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Fatherhood affects dendritic spines and vasopressin V1a receptors in the primate prefrontal cortex

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Like human fathers, male marmosets help raise their young, yet the ways in which fatherhood influences the brain remain largely unknown. We show that first-time and experienced marmoset fathers have enhanced density of dendritic spines on pyramidal neurons in prefrontal cortex as compared to non-fathers. In parallel, the abundance of vasopressin V1a receptors and the proportion of V1a receptor–labeled dendritic spines increase.

Marmosets are unusual among mammals in that fathers care extensively for their offspring, by carrying, protecting and feeding the young[1]. The complex activities of parenting may involve brain regions implicated in goal-directed behavior, such as the prefrontal cortex (PFC). Indeed, neuroimaging studies show that stimuli related to one's own child activate the anterior paracingulate and orbitofrontal areas of the PFC[2,3]. Notably, the PFC shows structural plasticity in adulthood[4,5] and contains receptors for several neuropeptides implicated in parental behavior[6], such as vasopressin, oxytocin and prolactin[7,8]. Here we examine whether fatherhood is associated with structural and neuropeptide receptor changes in the marmoset PFC.

We compared the brains of first-time and experienced marmoset fathers with those of adult male non-fathers living in mating pairs. All marmoset fathers in the study engaged in paternal care and carried their young (**Fig. 1a**). Fathers carried their infants on average over 70% of the time during the first month of the infant's life (**Supplementary Methods** online). We examined dendritic spine density in the dorsal part of cytoarchitectonic area FD[9] of the prefrontal cortex and found that fatherhood enhanced dendritic spine density on apical and basal trees of Golgi-impregnated pyramidal cells located in cortical layer II/III (**Fig. 1b,d**; PFC-apical, $F_{2,11} = 16.35$, $P = 0.001$; PFC-basal, $F_{2,11} = 12.43$, $P = 0.0026$). We observed no differences in these measures between first-time and experienced fathers. Fatherhood does not uniformly upregulate dendritic spine density, as this and related dendritic measures in the pyramidal cells of layer II/III of the occipital cortex (areas V1/V2) did not differ between fathers and controls (**Supplementary Table 1** online). Using the lipophilic tracer DiI, we confirmed the fatherhood-induced increase in dendritic spine density in the PFC (**Fig. 1c,d**; PFC-apical, $F_{2,11} = 43.27$, $P < 0.0001$; PFC-basal, $F_{2,11} = 104.5$, $P < 0.0001$). Because dendritic length on PFC pyramidal cells did not differ among the groups (**Supplementary Table 2** online), the enhancement in dendritic spine density most likely reflects an increase in the number of dendritic spines rather than a decrease in dendritic segment length.

Next, in the region showing changes in dendritic spine density, we examined receptors for several neuropeptides possibly involved in parenting: vasopressin (V1a, V1b), oxytocin and prolactin. We found that fatherhood enhanced the abundance of V1a receptors in the PFC (**Fig. 2a,c**; $F_{2,11} = 7.69$, $P = 0.011$). Neither the abundance of V1b,

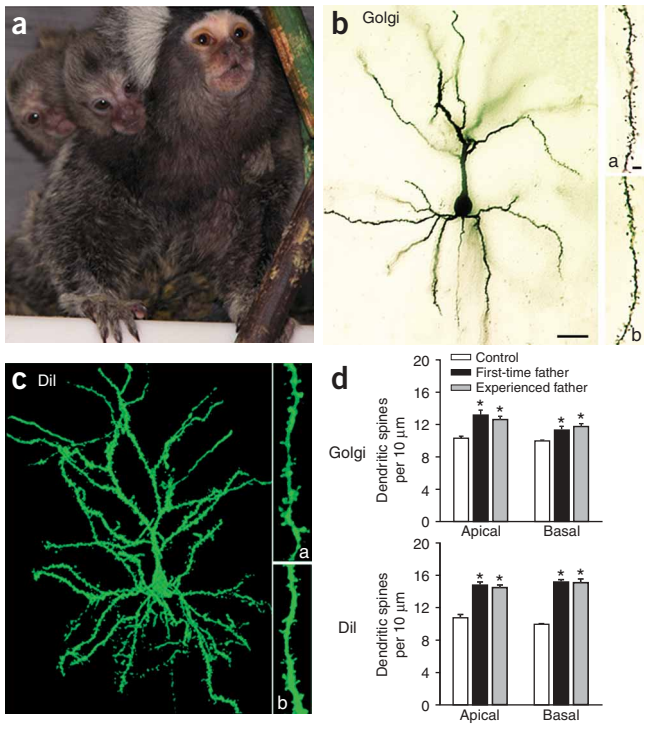

Figure 1 Fatherhood enhances dendritic spine density on prefrontal cortex pyramidal cells. (**a**) Marmoset father in our colony carrying infants. (**b**) Golgi-impregnated PFC pyramidal neuron of a marmoset father, with close-up views of apical (a) and basal (b) dendrites. (**c**) DiI-labeled PFC pyramidal neuron of a marmoset father, with close-up views of apical (a) and basal (b) dendrites. (**d**) Dendritic spine density of Golgi-impregnated (above) or DiI-labeled (below) PFC layer II/III pyramidal neurons. First-time and experienced fathers have greater dendritic spine density than controls. Error bars here and elsewhere represent s.e.m.; asterisks reflect significant differences from controls (Tukey post-hoc tests after one-way analysis of variance, $P < 0.05$). Scale bars in **b**: 20 µm (cell), 2 µm (dendrites). Same magnification in **c**.

