894.2                       | 30.5%         | 32.7%         | 279.1                  | 11.2%         | 28.9%         |
|                | M2             | 831.7                | 21.6%          | 1729.8                       | 18.3%         | 19.5%         | 168.6                  | 7.5%          | 17.5%         |
|                | M3             | 690.4                | 17.9%          | 1778.7                       | 18.8%         | 20.1%         | 231.8                  | 13.3%         | 24.0%         |
|                | M4 *           | 1040.2               | 27.0%          | 2461.1                       | 26.0%         | 27.8%         | 285.6                  | 11.5%         | 29.6%         |
|                | dendrite total | <b>3854.4</b>        | <b>100.0%</b>  | <b>8863.7</b>                | <b>93.6%</b>  | <b>100.0%</b> | <b>965.1</b>           | <b>43.5%</b>  | <b>100.0%</b> |
|                | soma           | <b>17.9 **</b>       |                | <b>610.1</b>                 | <b>6.4%</b>   |               | <b>1406.4</b>          | <b>56.5%</b>  |               |
|                | total          |                      |                | <b>9473.8</b>                | <b>100.0%</b> |               | <b>2371.5</b>          | <b>100.0%</b> |               |
|                | tree average   | 963.6                | 25.0%          | 2215.9                       |               |               | 241.3                  |               |               |
|                | SD             | 261.9                | 6.8%           | 562.0                        |               |               | 54.1                   |               |               |
|                | FS             | F1                   | 250.8          | 8.2%                         | 447.0         | 7.3%          | 7.8%                   | 35.5          | 2.9%          |
| F2             |                | 137.0                | 4.5%           | 240.9                        | 3.9%          | 4.2%          | 16.8                   | 1.4%          | 3.6%          |
| F3             |                | 373.6                | 12.2%          | 748.0                        | 12.1%         | 13.0%         | 66.7                   | 5.5%          | 14.4%         |
| F4             |                | 493.2                | 16.1%          | 977.0                        | 15.8%         | 16.9%         | 83.8                   | 6.9%          | 18.1%         |
| F5             |                | 690.4                | 22.5%          | 1270.6                       | 20.6%         | 22.0%         | 100.5                  | 8.3%          | 21.7%         |
| F6             |                | 496.2                | 16.2%          | 934.7                        | 15.2%         | 16.2%         | 72.9                   | 6.0%          | 15.7%         |
| F7             |                | 108.2                | 3.5%           | 192.3                        | 3.1%          | 3.3%          | 13.6                   | 1.1%          | 2.9%          |
| F8             |                | 201.4                | 6.6%           | 365.2                        | 5.9%          | 6.3%          | 27.1                   | 2.2%          | 5.9%          |
| F9 *           |                | 316.6                | 10.3%          | 588.5                        | 9.5%          | 10.2%         | 46.0                   | 3.8%          | 9.9%          |
| dendrite total |                | <b>3067.5</b>        | <b>100.0%</b>  | <b>5764.3</b>                | <b>93.5%</b>  | <b>100.0%</b> | <b>462.8</b>           | <b>38.1%</b>  | <b>100.0%</b> |
| soma           | <b>11.8 **</b> |                      | <b>400.1</b>   | <b>6.5%</b>                  |               | <b>751.5</b>  | <b>61.9%</b>           |               |               |
| total          |                |                      | <b>6164.4</b>  | <b>100.0%</b>                |               | <b>1214.3</b> | <b>100.0%</b>          |               |               |
| tree average   | 340.8          | 11.1%                | 640.5          |                              |               | 51.4          |                        |               |               |
| SD             | 191.8          | 6.3%                 | 368.2          |                              |               | 30.9          |                        |               |               |
| DB             | D1             | 2255.7               | 43.3%          | 4061.9                       | 40.4%         | 42.9%         | 414.4                  | 18.2%         | 42.9%         |
