The impact of paternal care on the postnatal development of inhibitory neuronal subpopulations in prefrontal and limbic brain regions was studied in the rodent Octodon degus. Comparing offspring from biparental families with animals raised by a single mother revealed region-specific deprivation-induced changes in the density of PARV- and CaBP-D28k expressing cells. Some deprivation-induced changes were only seen at P21: elevated CaBP-D28k-positive neurons in the orbitofrontal cortex, CA1, CA3, and dentate gyrus (DG) and elevated PARV-positive neurons in the lateral orbitofrontal, prelimbic/infralimbic (PL/IL), DG and CA1, nucleus accumbens, and amygdala. Some deprivation-induced changes were obvious in both age groups: increased CaBP-D28k-positive neurons in the nucleus accumbens shell and increased PARV-positive neurons in the ventral orbitofrontal. Some deprivation-induced changes were only seen in adulthood: increased CaBP-D28k-positive neurons in the amygdala and decreased PARV-positive neurons in the PL/IL and in CA3. In CA1, PARV-positive neurons were increased at P21 and decreased in adulthood. The functional significance of the deprivation-induced changes in PARV-positive neurons, which are involved in gamma oscillations and thereby affect information processing and which appear to be key players for critical period plasticity in sensory cortex development, as well as the behavioral implications remain to be further elucidated.

Keywords: biparental, Calbindin-D28K, calcium-binding proteins, enriched environment, limbic, Parvalbumin

Introduction

A variety of studies revealed that growing up without a supportive and caring father increases the risk of mental dysfunctions, low educational performance, and criminal activity (Baskerville 2002; O’Neill 2002; Erhard and Janig 2003; Boyce et al. 2006). The importance of paternal care and the survival benefits for his offspring is also emerging from studies in a variety of rodents (Wynne-Edwards 1987; Cantoni and Brown 1997; Libshaber and Eilam 2002; Wright and Brown 2002; Vieira and Brown 2003; Bredy et al. 2004; Schrading and Pillay 2004; Bredy et al. 2007; Helmeke et al. 2009; Pinkernelle et al. 2009). It appears likely that the behavioral differences of father-deprived individuals result from altered brain circuits, which have been formed under semideprived (i.e., without father) family conditions. This view is supported by findings in the biparental rodents Peromyscus californicus and Octodon degus, which revealed that paternal care significantly affects maturation of excitatory spine synapses in prefrontal cortical and limbic regions (Ovtscharoff et al. 2006; Bredy et al. 2007; Helmeke et al. 2009; Pinkernelle et al. 2009). Based on these findings, the aim of this study was to test the hypothesis whether paternal care also affects the development of inhibitory circuits, in order to maintain a "homeostatic" balance between excitation and inhibition within prefrontal and limbic pathways.

Various subpopulations of interneurons have been identified according to morphological, physiological, and neurochemical characteristics (Monyer and Markram 2004). Research on the functional development of visual cortex revealed evidence that γ-aminobutyric acidergic (GABAergic) neurotransmission triggers the opening and closing of sensitive developmental time windows and thereby interferes with the maturation of functional cortical columns (Hensch 2005). In view of the fact that sensory development essentially depends on environmental stimuli, as does the functional development of emotional pathways (prefrontal and limbic) (Poeggel et al. 2003; Helmeke et al. 2008, 2009), the present study aimed to identify the impact of paternal care on the development of inhibitory interneurons in limbic and prefrontal circuits. We did not attempt to analyze the full spectrum of GABAergic interneuron populations but rather selectively focused on the 2 populations expressing Parvalbumin (PARV) and Calbindin-D28K (CaBP-D28K), which predominantly innervate and inhibit the dendrites (CaBP-D28K-expressing neurons) or somata (PARV-expressing neurons) of pyramidal neurons, where a reduction of excitatory spine synapses has been found in father-deprived animals (Helmeke et al. 2009).

The diurnal trumpet-tailed rat O. degus (Fig. 1F) is an ideal animal model to study the impact of parental care on behavioral, endocrine, and neuronal development. Their precocity (gestational period of 90 days) allows to assess developmental changes of brain and behavior at early postnatal stages. Degus display complex social and familial systems, in which both the mother and father participate in the care for their offspring. The quantification of parent–offspring interactions revealed that paternal care comprises about 37% of total parent–offspring interactions (Helmeke et al. 2009; Pinkernelle et al. 2009), and that single degu mothers do not compensate the absence of the father by intensifying maternal care. Thus, the father-deprived family represents a semideprived social environment, which—by comparing with the biparental family situation—can unveil the contribution of paternal care on the development of brain and behavior of his offspring.

Materials and Methods

Animals

Octodon degus used in this study were bred in our colony at the Leibniz Institute for Neurobiology, Magdeburg. The families consisted of an
adult couple and their offspring. Until postnatal day (P) 21, the families were each housed in wire cages (5100 × 4200 × 6800 mm) equipped with climbing devices and longitudinal clay tubes used for nest building in an air-conditioned room with an average temperature of 22 °C. The animals were exposed to an artificial light/dark cycle (12 h/12 h; 6 AM to 6 PM). Fresh drinking water, rat diet pellets (SSNIFF special diets GmbH), and vegetables were available ad libitum. All experiments were performed in accordance with the European Community’s Council Directive of 24th November 1986 (86/609/EEC) and according to the German guidelines for the care and use of animals in laboratory research, and the experimental protocols were approved by an ethics committee.

