

Research report

Effects of diverse developmental environments on neuronal morphology in domestic pigs (*Sus scrofa*)

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Abstract

Potential effects of environmental rearing conditions on the brains of farm animals have not been examined experimentally, with the exception of one report for pig somatosensory cortex. The goal of the present experiment was to determine whether different developmental environments in use in agricultural production units affect neuronal morphology in the pig cerebral cortex. Littermate female pigs (gilts) were cross-fostered at birth and reared in either an indoor ($n = 6$) or outdoor ($n = 6$) production unit for 8 weeks. Additional littermates ($n = 6$) were sacrificed at 3 days of age to provide a developmental reference point. Brains were fixed by perfusion and stained by the Golgi-Cox method. The primary somatosensory, auditory and visual cortices were sectioned at $170 \mu\text{m}$, and layer IV stellate neurons ($n = 492$) were digitized and 3-dimensionally reconstructed. Measurements of dendritic length, membrane surface area, total number of segments, number of 1st- through 7th-order dendrites, spine density, soma area, and soma form factor were taken. In auditory cortex neurons, outdoor pigs compared to indoor pigs had (a) significantly more primary dendrites, (b) significantly greater spine density, and (c) trends of increases both in number of 2nd- and 3rd-order dendrites and in total dendritic length. In visual cortex neurons, indoor pigs had significantly more 7th-order dendrites, whereas in all three cortical areas, the indoor animals had more 5th-order dendrites. Multiple morphological differences occurred in stellate cell populations between the three sensory areas of the Week 8 pigs. Also, within different cortical areas, dendritic morphology changed substantially from 3 days to 8 weeks of age. Further investigations are needed to determine which environmental factors are critical in producing the observed changes in brain morphology and whether other brain effects may be produced by varying developmental environments. © 1998 Elsevier Science B.V.

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1. Introduction

Several decades of research have established that the brains of many species can be influenced by a host of environmental factors. Experiments have demonstrated effects of undernutrition [e.g., Refs. [7,8], environmental enrichment [e.g., Refs. [13,34]], and visual deprivation [e.g., Refs. [21,26,27,53,58,59]] on neuronal morphology.

Extrapolations from this work to additional species and environments, however, are problematic for a variety of reasons. First, observations to date have been limited virtually exclusively to species commonly used in the

laboratory, including most notably the rat, a lissencephalic animal. Secondly, past experiments have frequently employed extreme environmental manipulations which may not be representative of more limited and common ecological variations. Thirdly, the disparate brain regions, neuron types and neural measures examined in different experiments make it difficult to formulate any general predictions about which, if any, neural systems will be affected—or in what direction—by a given environmental situation.

Given such difficulties with extrapolations from the laboratory work to date, the present experiment was designed to explore the possibility of environmental impact on the brain organization of farm animals, specifically pigs (*Sus scrofa*). Furthermore, application of the environmen-

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tal enrichment paradigm to assess different farm production environments in common use might indicate whether standard husbandry practices may influence neural organization in farm animals. Multiple concomitant measures from different cortical sites in such analyses (see below) might also provide information relevant to a general formulation of how the environment may affect brain morphology.

The pig, *S. scrofa*, provides a promising model for such an exploration. For example, pigs are widely used in research [18,52] and have large, gyrencephalic brains. Pigs offer a variety of advantages such as similarities to human physiology including, but not limited to, the cardiovascular, renal and respiratory systems [e.g., Refs. [10,29,30,39]]. In addition, it is clear that swine behavior [5,6,35] and physiology [5] are affected by the types of environments employed on farms. Indeed, the developmental environments used in the present experiment have specifically affected immunological and behavioral responses of these pigs [46,47].

