Supplementary Methods

Animal Care and Treatment. Twelve adult male Common marmosets (Callithrix jacchus) were used for these studies. Four of the animals had no fatherhood experience, 4 had a single set of offspring, and 4 were fathers with multiple sets of offspring. Age did not differ statistically among the groups (group means: 4.2, 3.9, and 5.4 years, respectively). Animals were housed in male-female pairs, or mating pairs with subadult offspring, and evidence of mating was observed in all pairs, including controls. Each marmoset pair or family lived in a large cage, equipped with branches and a variety of objects, such as straw nests, wooden ladders and swings. A subset of families was videotaped and the videotapes were time sampled to quantify the proportion of time that infants were carried by their fathers ( > 10 daytime hours evenly distributed across the first month of life). The males were injected with an overdose of sodium pentobarbital and transcardially perfused using 4% paraformaldehyde. Animal procedures were approved by the Princeton University IACUC committee and were conducted in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Golgi impregnation. A modified Golgi-Kopsch protocol was used to process the tissue\(^1\). Small blocks of brain tissue containing the prefrontal cortex (PFC) or the occipital cortex (OC) were incubated in 4% potassium dichromate:dH\(_2\)O at room temperature in the dark, for 3 days. The solution was changed daily. The blocks were then rinsed in graded concentrations of silver nitrate and shaken in the dark in 1% silver nitrate:dH\(_2\)O for 1 week. Unilateral coronal sections were cut (125 µm thick) in dH\(_2\)O, rinsed in graded concentrations of ethanol, cleared, mounted on slides and coverslipped under Permount.

Slides were coded prior to data collection for this and other analyses. Five apical and five basal dendritic segments (each between 10 and 20 µm long) on five fully impregnated neurons in each region of interest were analyzed for dendritic spine density with a 100X oil objective on a BX-60 Olympus microscope using Neurolucida software (MicroBrightfield). To be selected for analysis, Golgi-impregnated pyramidal neurons had to satisfy the following criteria: 1) location within layers II/III of dorsal cytoarchitectonic area FD of the PFC\(^2,3\) (roughly corresponding to Walker’s areas 46, 9 and 12 in the macaque) or V1-V2\(^4\) of the OC; 2) thorough and consistent impregnation
(dark black/brown staining with no breaks in dendrites); and 3) isolation from surrounding cells. For every cell, the dendritic segments selected for analysis were 1) on secondary or tertiary dendrites; 2) located at least 50 µm away from the soma for apical dendrites and 30 µm for basal dendrites; and 3) located mostly in one plane of focus. Only spines extending away from the shaft, and thus easy to identify, were counted. This underestimates the number of spines located on the dendrite because some are obscured by the dendritic shaft. However, this exclusion is applied across all groups and does not appear to unfairly bias one population over another. Randomly selected dendrites satisfying these criteria on 5 cells of each type per animal were examined. The resulting data were averaged to get separate values for apical and basal dendritic spine density in every animal (N = # of animals here and elsewhere).

For dendritic length and branching analyses, 3 additional cells per region per animal were selected from the set of neurons satisfying the criteria described above. These cells were traced for every animal, providing an average length of apical and basal dendritic trees, as well as the number of dendritic bifurcations, or branch points, within a 125 µm-thick brain section. Neurons which met these criteria were randomly selected from each brain region; no more than two such neurons were analyzed per section.

**DiI labeling.** Crystals of a carbocyanine dye DiI (Molecular Probes, Eugene, OR) were implanted in the corpus callosum at the level of the caudal PFC. Tissue was incubated in Tris buffer in the dark for a minimum of 4 weeks, sectioned (125 µm thick) and imaged using a Zeiss Axiovert confocal laser-scanning microscope (510 LSM). This approach retrograde-labelled prefrontal cortex pyramidal neurons in layers II/III of area FD². Analyses of dendritic spine density, following the same parameters as described above for the Golgi method, were conducted on 2D projections made from Z-stacks of 0.5 µm-thick scans, containing apical and basal dendrites of fully labelled PFC pyramidal neurons. As with the Golgi-impregnated cells, we counted spines extending away from the shaft.