The following groups were analyzed, to avoid litter effects only 1 or 2 male pups were histologically analyzed from each family.

**Group I biparental family:**

1. Juvenile offspring ($N = 6$): The pups were raised by both parents and with siblings until postnatal day (P) 21.
2. Adult offspring ($N = 6$): The pups were raised together with their mother and siblings until the onset of puberty (P45). At P46, 2 male siblings of each litter were separated from their parents and housed together until P90 (adolescence).

**Group II single-mother family/father-deprived ($N = 6$):** Pups were raised without father, who was removed from the home cage 1 day after the birth of his offspring (P1).

1. Juvenile offspring: The pups were raised together with their mother and siblings until P21.
2. Adult offspring ($N = 6$): The pups were raised together with their mother and siblings until the onset of puberty (P45). On P46, 2 male siblings of each litter were separated from their parents and group housed until P90 (adolescence).

**Histology**

**Fixation and sectioning**

The animals were deeply anesthetized with Ketanest/Rompun (1:4, 7 mL/kg, intramuscularly) (Ketanest: Forke-Davis; Rompun: Bayer) and transcardially perfused with 50-mL Tyrode’s solution (Sigma-Aldrich) and 1% Liqueomin (Roche), followed by 150 mL freshy prepared 4% paraformaldehyde in 0.1 M sodium acetate buffer (pH = 6.5) and 300-400 mL freshly prepared 4% paraformaldehyde in 0.1 M sodium borate buffer (pH = 9.3). Brains were removed, weighed, and postfixed at 4 °C for 1 h in the last fixative. Series of 50 µm transverse vibratome (Leica Instruments GmbH) sections were collected in phosphate-buffered saline (PBS) as series in 24 well plates.

**Immunohistochemistry**

Alternating sections were incubated with 1) mouse anti-parvalbumin (PARV) antibodies (1:2500; Sigma-Aldrich) or 2) mouse anti-Calbindin-D28K (CaBP-D28K) antibodies (1:5000; SWANT) diluted in PBS containing 1% normal goat serum, 0.05% Triton-X-100 at 4 °C for 48 h. After rinsing, the sections were incubated with biotinylated secondary antibody (1:200), goat anti-mouse, respectively, followed by incubation with streptavidin-peroxidase complex (Sigma-Aldrich) and secondary antibody (1:200), goat anti-mouse, respectively, followed by incubation with streptavidin-peroxidase complex (Sigma-Aldrich) and final reaction in a DAB solution of 0.05% 3,3′-diaminobenzidine hydrochloride, 2% ammonium nickel sulfate (Riedel-de Haen AG), and 0.01% hydrogen peroxide in Tris-HCl buffer. In control sections, the primary antibody was omitted to test for unspecific background staining by the secondary and/or streptavidin-peroxidase detection system, none of these sections displayed unspecific staining.

**Quantitative Microscopy**

The general anatomy of the degu brain, as described in the degu brain atlas (Wright and Kern 1992), closely resembles that of the rat brain. For quantitative light microscopy, the following regions were analyzed: medial prefrontal cortex (mPFC, PrCm, ACd, and prelimbic/infralimbic [PL/IL]), orbitofrontal cortex (OFC: ventral orbitofrontal [VO], lateral orbitofrontal cortex [LO]), hippocampal CA1 and CA3 regions, dentate gyrus (DG), pregeminal aspects of the core nucleus accumbens shell (AcbSh) and core (AcbC) region of the nucleus accumbens (Acb) (Groenewegen et al. 1999; Zahm et al. 2003), basolateral (BLA), and the central (CeA) nucleus of the amygdala (Fig. 1A-E). These regions were selected due to their known functional role in the regulation of social and emotional behaviors (medial and orbitofrontal cortices), emotional-ity (Acb and amygdala), and learning and memory formation (hippocampus and prefrontal cortex). In the nucleus accumbens shell (AcbSh) region, only the intensely stained subregion was analyzed (Fig. 1 C).

Since the 2 marker proteins showed a layer-specific distribution in the medial prefrontal cortical subregions, the quantitative analysis was conducted separately for layer 2/3 and 5/6. Due to the specific cellular distribution of CaBP-D28K-immunoreactive neurons the CA1 region, the subregions stratum oriens and stratum pyramidale radius were analyzed separately. In the DG, where CaBP-D28K was detected in granule cells as well as in interneurons, only the immunoreactive interneurons were counted. Stratum lacunosum-moleculare of CA1 and stratum moleculare of the DG did not contain CaBP-D28K-immunoreactive neurons and thus were not analyzed.

For each animal and each region, an average of 3–5 sections (separated by a distance of 150 µm) were selected along the rostrocaudal extent, on which the brain region of interest displayed clear immunostained CaBP-D28K, clearly identifiable immunostained neurons. The regions of interest...
were scanned for both hemispheres using a computer-connected microscope equipped with a digital camera and analyzed (magnification \( \times 20 \) DG, CA1, CA3 mPFC regions, OFC-regions, and Acb; \( \times 10 \) BLA, CeA) using the NIS-Elements 2.3 (Laboratory Imaging Software, Nikon).

For all hippocampal layers, subregions of the amygdala, mPFC, OFC, and the Acb, the number of immunostained neurons were counted and related to the total area (in \( \mu m^2 \)) and used for statistical analysis.