Furthermore, the only examination of a farm animal's brain in relation to 'environmental enrichment' is work on the pig [32]. Grandin attempted to characterize potential effects of environmental conditions on the brains of Hampshire-sired pigs examined at 83- to 93 days of age and, in the process, gain further insight into a hypothesized sensory/arousal mechanism mediating such changes through investigating behavioral correlates. Briefly, Grandin's pigs were placed into 2 groups and exposed, respectively, to a 'complex' or a 'simple' environment. Sampling from an area of the somatosensory cortex (SSC) known to receive sensory input from the snout, an organ that is thought to be as sensitive as a human finger tip [61], and thus having a correspondingly extensive representation in the SSC, Grandin found that pigs in the simple environment had more extensive basilar dendrites and larger somata in layer II pyramidal neurons than pigs in the complex environment. Behaviorally, the pigs in the simple environment engaged in more belly-nosing of other pigs and were more excitable than complex environment pigs, but the latter pigs had more ground-rooting behaviors. So, while both conditions may have exercised the snout, through two different behaviors, Grandin concluded that rubbing and massaging another pig may be more stimulating to the SSC than physical enrichment, thereby offering a possible sensory mechanism for the anatomical results.

The present experiment was designed to extend the Grandin experiment to an examination of the effects of two developmental environments. Given the exploratory nature of the work, the choice of brain regions, neuronal types and morphological features measured were all influenced by the analogous experimental literature with non-farm animals as well as by the Grandin experiment. Based on the considerable evidence that environmental influences are often expressed in sensory systems [e.g., for review, see Ref. [9]], three different sensory cortices were exam-

ined. Given the indication in the experiment of Grandin that somatosensory cortex is influenced by the pig's environment, primary somatosensory cortex as well as two independent sensory regions implicated in plastic changes in laboratory species (i.e., visual and auditory cortex, VC and AC, respectively) were examined.

To realize the statistical power of working with a large sample of a single neuronal type, the present study concentrated on the stellate population found in layer IV. These cells occupy an important place in the relay circuit between thalamic input and the cerebral cortex as local interneurons [40,49,62,63]. Briefly, this circuit operates through axo-dendritic synapses from thalamic neurons onto stellate interneurons. This intrinsic interneuron then inhibits neighboring pyramidal cells in other layers via axo-dendritic synapses [63]. This increase in IPSPs at the axon hillock of the affected pyramidal neurons decrease their ability to depolarize and project signals to other areas of the cortex. Additionally, immunocytochemical and autoradiographic techniques have shown that these stellate cells are largely GABA-ergic [e.g., Ref. [51]]. Substantial evidence exists, then, that this neuron may form the basis of feed-forward inhibition that shapes the receptive field properties of sensory cortical neurons. For these reasons, this neuron type was a reasonable choice to begin to examine subtle differences in environmental sensory inputs.

The initial goal of this project was to provide information regarding stellate neuronal morphology in the pig. We hypothesized that the neocortex of the pig would demonstrate plasticity, with alterations in neuronal morphology reflecting the different sensory experiences of the two developmental environments. An additional goal was to explore potential differences in development across three sensory brain areas.

2. Materials and methods

2.1. Animals

Subjects were 18 littermate domestic gilt pigs (*S. scrofa*), raised at the Texas Tech University (Lubbock, TX) Swine Research Center. After birth, the littermates (3 per sow) were randomly assigned to one of three experimental conditions; Indoor, Outdoor, or Day 3. The first littermate remained in one environmental condition with the mother sow. A second littermate was cross-fostered to a sow in the other condition, so one genetic littermate was represented in each environment. A third littermate was sacrificed near birth (day 1–3) to provide information on an initial baseline for the indoor and outdoor conditions. The animals assigned to indoor or outdoor conditions were euthanized at 8 weeks of age (average weight = 15 kg). This strategy was used for 2 indoor and 4 outdoor mother sows for a total of 6 litters per condition. Gilts reared with

their genetic mothers did not differ from gilts crossfostered to another sow [MANOVA on genetic vs. crossfostering effects across all dependent variables; Rao's $R(13, 478) = 0.66$ ($p > 0.80$)]. This experiment was approved by the Texas Tech University Animal Care and Use Committee.