|                | D2             | 230.6                | 4.4%           | 397.4                        | 4.0%          | 4.2%          | 39.5                   | 1.7%          | 4.1%          |
|                | D3             | 498.0                | 9.6%           | 904.7                        | 9.0%          | 9.6%          | 89.2                   | 3.9%          | 9.2%          |
|                | D4 *           | 50.6                 | 1.0%           | 80.0                         | 0.8%          | 0.8%          | 6.6                    | 0.3%          | 0.7%          |
|                | D5             | 2169.8               | 41.7%          | 4014.5                       | 39.9%         | 42.4%         | 415.8                  | 18.2%         | 43.1%         |
|                | dendrite total | <b>5204.7</b>        | <b>100.0%</b>  | <b>9458.5</b>                | <b>94.1%</b>  | <b>100.0%</b> | <b>965.6</b>           | <b>42.3%</b>  | <b>100.0%</b> |
|                | soma           | <b>17.9 **</b>       |                | <b>597.7</b>                 | <b>5.9%</b>   |               | <b>1316.8</b>          | <b>57.7%</b>  |               |
| total          |                |                      | <b>10056.2</b> | <b>100.0%</b>                |               | <b>2282.5</b> | <b>100.0%</b>          |               |               |
| tree average   | 1040.9         | 20.0%                | 1891.7         |                              |               | 193.1         |                        |               |               |
| SD             | 1081.9         | 20.8%                | 1981.5         |                              |               | 204.8         |                        |               |               |
| LB             | L1             | 926.1                | 21.6%          | 1597.1                       | 18.7%         | 20.1%         | 151.9                  | 8.4%          | 19.5%         |
|                | L2             | 850.3                | 19.8%          | 1480.5                       | 17.4%         | 18.7%         | 142.4                  | 7.9%          | 18.3%         |
|                | L3             | 1109.8               | 25.9%          | 2334.1                       | 27.4%         | 29.4%         | 245.5                  | 13.6%         | 31.5%         |
|                | L4 *           | 25.8                 | 0.6%           | 31.3                         | 0.4%          | 0.4%          | 1.4                    | 0.1%          | 0.2%          |
|                | L5             | 1375.2               | 32.1%          | 2488.4                       | 29.2%         | 31.4%         | 237.7                  | 13.1%         | 30.5%         |
|                | dendrite total | <b>4287.1</b>        | <b>100.0%</b>  | <b>7931.3</b>                | <b>93.1%</b>  | <b>100.0%</b> | <b>778.9</b>           | <b>43.0%</b>  | <b>100.0%</b> |
|                | soma           | <b>26.7 **</b>       |                | <b>588.5</b>                 | <b>6.9%</b>   |               | <b>1030.8</b>          | <b>57.0%</b>  |               |
| total          |                |                      | <b>8519.9</b>  | <b>100.0%</b>                |               | <b>1809.7</b> | <b>100.0%</b>          |               |               |
| tree average   | 857.4          | 20.0%                | 1586.3         |                              |               | 155.8         |                        |               |               |
| SD             | 507.0          | 11.8%                | 975.0          |                              |               | 98.5          |                        |               |               |
| All cells      | dend average   | <b>4103.4</b>        |                | <b>8004.4</b>                | <b>93.6%</b>  |               | <b>793.1</b>           | <b>41.7%</b>  |               |
|                | SD             | <b>891.0</b>         |                | <b>1620.3</b>                | <b>0.4%</b>   |               | <b>237.1</b>           | <b>2.5%</b>   |               |
|                | soma average   | <b>18.6 **</b>       |                | <b>549.1</b>                 | <b>6.4%</b>   |               | <b>1126.4</b>          | <b>58.3%</b>  |               |
|                | SD             | <b>6.1</b>           |                | <b>99.7</b>                  | <b>0.4%</b>   |               | <b>296.8</b>           | <b>2.5%</b>   |               |