**Statistical Analysis**

All measurements were made by an experimenter blind to the experimental conditions of the animals. For the statistical calculation, the counts of the serial sections were averaged to obtain a single value per brain region. Since no hemispheric effects and interactions were detectable, the hemispheric data were pooled and a two-way analysis of variance (ANOVA) (SigmaPlot 11, Systat Software) was applied with rearing conditions and age as factors. Holmes-Sidak post hoc tests were used to compare selected groups. The significance level was set at \( P < 0.05. \)

**Preparation of Figures**

Digital images were processed by using Adobe Photoshop 7.0 (Adobe Systems Incorporated) and assembled into montages. Only general adjustments of color, contrast, and brightness were made.

**Results**

Three-way ANOVA revealed no significant lateralization effects for all brain regions, which were analyzed.

**Medial Prefrontal Cortical Regions**

Due to the different distribution of immunopositive neuronal structures in the medial prefrontal cortical subregions, the quantitative analysis was conducted in a layer-specific manner. In general, for the CaBP-D28k-immunoreactive neurons, we observed in both age groups and treatment groups a higher density in layers 2/3 compared with layers 5/6 in the PrCm, Acd, and in the PL/IL (Fig. 2). For PARV-immunopositive neurons, a slightly higher density was found in the lower layers compared with the upper layers.

**Calbindin-D28k**

Neither at P21 nor at P90 significant differences could be observed between biparentally and father-deprived animals in all layers of the PrCm, Acd, and in the PL/IL (Fig. 2). Two-way ANOVA revealed no significant effect in PrCm 2/3 (age: \( F_{1,22} = 4.13, P = 0.054 \); deprivation: \( F_{1,22} = 0.01, P = 0.926 \); age \( \times \) deprivation: \( F_{1,22} = 1.72, P = 0.203 \)), PrCm 5/6 (age: \( F_{1,22} = 3.27, P = 0.084 \); deprivation: \( F_{1,22} = 0.87, P = 0.361 \); age \( \times \) deprivation: \( F_{1,22} = 1.53, P = 0.229 \)), Acd 2/3 (age: \( F_{1,22} = 3.91, P = 0.06 \); deprivation: \( F_{1,22} = 0.66, P = 0.817 \); age \( \times \) deprivation: \( F_{1,22} = 2.63, P = 0.119 \)), Acd 5/6 (age: \( F_{1,22} = 4.65, P = 0.042 \); deprivation: \( F_{1,22} = 0.11, P = 0.747 \); age \( \times \) deprivation: \( F_{1,22} = 3.05, P = 0.095 \)), PL/IL 2/3 (age: \( F_{1,22} = 1.17, P = 0.291 \); deprivation: \( F_{1,22} = 0.04, P = 0.838 \); age \( \times \) deprivation: \( F_{1,22} = 4.01, P = 0.058 \)), PL/IL 5/6 (age: \( F_{1,22} = 0.00, P = 0.998 \); deprivation: \( F_{1,22} = 3.59, P = 0.071 \); age \( \times \) deprivation: \( F_{1,22} = 0.25, P = 0.623 \)).

**Parvalbumin**

In general, in all medial prefrontal cortical subregions, the density of PARV-positive neurons tends to decrease between P21 and P90 in both animal groups, this developmental reduction was more pronounced in the fatherless group.

A significant effect of father deprivation was detected in the PL/IL subregion, whereas in all other subregions, no effect of father deprivation but sometimes age effects could be detected.

Two-way ANOVA revealed: PrCm 2/3 (age: \( F_{1,23} = 13.47, P < 0.001 \); deprivation: \( F_{1,23} = 0.89, P = 0.355 \); age \( \times \) deprivation: \( F_{1,23} = 0.49, P = 0.49 \)), PrCm 5/6 (age: \( F_{1,23} = 2.15, P = 0.156 \); deprivation: \( F_{1,23} = 2.09, P = 0.167 \); age \( \times \) deprivation: \( F_{1,23} = 0.81, P = 0.365 \)), Acd 2/3 (age: \( F_{1,23} = 23.19, P < 0.001 \); deprivation: \( F_{1,23} = 0.13, P = 0.718 \); age \( \times \) deprivation: \( F_{1,23} = 5.19, P = 0.032 \)), Acd 5/6 (age: \( F_{1,23} = 8.40, P = 0.008 \); deprivation: \( F_{1,23} = 0.29, P = 0.594 \); age \( \times \) deprivation: \( F_{1,23} = 4.55, P = 0.048 \)). In adulthood, the father-deprived animals showed significantly higher densities of PARV-expression neurons in layers 2/3 of the PL/IL. In adulthood, the father-deprived animals showed significantly reduced densities of PARV-immunoreactive neurons in layers V/VI of the PL/IL prefrontal regions (Fig. 3).
In contrast, the fatherless juvenile (P21) animals displayed significantly elevated densities of CaPB-D28k-positive neurons in the shell region. Two-way ANOVA revealed the following effects: age: $F_{1,23} = 9.32$, $P = 0.006$; deprivation: $F_{1,23} = 13.69$, $P < 0.001$; age x deprivation: $F_{1,23} = 0.40$, $P = 0.532$). At P21, the father-deprived animals displayed significantly elevated densities of CaPB-D28k-positive neurons in the shell region compared with age-matched biparental controls ($P = 0.006$). This difference seen in the juvenile groups was still maintained in adulthood ($P = 0.038$, Fig. 4), even though the biparental controls also showed a significantly increased density of CaPB-D28k-positive neurons between P21 and P90 ($P = 0.011$). The juvenile fatherless animals have already reached a similar density of CaPB-D28k-positive neurons in the shell region as the biparental control group in adulthood.