2.2. Environments

Each environment included two age-determined phases. In Phase 1 of the indoor treatment, the mother sows were placed in a standard, environmentally controlled indoor production unit consisting of a 1.5 m \times 2.1 m farrowing crate typically used in the United States. Other, non-experimental piglets were also in the pen so that each sow had, on average, 10 piglets. This environment had cement slatted or plastic-coated expanded-metal flooring and was maintained with an indoor temperature of 26°C plus zone heat with a heat bulb, standard photoperiod (14:10 L:D), and mechanical ventilation. Pigs had a creep area (0.46 m \times 2.1 m) with a heat lamp and creep food available from Day 7 on. Phase 2 began after weaning (28 days) when the female pigs were placed, with other piglets, in an indoor nursery (6.5 m²) with a slightly cooler environment for an additional 4 weeks.

The second environmental condition (also in 2 growth phases) simulated a typical outdoor production unit in use in the United States and Great Britain. Here, in Phase 1, piglets were exposed to the outdoor Texas weather (February–March, mean daytime:nighttime temperature, 13°C:4°C), including rain, wind, and sun. Small huts in the field provided a zone-heated microenvironment where an electric fence enclosed the pig area (3 acres, 16 sows). Other features of the outdoor system included straw bedding, mud wallows, feeding stations, and nipple waterers. In addition, the outdoor environment also gave piglets free access to neighboring litters. After weaning, for Phase 2, the pigs were placed in an outdoor nursery (6.5 m²) with otherwise similar conditions. Contact with humans was minimal in both environments.

2.3. Histology

After a lethal overdose of sodium pentobarbital, each animal was exsanguinated. The head was then removed and perfused through the carotid arteries with 10% formalin at room temperature [31]. The brains were then removed in 2 pieces (right and left hemispheres), coded to prevent experimenter knowledge of treatment conditions, and fixed in a jar of 10% formalin solution for at least 6 weeks. While in formalin, the coded brains were shipped from Lubbock to West Lafayette, where the histology, microscopy, digitizing and statistics were performed.

Photographs, used to record sampling sites, were taken of each brain (left hemisphere). Each brain then had 3 blocks (1 \times 1 \times 2 cm) taken from it; one each from the primary AC, SSC, and VC. In an attempt to control for any

variance associated with hemispheric differences, only the left hemisphere was sampled. For the SSC, morphological studies by Campbell [14] and electrophysiological studies [1,65] were used to identify the area representing the snout. For the AC and VC, the previous references as well as an electrophysiological survey [3] and cross-species comparative studies of the major sulci (ectosylvian, caudal suprasylvian, and lateral) in the dog and the cat [12,14,38,49,64,65] served as guides (Fig. 1).

After the cortical blocks were excised, they were rolled in gauze and placed individually in 150 ml of a modified Golgi–Cox solution in the dark [31]. Twenty-four hours later, the block was rerolled in gauze, placed in a fresh 150 ml of Golgi–Cox and returned to the dark. After 21 days, the tissue was taken out of the solution and placed in 70% alcohol for 2 h. The block was then cut into sequential, coronal sections on the vibratome at 170 μ m, with every third section cut at 60 μ m for counterstaining. Tissue processing was done using the protocol by Glaser and Van der Loos [24] with minor changes. Sections were then mounted using DPX, coverslipped, and allowed to dry. This procedure yielded many well stained neurons in all cortical layers (Fig. 2). A 0.3% Cresyl Violet counterstain was used to distinguish layer IV in the brain areas previously identified (Fig. 3).

2.4. Data collection

Several criteria were developed for choosing the stellate cells. First, cells had to correspond morphologically to the class of 'relatively spiny stellate cells,' a stellate subpopulation characterized by Lund [40] and Mates and Lund [44]. These neurons have round somata, somewhat spinous dendrites, a significant number of lateral dendritic branches, but no extended apical process. Second, neurons had to be located in layer IV as discussed by Campbell [14] and others who have defined cortical lamination by cell morphology and density through Nissl stains. When layer