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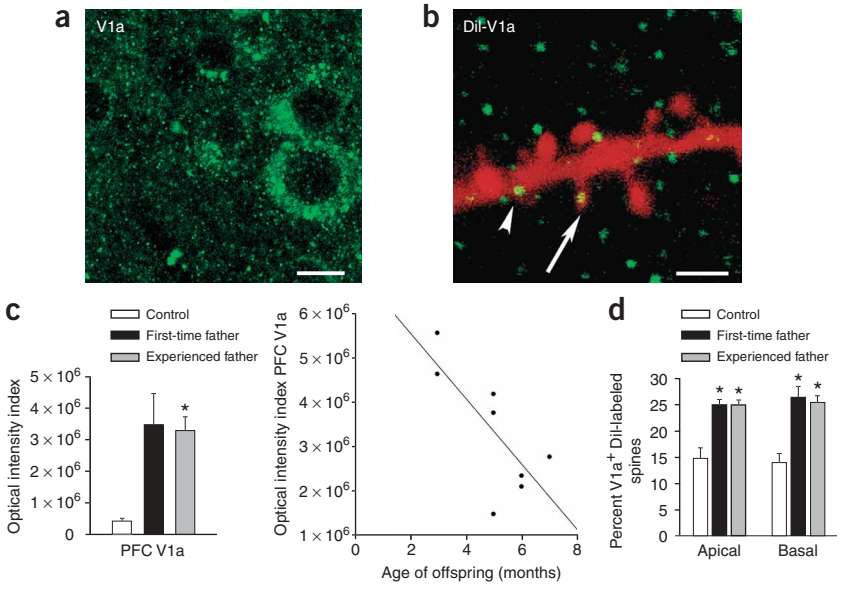

Figure 2 Fatherhood induces vasopressin V1a receptor changes in the prefrontal cortex. (**a**) Vasopressin V1a receptor immunolabeling in the PFC of a marmoset father. (**b**) DiI-labeled dendritic segment from a PFC pyramidal cell (red), labeled with vasopressin V1a receptor antibody (green). Some V1a receptor immunolabeling is located on dendritic spines (arrow) or the dendritic shaft (arrowhead). (**c**) Optical intensity levels of V1a receptor are greater in fathers relative to controls (left), but V1a receptor abundance declines with the age of the offspring (right). (**d**) A greater proportion of dendritic spines show immunolabeling for the V1a receptor in the PFC of marmoset fathers as compared to controls. V1a staining was abolished by preincubation with the immunizing peptide. Scale bars: **a**, 10 μm; **b**, 2 μm.

oxytocin and prolactin receptors in the PFC nor the abundance of V1a receptors in the occipital cortex were affected (**Supplementary Table 3** online). Marmoset infants are usually carried until they are 3 months old, but carrying can continue for months thereafter[1]. V1a receptor abundance was negatively correlated with the age of the father's youngest offspring (**Fig. 2c**, two-tailed Pearson's $r = -0.749$, $P = 0.033$), suggesting that the receptor enhancement may be temporary and driven by recent contact with infants. We examined the cellular location of V1a receptors on DiI-labeled pyramidal neurons and found receptor expression on the cell bodies, dendritic shafts and individual dendritic spines (**Fig. 2b**). Fatherhood increased the proportion of dendritic spines that were immunoreactive for V1a receptors (**Fig. 2d**; V1a spines-apical, $F_{2,10} = 17.19$, $P = 0.001$; V1a spines-basal, $F_{2,10} = 14.62$, $P = 0.002$).

Given these fatherhood-induced changes in V1a receptors in the PFC, we investigated the possible source of vasopressin to this region. Consistent with earlier findings[10], we detected vasopressin immunolabeling in the hypothalamic paraventricular and supraoptic nuclei (**Fig. 3a**,**b**), neither of which is known to project to the PFC. We also found vasopressin immunolabeling in the PFC pyramidal cell bodies, including those located in layers II/III (**Fig. 3c**). V1a receptor binding excites cortical neurons[11], and excitation can induce spine growth[12]; therefore, activity at the V1a receptor could precede the formation of new dendritic spines. Because most PFC pyramidal layer II/III neurons extend local and long-range intralayer collaterals[13], the presence of vasopressin in their somata implies that fatherhood may enhance dendritic spine density on the same population of neurons that synthesizes vasopressin and expresses the V1a receptor.

Altogether, we provide evidence for significant structural reorganization in the prefrontal cortex of infant-carrying primate fathers and a parallel enhancement in the abundance of vasopressin V1a receptors. These preliminary results raise questions for future studies. First, what are the neural mechanisms underlying this reorganization? In this regard, if our results demonstrating V1a receptor immunolabeling changes with fatherhood reflect changes in the number of binding sites, then it is possible that the structural changes are causally linked to alterations in vasopressin signaling. Second, given that marmosets breed cooperatively, do similar effects occur in the brains of nonparental caregivers? And finally, what are the functional consequences of the structural and vasopressin V1a receptor changes in fathers?

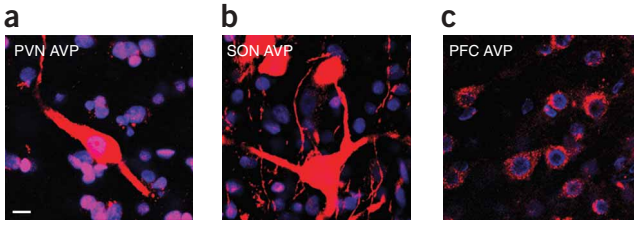

Figure 3 Vasopressin is observed not only in the hypothalamus but also in the prefrontal cortex. (**a**–**c**) Vasopressin (AVP) immunolabeling in the hypothalamic paraventricular nucleus (PVN) (**a**), in the supraoptic nucleus (SON) (**b**) and in layer II/III pyramidal neurons (**c**) in the PFC of a marmoset father (AVP, red; Hoechst DNA stain, blue). Scale bar: 10 μm (applies to all panels).

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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