\*with cut end, \*\*long axis.

## 方法

**Slice recording.** Sections of frontal cortex of young Wistar rats (19-23 days postnatal) were cut to a thickness of 300  $\mu\text{m}$  in oblique horizontal cutting plane and immersed in a buffered solution containing (in  $\mu\text{M}$ ) NaCl, 124.0; KCl, 3.0;  $\text{CaCl}_2$ , 2.4;  $\text{MgCl}_2$ , 1.2;  $\text{NaHCO}_3$ , 26.0;  $\text{NaH}_2\text{PO}_4$ , 1.0; and glucose, 10.0, aerated with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . Cortical cells were targeted for whole-cell recording on a fixedstage at 30  $^{\circ}\text{C}$  using a 40x water immersion objective. The electrode solution consisted of (in  $\mu\text{M}$ ): potassium methylsulfate, 120; KCl, 5.0; EGTA, 0.5;  $\text{MgCl}_2$ , 1.7; Na2ATP, 4.0; NaGTP, 0.3; HEPES, 8.5; and biocytin, 17.

**Histology.** Tissue slices were fixed in 4% paraformaldehyde, 1.25 or 0.05% glutaraldehyde, and 0.2% picric acid in a 0.1 M phosphate-buffered (PB) solution and resectioned into 50  $\mu\text{m}$  thick slices. Slices were incubated with avidin-biotinperoxidase complex (VECTASTAIN Elite ABC Kit PK-6100, Vector Laboratories, Burlingame, U.S.A.), in Tris-HCl buffered saline (TBS) with or without 0.04% Triton X-100 (TX), and reacted with 3,3-diaminobenzidine tetrahydrochloride (DAB) (0.05%) and  $\text{H}_2\text{O}_2$  (0.003%). Slices were then postfixed in 1%  $\text{OsO}_4$  in PB, dehydrated and flat embedded on silicon-coated glass slides in Epon. The osmium/epon fixation process generates, 10% distortion in x, y, and z directions<sup>16</sup>. The shrinkage was not corrected in the analysis. They were reconstructed with NeuroLucida (MicroBrightField, Williston, VT) with a 60x objective lens. We selected 4 interneurons representing each of 4 nonpyramidal cell subtypes: a regular spiking nonpyramidal (RSNP) MA cell, a fast spiking FS cell, an RSNP DB cell, and a BSNP LB cell. Neurons were selected based on the intactness of their dendritic trees, having only minimal dendritic severing during slice preparation (Fig. 1b). The stained cells were photographed at 0.5 $\mu\text{m}$  focus steps using a 100x objective, and dendritic segments identified for EM observation were marked on those photo images. Each subtype had the stereotypical morphological properties of axonal and dendritic arborizations previously associated with those subtypes. From these neurons we selected 14 to 29 dendritic segments to make 3D reconstructions using Visilog software (Noesis, Ve lizy, France). Segments without bifurcations were chosen every 50 $\mu\text{m}$  along dendrites lacking artificially cut ends. We also chose bifurcation points from all subtypes to reconstruct. Cells were then serially sectioned into 90 nm thick slices using an ultramicrotome (Reichert Ultracut S, Leica Microsystems, Wetzlar, Germany). Ultrathin serial sections mounted on formvar-coated single-slot grids (NOTCH-NUM Grids, 132  $\mu\text{m}$  slot, SynapTek) were stained with lead citrate. The segments for EM observation were identified by comparison of EM images and light microscopic images. Electron micrographs were taken with a Hitachi H-7000 electron microscope, using tilting of up to 60 $\mu\text{m}$ . The thickness of ultra thin sections was calibrated by a color laser 3D profile microscope (VK-9500; Keyence, Japan). EM images of the segments and associated structures were captured using a CCD camera (Kodak Megaplug 1.4i, Princeton Instruments, USA) and reconstructed using a 3-D reconstruction system with the software developed by Noesis (Ve lizy, France) as an extension of their Visilog program. The mean length of the segments was 16-18  $\mu\text{m}$  (minimum 3.7 $\mu\text{m}$ , maximum 34.3 $\mu\text{m}$ ) and 7-13% of the total length of the nonpyramidal cell s dendrites was reconstructed 3 dimensionally (Table 1). These segments were reconstructed from 35 145 successive ultrathin sections (90nm thickness). Dendritic cross-sectional area and circumference were measured as averaged value of a short part of the reconstructed dendrite segment, cross-sectional area was determined as volume divided by length of a portion of the segment, and circumference was defined as surface area divided by length of the same portion (Fig. 4a). The average length of each segment portion was 724.1 $\pm$ 407.1 nm (range: 183.1-2737 nm, n=164).