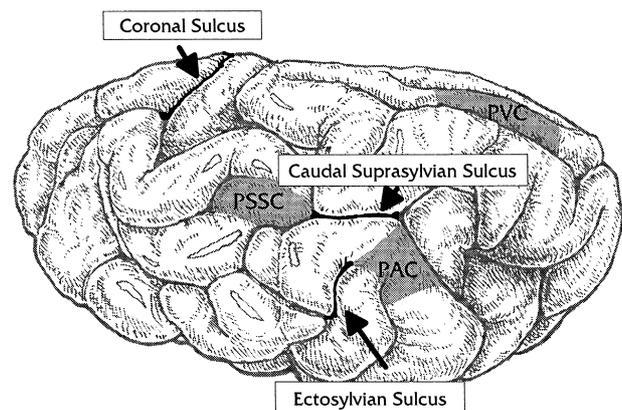


Fig. 1. This schematic of the pig brain from a dorsolateral perspective shows where brain blocks were located. Important (labeled) sulci used as landmarks included the ectosylvian, caudal suprasylvian, and lateral sulci.

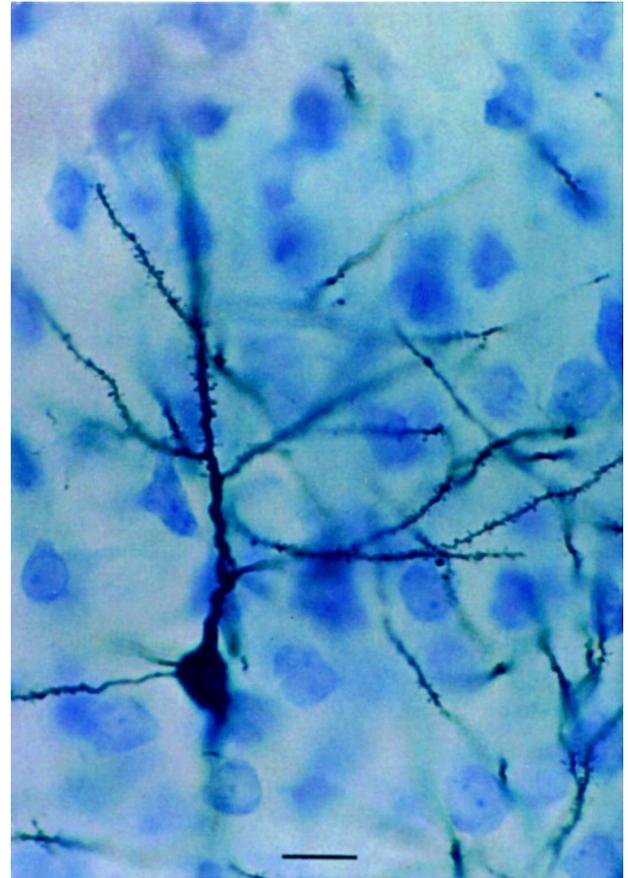
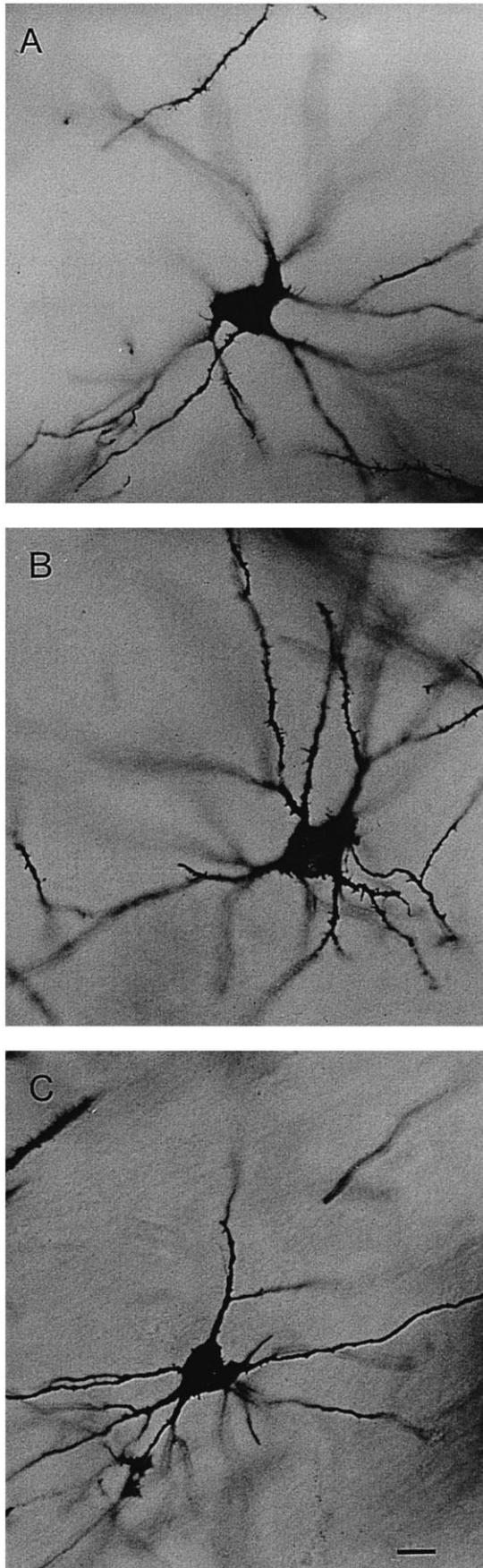