Figure 2. Prefrontal cortical regions: Comparison of the density of CaPB-D28k-immunoreactive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). Due to the different distribution of immunopositive neuronal structures in the medial prefrontal cortical subregions, the quantitative analysis was conducted in a layer-specific manner. Levels of significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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**Parvalbumin**

Two-way ANOVA revealed the following effects: core region (age: $F_{1,23} = 1.55, P = 0.226$; deprivation: $F_{1,23} = 10.44, P = 0.004$; age × deprivation: $F_{1,23} = 1.51, P = 0.232$), shell region (age: $F_{1,22} = 0.34, P = 0.565$; deprivation: $F_{1,22} = 12.39, P = 0.002$; age × deprivation: $F_{1,22} = 3.11, P = 0.092$). At the age of P21, the AcbC ($P = 0.005$) and AcbSh ($P = 0.006$) of fatherless juvenile animals displayed significantly elevated numbers of PARV-positive neurons compared with control animals (Fig. 4), whereas in adulthood, this difference was no longer significant.

**Hippocampus (CA1, CA3 Regions and Dentate Gyrus)**

Two-way ANOVA revealed the following effects: stratum oriens (age: $F_{1,20} = 13.11, P = 0.002$; deprivation: $F_{1,20} = 0.83, P = 0.374$; age × deprivation: $F_{1,20} = 6.20, P = 0.022$), stratum pyramidale/radiatum CA1 (age: $F_{1,20} = 2.57, P = 0.125$; deprivation: $F_{1,20} = 1.22, P = 0.282$; age × deprivation: $F_{1,20} = 0.05, P = 0.818$), CA3 region (age: $F_{1,20} = 9.37, P = 0.006$; deprivation: $F_{1,20} = 9.43, P = 0.006$; age × deprivation: $F_{1,20} = 1.74, P = 0.202$), DG (age: $F_{1,20} = 0.06,$

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**Figure 3.** Prefrontal cortical regions: Comparison of the density of PARV-immunoreactive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). Due to the different distribution of immunopositive neuronal structures in the medial prefrontal cortical subregions, the quantitative analysis was conducted in a layer-specific manner. Levels of significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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Parvalbumin

Two-way ANOVA revealed the following effects: core region (age: $F_{1,23} = 1.55, P = 0.226$; deprivation: $F_{1,23} = 10.44, P = 0.004$; age × deprivation: $F_{1,23} = 1.51, P = 0.232$), shell region (age: $F_{1,22} = 0.34, P = 0.565$; deprivation: $F_{1,22} = 12.39, P = 0.002$; age × deprivation: $F_{1,22} = 3.11, P = 0.092$). At the age of P21, the AcbC ($P = 0.005$) and AcbSh ($P = 0.006$) of fatherless juvenile animals displayed significantly elevated numbers of PARV-positive neurons compared with control animals (Fig. 4), whereas in adulthood, this difference was no longer significant.

Hippocampus (CA1, CA3 Regions and Dentate Gyrus)

Calbindin-D28k

Two-way ANOVA revealed the following effects: stratum oriens (age: $F_{1,20} = 13.11, P = 0.002$; deprivation: $F_{1,20} = 0.83, P = 0.374$; age × deprivation: $F_{1,20} = 6.20, P = 0.022$), stratum pyramidale/radiatum CA1 (age: $F_{1,20} = 2.57, P = 0.125$; deprivation: $F_{1,20} = 1.22, P = 0.282$; age × deprivation: $F_{1,20} = 0.05, P = 0.818$), CA3 region (age: $F_{1,20} = 9.37, P = 0.006$; deprivation: $F_{1,20} = 9.43, P = 0.006$; age × deprivation: $F_{1,20} = 1.74, P = 0.202$), DG (age: $F_{1,20} = 0.06,$
In the stratum pyramidale/radiatum of CA1, no significant differences of CaPB-D28k-positive neurons were found in juvenile (P21) and adult (P90) father-deprived and biparentally raised animals (Fig. 5). In the stratum oriens of juveniles, elevated densities of CaPB-D28k-positive neurons (P = 0.026) were found compared with biparental controls, which during development significantly decreased until adulthood (P < 0.001) (Fig. 5).

Figure 4. Nucleus accumbens: Comparison of the density of CaPB-D28k-immunoreactive and PARV-positive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). (A) and (B) illustrates the significant increase of CaPB-D28k-immunoreactive neurons in father-deprived animals (−father, B) compared with biparental controls (+father, A) at P21; quantification is shown in (E). Note the increased staining intensity of neuropil in the father-deprived animals (B) compared with biparental controls (A). (C) and (D) illustrates the significant increase of PARV-immunoreactive neurons in father-deprived animals (−father, D) compared with biparental controls (+father, C) at P21; quantification is shown in (E). Note the increased staining intensity of neuropil in the father-deprived animals (D) compared with biparental controls (C). Scale bars A-D = 50 μm. Levels of significance: *P < 0.05; **P < 0.01; ***P < 0.001.
In the CA3 region, a significant elevation of CaBP-D28k-positive neurons was observed in the juvenile father-deprived group compared with age-matched biparentally raised animals ($P = 0.006$). Between P21 and P90, a significant decrease of CaBP-D28k-positive neurons was found in the father-deprived animals ($P = 0.006$), which resulted in similar densities of CaBP-D28k-positive neurons in both adult animal groups (Fig. 5).