**Immunohistochemistry.** Three male Wistar rats (6 weeks of age, 140–160g) were anesthetized with an overdose of Nembutal and perfused through the heart with 10ml of a solution of 250 $\mu\text{M}$  sucrose, 5 $\mu\text{M}$   $\text{MgCl}_2$  in 0.02M phosphate buffer (pH 7.4) (PB), followed by 300 ml of 4% paraformaldehyde containing 0.2% picric acid and 0.1% glutaraldehyde in 0.1M PB. Brains were then removed and oblique horizontal sections (50  $\mu\text{m}$  thick) of frontal cortex were cut on a vibrating microtome (Leica VT1200S, Nussloch, Germany). Tissue sections were dehydrated in 10% sucrose in PB, followed by 25% sucrose and 10% glycerol in PB for 2 hours, frozen in liquid nitrogen. The sections were then incubated in 0.1M PB containing 1% sodium borohydrate for 30 minutes and in 0.05M Tris-buffered saline (TBS) containing 1%  $\text{H}_2\text{O}_2$  for 30 minutes before incubation with primary antiserum against either somatostatin developed in rabbit (generous gifts from Dr. R Benoit, mixture of S309 [1:1000] and S320 [1:4000]), parvalbumin developed in mouse (1:4000, P-3171, Sigma-Aldrich, Saint Louis, MO, U.S.A.), or calcitonin developed in mouse (monoclonal; Chemicon, Temecula, CA, U.S.A., MAB1568; 1:2500) diluted in TBS containing 10% normal goat serum and 2% bovine serum albumin overnight at 4 $^{\circ}\text{C}$ . The sections were then incubated in biotin-conjugated secondary antiserum (1:200, BA-1000 or BA-2000, Vector Laboratories) followed by the ABC (Vector Laboratories), and staining with DAB with nickel (0.02% DAB, 0.3% nickel in 0.05M Tris HCl buffer). The stained sections were post-fixed for 40 minutes in 1.5% potassium ferrocyanide and 1% osmium tetroxide, followed by 1 hour in 1% osmium tetroxide alone and dehydrated in graded dilutions of ethanol with 1% uranyl acetate added at the 70% ethanol dehydration state. After flat-embedding in Epon, the sections were observed and photographed under the light microscope as described under ‘Histology’. Then slices were serially re-sectioned at a thickness of 90nm using the ultramicrotome. The labeled dendrites and associated structures were photographed and digitized from EM negatives using a scanner (GT-9800F, Epson, Suwa, Japan). The structures were reconstructed using the 3D reconstruction software, Reconstruct; <http://synapses.clm.utexas.edu/tools/index.stm>.

**FIB/SEM image capture (Microtubule analysis).** Two male Wistar rats (8 weeks of age, 240g) were anesthetized with an overdose of Nembutal and perfused through the heart with 10 ml of a solution of 250 $\mu\text{M}$  sucrose, 5  $\mu\text{M}$   $\text{MgCl}_2$  in 0.02 M phosphate buffer (pH 7.4) (PB), followed by 300 ml of 2% paraformaldehyde containing 0.2% picric acid and 2% glutaraldehyde in 0.1M PB at 37 $^{\circ}\text{C}$ . Warming up the perfusion solution to body temperature preserves the structure of microtubules. Brains were then removed and oblique horizontal sections (50 $\mu\text{m}$  thick) of frontal cortex were cut on a vibrating microtome (Leica VT1200S, Nussloch, Germany). The sections were post-fixed for 40 minutes in 1.5% potassium ferrocyanide and 1% osmium tetroxide, followed by 1 hour in 1% osmium tetroxide alone and dehydrated in graded dilutions of ethanol with 1% uranyl acetate added at the 70% ethanol dehydration state. Tissue was then flat-embedded in Epon.

For electron microscopy observation, the Epon blocks containing cortical layer II/III were glued to an aluminium stub using silver paint. Besides the top surface, the whole block was covered by silver paint to avoid charging the epoxy. The top surface to be studied was coated by several 10 nm thick layers of iridium using a sputter coater. For 3D analysis the mounted blocks were transferred in a combined FIB/SEM (Focused Ion Beam / Scanning Electron Microscope) system using the Carl Zeiss Auriga40 Crossbeam(Carl Zeiss NTS GmbH, Oberkochen, Germany). In a combined FIB/SEM system, very site specific regions of interest near the surface of embedded tissue in epoxy can be directly cross-sectioned and imaged for three dimensional analysis. The z-resolution is given by FIB slice thickness which can be in the nanometer scale. Regions of interest were identified by SEM imaging the top surface of the block and by comparison with light microscopy images. The top of the regions was protected by ion beam induced deposition of platinum (25um\*25um area, 1 nA ion beam current, 900 sec deposition time). A view channel for SEM observation was coarse milled and the region was separated from the bulk by two side cuts using a 16nA ion beam. With a 2nA ion beam, the first coarse cross-sectioning was medium polished. Using a 600 pA ion beam probe, the surface of the crosssection was further fine polished and slices of 10 nm thickness were milled. After each slice, the milling was paused and the freshly exposed surface of the cross-section was imaged at 1.5kV acceleration potential using the in-column energy selective backscattered electron (EsB) detector. The retarding potential of the EsB detector was set to 1314V (data cube A) or 1014V (data cube B). By low kV imaging and detection of the low loss back-scattered electrons of the stained tissue surface, imaging was achieved. Using contrast inversion, TEM-like contrast and comparable imaging information was achieved. The acquired images have a 2048\*1536 store resolution, and a image pixel size of 5 nm (data cube A) or 7 nm (data cube B). The cross-section was imaged by the SEM at 54 deg stage tilt and the tilt of the cross-section vs. the electron beam was corrected (tilt angle of 36 deg was used for correction). The milling time per slice was between 15.8 and 17.5sec. The imaging time per slice was about 52sec. The serial image alignment and tracing the microtubules were done using Fiji image processing package: <http://fiji.sc/wiki/index.php/Fiji>, plugins "Register Virtual Stack" or "TurboReg" for the alignment, and "TrakEM2" for the tracing. The structures were reconstructed using the 3D reconstruction software, Reconstruct (available at <http://synapses.clm.utexas.edu/tools/index.stm>)