**Immunofluorescence.** Unilateral coronal sections of the PFC (40 µm-thick) were incubated in rabbit anti-vasopressin V1a and V1b, anti-oxytocin (all 1:200, Alpha
Diagnostic), anti-prolactin (1:200, Zymed) receptor or guinea pig anti-AVP (1:200, Peninsula Laboratories) antibodies with 0.3 \% Triton X-100 in Tris buffered saline (TBS) for 48 hours at 4°C. Unilateral coronal sections of the OC (40 µm-thick) were incubated in rabbit anti-vasopressin V1a antibody with 0.3 \% Triton X-100 in TBS for 48 hours at 4°C. Then, the tissue was incubated for 1 hour in the dark with goat anti-rabbit or goat anti-guinea pig antibody conjugated to Alexa 488 (1:500; Molecular Probes), mounted, dried and coverslipped. Omission of the primary antibody blocked the staining in all cases; for the vasopressin V1a receptor, pretreating the antibody with the blocking peptide (Alpha Diagnostic) also prevented immunolabeling.

Quantitative immunohistochemical analysis of staining intensity for neuropeptide receptors was carried out in areas showing changes in dendritic spine density and in the OC, as previously described\textsuperscript{5-6}. Briefly, a Zeiss Axiovert confocal microscope with 510 LSM software was used to determine the average pixel intensity and cross-sectional area of immunolabeling in layer II/III of dorsal cytoarchitectonic area FD\textsuperscript{2} or V1/V2 regions of the OC\textsuperscript{4}. Holding scanning parameters constant, ten 1-µm-thick sites (each site was 3.32 mm\textsuperscript{2} in area) were sampled for each marker in every region of interest in each animal. Background labeling was subtracted using the following procedure. Pixel values of a randomly chosen representative image were expressed as a gray scale (0-255), and photometric offset value was raised until lower intensity background labeling was excluded. This constant value was subtracted from every image in the data set to exclude background labeling. For every scan, average pixel intensity after offset was multiplied by the area of pixels falling over the threshold and summed over the sampling sites, yielding an index of immunolabeling intensity. For all markers and regions, this value was gathered for each animal and used for statistical comparisons.

PFC sections labelled with DiI were used for double-labeling with V1a receptor. DiI is a lipophilic dye that diffuses from labelled cells with damaged membranes, so incubation times for vasopressin V1a receptor antibodies were shortened in order to minimize the time from sectioning DiI-labelled tissue to analysis. Sections were sliced in Tris buffer at the thickness of 100 µm, incubated overnight in the dark in rabbit anti-vasopressin V1a with 10 \% Tween-20 at room temperature, followed by 1 hour long
incubation in goat anti-rabbit anti-body conjugated to Alexa 488 (1:500). At least 3 PFC pyramidal neurons and over 100 dendritic spines on secondary and tertiary apical and basal dendritic trees were separately examined for every animal ( > 200 total dendritic spines per animal) using a Zeiss Axiovert confocal laser-scanning microscope (510 LSM), yielding a percentage of Dil-labelled dendritic spines which were labelled with vasopressin V1a receptor antibody. Dil-labelled cells and dendrites for this analysis were selected following the same parameters as for dendritic spine density, without examining the V1a staining. For every selected dendrite, a 10-20 μm-long segment was fully analyzed and all dendritic spines extending away from the shaft were included.

*Image Acquisition and Processing.* Photomicrographs of Golgi-impregnated tissue were obtained using ImagePro Plus software (Media Cybernetics) on a computer attached to an Olympus BX-50 microscope, with 40X or 100X oil immersion objectives. Confocal photomicrographs were obtained on Zeiss Axiovert 100 confocal microscope (40X water immersion objective; Enterprise, Argon and HeNe lasers) attached to a computer with 510 LSM software (Zeiss). 2D projections were created in 510 LSM from 3D Z-stacks of 0.5-1 μm-thick scans throughout regions or objects of interest, at appropriate magnifications. Minor cropping, whole field brightness/contrast adjustments and final figure layout were conducted on full resolution images using Adobe Photoshop CS.

**References**