In the DG ($P = 0.02$) and hilus ($P = 0.017$), significantly elevated densities of CaBP-D28k-immunoreactive neurons were observed in the juvenile father-deprived group compared with control animals (Fig. 5), in adulthood, this difference was no longer observed.

**Parvalbumin**

Two-way ANOVA revealed the following effects: CA1 region (age: $F_{1,20} = 53.49$, $P < 0.001$; deprivation: $F_{1,20} = 0.28$, $P = 0.604$; age × deprivation: $F_{1,20} = 13.91$, $P < 0.001$), CA3 region (age: $F_{1,20} = 69.07$, $P < 0.001$; deprivation: $F_{1,20} = 1.08$, $P = 0.310$; age × deprivation: $F_{1,20} = 15.24$, $P < 0.001$), DG (age: $F_{1,20} = 25.48$, $P < 0.001$; deprivation: $F_{1,20} = 1.34$, $P = 0.260$; age × deprivation: $F_{1,20} = 7.86$, $P = 0.011$), hilus (age: $F_{1,20} = 31.77$, $P < 0.001$; deprivation: $F_{1,20} = 0.40$, $P = 0.535$; age × deprivation: $F_{1,20} = 4.79$, $P = 0.041$). In the CA1 region at P21, significantly more parvalbumin-expressing cells were found in the fatherless group ($P = 0.035$) compared with the biparental controls. During postnatal development between P21 and P90, the number of parvalbumin-expressing cells decreased significantly (with father: $P = 0.02$, without father: $P < 0.001$). At P90, significantly less PARV cells were found in the CA3 region, a significant elevation of CaPB-D28k-positive neurons was found in the fatherless reared animals compared with degus, reared with father ($P < 0.001/BLA$, $P = 0.01/CeA$). In the BLA, the elevated number of parvalbumin-stained cells in the fatherless group decreased until P90 ($P < 0.001$), where no significant differences were seen between the 2 rearing groups in the BLA, and no difference between the 2 rearing groups was seen in the CeA (Figs 7 and 8).

**Ratio of Calbindin-D28k-Positive/Parvalbumin-Positive Neurons**

It has been shown that PARV- and CaBP-D28k-immunoreactive neurons transduce their inhibitory function at different compartments of principal neurons, that is, PARV-neurons preferentially innervate soma and axon, while CaBP-D28k neurons terminate on their apical dendrites. Therefore, changes in the relation between these 2 inhibitory subpopulations in the father-deprived animals in comparison with the biparental controls are of interest to further understand the changes in inhibitory balance along the different neuronal compartments.

**Medial prefrontal cortical regions**

In layer 2/3 of all medial prefrontal subregions, CaBP-D28k-expressing neurons are the dominant interneuron subtype, whereas in layers 5/6, PARV-expressing neurons are dominant.

In layers 2/3 of the PrCm, AgCd, and PL/IL, the ratio of the CaBP-D28k-positive neurons to PARV-positive neurons did neither show developmental changes, that is, between P21 and P90 nor were differences between biparental and father-deprived animals observed. In layers 5/6 of the PrCm and AgCd, no developmental shift was seen in the biparental group. However, in the father-deprived group, the ratio shifts in favor of CaBP-D28k expressing cells until P90. In PL/IL layer 5/6, father deprivation leads to a relative decrease of CaBP-D28k-positive neurons compared with PARV-positive neurons at P21. This PARV/CaBP-D28k ratio is reversed at P90, due to a dramatic decrease in the number of PARV cells in the fatherless group (Fig. 9).

**Orbitofrontal regions**

In the VO and LO of biparental animals, the CaBP-D28k-expressing cells represent the dominant subpopulation, whereas in the father-deprived group, the 2 subpopulations are equally represented.

In the VO and LO of father-deprived animals, the PARV: CaBP-D28k ratio shifts in favor of PARV-expressing cells at P21, this difference is still evident at P90 (Fig. 9).

**Amygdala (BLA, CeA)**

**Calbindin-D28k**

Two-way ANOVA revealed the following effects: BLA (age: $F_{1,21} = 1.09$, $P = 0.308$; deprivation: $F_{1,21} = 8.10$, $P = 0.01$; age × deprivation: $F_{1,21} = 1.53$, $P = 0.230$), CeA (age: $F_{1,23} = 0.40$, $P = 0.534$; deprivation: $F_{1,23} = 17.33$, $P < 0.001$; age × deprivation: $F_{1,23} = 1.54$, $P = 0.227$). In the BLA and in the CeA, no difference between the 2 rearing groups was observed at P21. At P90, the BLA and CeA of fatherless animals displayed significantly reduced numbers of CaBP-D28k-immunopositive neurons compared with age-matched controls ($P = 0.01$) as well as the CeA ($P < 0.001$) (Figs 7 and 8).
Nucleus accumbens. In the AcbC and in the adult AcbSh, the CaBP-D28k-expressing neurons are the dominant interneuron subpopulation.