**Statistics.** We used Mann Whitney U test for statistical analysis and ANOVA (followed by Tukey test) for ellipticity index of 4 subtypes of nonpyramidal cells for Fig. 3c and Table 1, and Wilcoxon signed-rank test (non-parametrics) for Fig. 4e.

三维重建树突的 Rall 模型和分枝部分。

1)利用周长和横截面积将 Rall 模型方程转化为简单方程。

$$G_N(p) = G_N(d_1) + G_N(d_2) \quad (5)$$

Rall 的电导匹配假设，其中  $G_N(p)$  为母树突的输入电导， $G_N(d)$  为子树突的输入电导。电导是由下式定义

$$G_N(\text{inf inite cable}) = \frac{2\pi r^{3/2}}{\sqrt{R_m R_i / 2}} \quad (6)$$

式中， $R_i$  为细胞内比电阻率( $\Omega\text{-cm}$ )， $R_m$  为膜电阻率( $\Omega\text{-cm}^2$ )， $r$  为树突半径。Rall 模型(式(5))可定义为

$$\frac{2\pi r_p^{3/2}}{\sqrt{R_m R_i / 2}} = \frac{2\pi r_{d1}^{3/2}}{\sqrt{R_m R_i / 2}} + \frac{2\pi r_{d2}^{3/2}}{\sqrt{R_m R_i / 2}} \quad (7)$$

化简后可得

$$r_p^{3/2} = r_{d1}^{3/2} + r_{d2}^{3/2} \quad (8)$$

$c$  为树突的周长，用  $c=2\pi r$  表示。 $a$  为枝晶的横截面积，用  $a=\pi r^2$  表示。把这些放在一起

$$c \times a = 2\pi r \times \pi r^2 = 2\pi^2 r^3 \quad (9)$$

开平方根可得

$$\sqrt{c \times a} = \sqrt{2\pi^2 r^3} = \sqrt{2}\pi r^{3/2} \quad (10)$$

两边同除以  $\sqrt{2}\pi$  可得

$$r^{3/2} = \frac{\sqrt{c \times a}}{\sqrt{2}\pi} \quad (11)$$

方程 (8) 可变换为如下形式

$$\frac{\sqrt{c_p \times a_p}}{\sqrt{2}\pi} = \frac{\sqrt{c_{d1} \times a_{d1}}}{\sqrt{2}\pi} + \frac{\sqrt{c_{d2} \times a_{d2}}}{\sqrt{2}\pi} \quad (12)$$

化简后可得

$$\sqrt{c_p + a_p} = \sqrt{c_{d1} + a_{d1}} + \sqrt{c_{d2} + a_{d2}} \quad (13)$$

2)利用横截面积和椭圆度指数 E 将式(8)转化为式，利用椭圆度指数 E，得到半径 r 与横截面积 a 的简单关系如下

$$E = \frac{a}{\pi r^2} ; r = \sqrt{\frac{a}{\pi E}} \quad (14)$$

方程 (8) 可变换为如下形式

$$\left(\frac{a_p}{E_p}\right)^{\frac{3}{4}} = \left(\frac{a_{d1}}{E_{d1}}\right)^{\frac{3}{4}} + \left(\frac{a_{d2}}{E_{d2}}\right)^{\frac{3}{4}} \quad (15)$$

仿真分析。使用神经元 7.127 进行模拟。真实的形态学模型忠实地反映了我们在非锥体细胞的 4 个亚型中发现的形态学结果。利用图 2b 和 4b 所示的枝晶尺寸规则（补充图 3），对切片制备过程中产生的一些人工切割端进行了人为延伸。所有模型均为被动型（膜电阻：25000  $\Omega\text{cm}^2$ ，细胞内电阻：200  $\Omega\text{cm}$ ，膜容量：100F/ $\text{cm}^2$ ）。泄漏电导的平衡电位设置为 -58 mV（MA 细胞）、-72 mV（FS 细胞）、-62 mV（DB 细胞）和 -63 mV（LB 细胞），这些值反映了实验测定的这些神经元亚型的平均静息膜电位。突触电流模拟为 a 突触（2nS，t=1ms），注入模型细胞的所有树突状室。为了使用神经元（使用圆柱形电缆）模拟椭圆截面树突，我们首先用横截面与实际形态相匹配的锥形圆柱体对每个非锥体神经元进行建模，然后修改每个腔室中的泄漏电导和膜电容，使其与估计值相等在我们的椭圆树突中（图 3e）。校正系数按以下步骤计算。我们通过线性回归从 EM 数据中得到了从远端树突的总长度（R）得出横截面积（S）和周长（L）的函数。

DB: S= 365.833×R+1122000, L=1.51953×R+11540.9

MA: S=1508.73×R+16958.1、L=4.61523×R+11511.3

FS: S=831.893×R+183164、L=3.26633×R+11600.4

LB: S=890.593×R+143202, L=4.10123×R+11161.2

2 模型直径（D）根据 EM 或以上函数的截面积计算，如果没有 EM 数据可用。

3 为了使圆形模型等效于具有椭圆形状模型，我们通过乘以修正系数 L/（ $\pi D$ ）来校正被动泄漏电导和膜电容，使这两个特性更大。我们还通过使较小的子树突的直径比原始尺寸大 1.5 倍（即横截面积比原来大 2.25 倍），构建了每个神经元的电导失配模型，较大的子枝晶直径为原始尺寸的一半（即横截面积变为四分之一）。电导失配模型的电导匹配指数比实际形态的模型具有更大的标准差和更宽的范围，尽管平均值相似（表 2）。为了测量模型细胞的体支和树突状支的体积和表面积，我们假设这些分支是截短的圆锥，其中体积（ $V_b$ ）用下式计算

$$V_b = L_b \times (a_p + a_d + \sqrt{a_p \times a_d}) / 3 \quad (16)$$

其中  $L_b$  为分支的长度， $a_p$  为分支近端截面积， $a_d$  为分支远端截面积。用表面积( $S_b$ )计算

$$S_b = L_b \times (c_p + c_d) / 2 \quad (17)$$

式中， $c_p$  为近侧周长， $c_d$  为远侧周长。细胞体也被认为是椭圆形的；计算体积( $V_s$ )的长椭球体

$$V_s = 4/3\pi a^2 b \quad (18)$$

其中 a 为赤道半径(沿 x、y 轴)，b 为光镜测得的极半径。计算表面积( $S_s$ )

$$S_s = 2\pi(a^2 + ab\alpha / \sin \alpha) ; \alpha = \cos^{-1}(a/b) \quad (19)$$

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## Acknowledgements

We thank Y. Itoh, N. Yamaguchi, S. Hatada and A. Sekigawa for technical assistance. Dr. R. Benoit for generous gifts of somatostatin antisera. Dr. Maki K. Yamada for fruitful advice to visualize microtubule. We thank Drs. Nelson Spruston, Charles J. Wilson, Rafael Yuste and Jesse H. Goldberg for valuable comments on the manuscript. This work was supported by KAKENHI (22120518, 20500300); Hayama Center for Advanced Studies (HCAS); and the Naito Foundation Subsidy for Natural Science Researches.

## Author contributions

Y. Kubota designed the project and conducted the electron microscopy. Y. Kawaguchi did all of slice experiments. F. Karube did LM reconstruction and data analysis. Y. Kubota and F. Karube reconstruct images from serial EM photos. M. Nomura conducted all simulation studies and Y. Kubota analyzed the results. A. Mochizuki provided analysis for Fig. 4e. A. T. Gulledge aided in data analysis and interpretation. A. Schertel operated FIB/SEM for serial image data set acquisition. All authors wrote the paper.

## Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests The author(s) declare no competing financial interests.

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How to cite this article: Kubota, Y. et al. Conserved properties of dendritic trees in four cortical interneuron subtypes. *Sci. Rep.* 1, 89; DOI:10.1038/srep00089 (2011).