In the AcbC, the ratio of CaBP-D28k-positive/Parvalbumin-positive neurons shows only minor changes during postnatal development, that is, between P21 and P90 or in response to paternal deprivation (Fig. 10).

At P21, the PARV-expressing neurons are the dominant type of interneurons in the biparental group, whereas in the fatherless group, the ratio between PARV- and CaBP-D28k-expressing neurons is almost equal. In the AcbSh of the biparental group, a developmental shift in favor of CaBP-D28k-expressing cells was seen between P21 and P90. In the father-deprived group, an elevated percentage of CaBP-D28k-expressing cells is

Figure 5. Hippocampus: Comparison of the density of CaBP-D28k-immunoreactive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). (A) and (B) illustrates the significant increase of CaBP-D28k-immunoreactive neurons (typical cell shown as insert in B) in the CA3 subregion of father-deprived animals (–father, B) compared with biparental controls (+father, A) at P21, quantification is shown in (E). (C) and (D) illustrates the significant increase of CaBP-D28k-immunoreactive neurons (arrowheads, typical cell shown as insert in D) in the hilus of father-deprived animals (–father, D) compared with biparental controls (+father, C) at P21, quantification is shown in (E). Scale bars A–D = 100 μm, inserts = 50 μm. Levels of significance: *P < 0.05; **P < 0.01; ***P < 0.001.
already evident at P21, and at P90, nearly the same ratio of PARV- and CaBP-D28k-expressing cells was observed in both groups (Fig. 10).

**Hippocampal formation.** In the CA1, CA3, and DG, the PARV-expressing neurons are the dominant interneuron population. In the biparental group, this ratio of CaBP-D28k-positive

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Figure 6. Hippocampus: Comparison of the density of PARV-immunoreactive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). (A) and (B) illustrate the significant decrease of PARV-immunoreactive neurons (typical cell shown as inset in A) in the CA1-subregion of father-deprived animals (−father, B) compared with biparental controls (+father, A) at P90, quantification is shown in (E). (C) and (D) illustrate the significant decrease of PARV-immunoreactive neurons (typical cell shown as inset in C) in the CA3-subregion of father-deprived animals (−father, D) compared with biparental controls (+father, C) at P90, quantification is shown in (E). Abbreviations: alv, alveus or str. oriens; pyr, str. pyramidale; rad, str. radiatum. Scale bars A–D = 100 μm, inserts in A and C = 20 μm. Levels of significance: *P < 0.05; **P < 0.01; ***P < 0.001.
neurons to PARV-positive neurons is only slightly changed between P21 and P90. In the father-deprived group, the CA1 and CA3 regions show an increase of CaBP-D28k-positive neurons in relation to PARV-positive neurons at P90 resulting in an almost equal ratio between the 2 cell types (Fig. 10).

In the hilus, the PARV-expressing neurons are the dominant interneuron population. In the biparental group, the ratio of the 2 Ca-binding proteins shifts in favor of CaBP-D28k-expressing cells between P21 and P90, this effect is mainly due to a doubling of CaBP-D28k-positive cells accompanied by a decrease of PARV neurons. In the fatherless group, the shift in favor of the CaBP-D28k cells is already detectable at P21. This ratio increases until P90, which is due to pronounced decrease of PARV-positive cells, which nevertheless remains the dominant interneuron population (Fig. 10).

**Amygdala.** In the CeA and BLA, the CaBP-D28k-expressing subpopulation is the dominant interneuron type. In the CeA and BLA of the biparental group, the ratio of PARV-positive/CaBP-D28k-expressing neurons did not change between P21 and P90. In the father-deprived group, the ratio of the CaBP-D28k-positive/PARV-positive neurons shifts in favor of PARV-expressing cells at P21, this ratio is maintained into adulthood (P90, Fig. 10).

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**Figure 7.** Basolateral nucleus of the amygdala: Comparison of the density of CaPB-D28k-immunoreactive and PARV-positive neurons in the offspring of father-deprived (black bars) and biparentally raised (white bars) at the age of 3 weeks (P21) and in adulthood (P90). (A) and (B) illustrates the significant increase of PARV-immunoreactive neurons in the BLA of father-deprived animals (−father, B) compared with biparental controls (+father, A) at P21, quantification is shown in (E). Note the increased staining intensity of neuropil in the father-deprived animals (B) compared with biparental controls (A). (C) and (D) illustrates the significant decrease of CaBP-D28k-immunoreactive neurons in the BLA of father-deprived animals (−father, D) compared with biparental controls (+father, C) at P90, quantification is shown in (E). Abbreviations: CeA, central nucleus; BLA, basolateral nucleus; LA, lateral nucleus. Scale bars A–D = 100 μm. Levels of significance: *P < 0.05; **P < 0.01; ***P < 0.001.
Discussion

Our study revealed that father-deprived degus display region-specific changes in the density of PARV- and CaBP-D28k-expressing cells compared with biparental controls. A transient increase (only seen in young animals) of CaBP-D28k-positive neurons in the OFC, CA1, CA3, and DG and of PARV-positive neurons in the LO, PL/IL layers 2/3, DG and CA1-region, Acb, and amygdala was observed. In adulthood, a decrease of CaBP-D28k-positive neurons in the amygdala and of PARV-positive neurons in the PL/IL layers 5/6 and in the CA3-region was seen. In the CA1-region, the density of PARV-positive neurons was elevated at P21 and then decreased in adulthood, whereas a lasting elevation of CaBP-D28k-positive neurons was seen in the AcbSh and PARV-positive neurons remained elevated in the VO.

Role of Ca-Binding Proteins for the Physiological Activity of Interneuron Subpopulations

The neuronal expression of the Ca-binding proteins Parvalbumin and Calbindin D28K has been discovered decades ago. Even though functional links between the expression of a specific Ca-binding protein, physiological activity patterns, and morphological features have been evaluated in a variety of studies, these correlations as well as the intracellular function of these Ca-binding proteins are still poorly understood. Subpopulations of local circuit neurons have been defined according to morphological, physiological, and neurochemical characteristics (Monyer and Markram 2004; Woodruff and Sah 2007), which serve specific inhibitory functions within the network. The subpopulations, which have been analyzed in the present study exert their inhibitory functions either at the soma and axon hillock (PARV-positive) or at the dendrites...
(CaBP-D28k-positive) of spine-bearing neocortical, hippocampal, and amygdalar pyramidal neurons.

Results from transgenic and knockout models did not reveal a coherent picture of the relevance of these Ca-binding proteins for GABAergic inhibitory neuronal networks (Schwaller 2009). Even though it appears that PARV and CaBP-D28k are not essential for the basic properties of particular subtypes of cells (Baimbridge et al. 1992), experiments in PARV–/– and CaBP-D28k–/– knockout mouse mutants indicate that these proteins are important for the fine-tuning of inhibitory networks. A few studies indicate that the expression of PARV and CaBP-D28k might be inversely correlated to inhibitory activity, while others favor the interpretation that protein expression indicates elevated inhibitory activity. Since CaBP-D28k and PARV have different calcium-binding kinetics, the inhibitory response of an interneuron upon activation may be dampened and/or delayed due to the intracellular Ca-buffering and thereafter, by releasing Ca2+ from the proteins, can prolong synaptic GABA release (Vreugdenhil et al. 2003). Such “tonic inhibitory currents” (Farrant and Nusser 2005) control neuronal excitability, in particular the signal to noise ratio.

Changes in the Composition of Neurochemically Characterized Interneuron Subpopulations during Normal Development

To our knowledge, this is the first study, which shows changes 1) in the density of PARV- and CaBP-D28k-expressing neurons and 2) changes in the CaBP-D28k/PARV ratio during postnatal development. The AcbSh was the only region where the density of CaBP-D28k-expressing neurons almost doubled between P21 and adulthood. Within the same time span, the density of PARV-immunoreactive neurons was significantly decreased in the PrCm, CA1, CA3, and in the hilus of biparental controls, which resulted in an increase of the CaBP-D28k/PARV ratio in the PL/IL and hilus. In the AcbSh, the ratio of CaBP-D28k-/PARV-expressing cells was reversed during development (P21: PARV > CaBP-D28k; adult: CaBP-D28k > PARV).

The developmental decrease in PARV-positive neurons is interesting since these basket cells have been recently...
identified as the key player for critical period plasticity in the visual cortex (Hensch 2005). PARV-expressing cells form the largest inhibitory interneuron subpopulation in the cortex (~40% of the GABA cell population), they arise from the medial ganglionic eminence during late embryonic development and spread into the cortex, hippocampus, thalamus, and amygdala. During postnatal development, PARV-immunoreactive interneurons emerge as a subpopulation within the CaBP-D28k-positive cells, which until adulthood split into 2 separate neuron populations (Alcantara, Soriano, and Ferrer 1996). The mechanisms controlling inhibitory interneuron diversification their allocation to distinct cortical areas or their innervation sites (dendrites or soma/axon) and their role in prefrontal cortical development are not understood in detail. The continuing refinement of GABA circuits and changes in the ratio of PARV-/CaBP-D28k-expressing neurons in response to chronic deprivation or repeated perinatal stress (Giachino et al. 2007; Helmeke et al. 2008) indicates that these maturational events are significantly modulated by experience.

**Paternal Deprivation Alters Neurochemically Characterized Interneuron Subpopulations**

The importance of the sensory environment and physical exercise during “experience-expectant” neuronal development of sensory and motor system development has been demonstrated in a variety of model systems. In humans and other animals, the parents provide the earliest socioemotional environment, and recent studies show that, in addition to sensory stimulation and physical exercise, socioemotional stimulation, in particular during the interaction with the mother, is essential for the development of endocrine functions, brain wiring, and behavior.

The semideprived environment in the fatherless families allowed us to test the hypothesis that paternal care essentially affects the development of inhibitory neurons and to identify affected brain regions. In the majority of brain regions, the deprivation-induced changes were transient, that is, visible in 3-week-old animals but no longer in adulthood: in father-deprived 3-week-old animals, the CaBP-D28k-positive neurons were elevated in the OFC and hippocampus, PARV-positive neurons were elevated in the medial PFC, hippocampus, Acb and in the amygdala. However, it is important to note that even small and transient adjustments within inhibitory circuits during specific developmental time windows can affect long-term development of the same or other afferent/efferent brain regions. Other changes found in the father-deprived animals were only evident in adulthood, for example, CaBP-D28k-positive neurons in the amygdala, and PARV-positive neurons in the PL/IL and CA3 were decreased. In the CA1 region, the short-term and long-term changes in response to father deprivation were reversed, that is, PARV-positive neurons were elevated at P21 and decreased in adulthood compared with biparental controls.

In particular, interneurons defined by the fast-spiking phenotype and expression of PARV have been suggested to be involved in gamma (30–80 Hz) oscillations, which are hypothesized to enhance information processing (Sohal et al. 2009). Gamma oscillations are thought to provide the temporal structure that is necessary for synaptic plasticity, and gamma oscillation deficits may disturb developmental synaptic reorganization events. The establishment of balanced excitation and inhibition is critical during cortical development as well as for adult cortical function, and it has been shown that excitation/inhibition circuit dynamics can dictate the timing of critical periods (Hensch 2005). Even though this requires physiological confirmation, our neuromorphological findings in the OFC indicate that the father-deprived animals may end up with hypofunctional OFC-limbic circuits, since the upregulation of CaBP-D28k-immunoreactive neurons in the OFC of father-deprived animals may amplify the reduction in excitatory spine synapses (Helmeke et al. 2009; Pinkernelle et al. 2009), and the increase of PARV-expressing neurons may dampen the output (soma and axon) of principal neurons.

**Methodological Considerations**

Deprivation-induced changes might reflect 1) a true change in the number/density of PARV- or CaBP-D28k-immunoreactive neurons (e.g., via apoptosis/neurogenesis, impaired migration, switch between CaBP-D28k- and PARV-expression) and/or 2) altered physiological activity levels of these neuron populations, which might be reflected by their expression of CaBP-D28k or PARV. An involvement of apoptosis and/or suppressed neurogenesis cannot be completely ruled out, since apoptosis as well as neurogenesis in the DG of rats was shown to be affected by changes of the postnatal socioemotional environment (Zhang et al. 2002; Oomen et al. 2009). Altered migration of GABAergic neurons appears unlikely since it takes place prenatally, that is, prior to the time period investigated here. A switch in neuronal PARV- and CaBP-D28k-expression might also contribute, since a phenotypic shift, that is, reducing the expression of CaBP-D28k and upregulating PARV expression has been described in rats during the first 3 postnatal weeks (Alcantara, de Lecea, et al. 1996). In addition, the expression of PARV- or CaBP-D28k is activity dependent (e.g., (Jacopino and Christakos 1990; Lowenstein et al. 1991), thus, neurons in which the concentration of a given Ca-binding protein falls below or rises above the immunocytochemical detection limit may histologically disappear or appear, respectively. With regard to this, it is worth to mention that in our experiments we hardly observed any CaBP-D28k-immunoreactive pyramidal neurons, as has been described for some cortical regions. Increase in thickness and volume of a given brain region might not play a major role (even though it cannot be completely excluded) since the 2 groups did not differ in brain weights neither at P21 nor in adulthood (Ovtscharoff et al. 2006; Helmeke et al. 2009).

**Behavioral Implications of Altered Inhibitory Networks in Father-Deprived Animals**

Findings in PARV- and CaBK-D28k-knockout mice (Schwaller 2009) indicate that changes (or the complete lack) of PARV- and CABK-D28k-expression, respectively, do not result in dramatic behavioral changes. However, it appears likely that altered activity levels of specific inhibitory interneuron subpopulations in specific prefrontal and limbic brain areas may result in altered cognitive and emotional competence. Altered density of PARV- and CaBP-D28k-expressing neurons in the amygdala, hippocampus, Acb and medial and orbitofrontal prefrontal cortex of father-deprived animals may affect learning and memory formation and emotionality. As previously mentioned, the activity of the PARV-containing class of inhibitory interneurons has been linked to the production of cortical network oscillations, and evidence is emerging that gamma-band oscillations (30–80 Hz) are an essential mechanism for cortical information transmission and processing.
(Sohal et al. 2009). In humans, it has been shown that resting-state gamma-band oscillations increase during development (Uhlhaas et al. 2010), and a functional role of early gamma-band activity in the maturation of cognitive functions has been proposed. Disturbance of gamma oscillation patterns may contribute to the onset of schizophrenia and autistic spectrum disorders, since changes of PARV- and CABEL-D28k-expressing neurons have been found in cortical and limbic brain regions of these patients (Danos et al. 1998; Beasley et al. 2002; Reynolds et al. 2002; Zhang and Reynolds 2002; Levitt et al. 2004; Lewis et al. 2005; Torrey et al. 2005; Bernstein et al. 2007; Lawrence et al. 2010). Whether and in which way the deprivation-induced changes of GABAergic inhibitory interneurons are related to behavioral changes or mental dysfunctions in our animal model has at this point to remain speculative.

Conclusions

The present study is the first experimental identification of the paternal contribution to brain development and revealed that paternal deprivation alters the regional density as well as the homoeostatic balance of distinct interneuron populations in an age- and region-specific manner. While the presence of the male’s role in this biparental species makes it a valuable model for the investigation of the importance of “paternal” care, future research will address questions such as whether by replacing the father with another family member or social partner can mitigate the observed effects on brain and behavior and the analysis of sex-specific vulnerability toward paternal deprivation, as has been found in other species (Bredy et al. 2004).

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Notes

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