Fig. 3. High quality Nissl staining guided successful layer IV sampling. This neuron is a layer III, Golgi–Cox stained, pyramidal cell found in the somatosensory cortex. Scale bar equals 20 μ .

boundaries were ambiguous, neurons had to fall into an arbitrarily determined fourth zone. For this purpose, the cortical mantle was divided into 6 equal zones (representing the generally accepted layers of the cortex) where layer IV was defined as the fourth zone. Third, once a neuron was identified as belonging to this stellate category in the appropriate cell layer, it also had to be fully impregnated (smooth, continuous, and dense black staining) by the Golgi–Cox solution. Fourth, all dendrites of the neuron had to be completely contained within the section on the basis of two criteria. Namely, inspections of each dendrite at full magnification (480 \times) had to establish that the process (a) did not extend to any surface of the section, and (b) tapered continuously and smoothly to an extremely fine tip, without evidence of truncation.

Neurons meeting the three criteria were then digitized (480 \times ; 40 \times dry objective, 12 \times ocular) using the Eutec-

Fig. 2. These photographs were taken of Golgi–Cox stained neurons. (A) This layer III–IV stellate cell with relatively spiny dendrites was located in the auditory cortex and was of the type we sampled from. (B) This spiny stellate neuron was found in layer IV of the visual cortex. (C) This layer II pyramidal neuron was encountered in the somatosensory cortex. (Scale bar equals 10, 10, and 15 μ , respectively for A, B, and C.)

Table 1
Number of neurons per brain region per condition in a sample of 492 stellate cells

	Sensory region		
	Auditory	Somatosensory	Visual
<i>Condition</i>			
Day 3	58	47	47
In	68	57	59
Out	51	50	55

tics 3-dimensional reconstruction software [15]. A total of 492 neurons were characterized in this way (see Table 1). An experimentally determined correction factor (1.521) was applied to all z values to compensate for refractive compression from the use of a dry objective [25].

2.5. Data analysis

Dependent measures were taken on 13 parameters: *Soma area*, calculated as the area of the cell body in the x and y planes, is an aspect of a neuron commonly thought to reflect metabolic demands [23] and is a useful index of growth in the nervous system [66]. *Total dendritic length* has been shown to differ in rats housed in different environments [33,36,37]. This measure has also been useful in exploring brain maturation [56]. *Membrane surface area* (the reconstruction software encodes each dendrite as a series of diameter measurements separated by known distances and calculates the area of each successive element of the dendrite as a tapered cylinder) has been advantageous in describing postnatal changes in neurons [48]. *Spine density*, a measure of the proportion of excitatory

synapses per micron dendrite, may be affected by environmental paradigms [28,55]. Developmental studies have further demonstrated that spines undergo a rearrangement (characterized by initial overproduction and later paring) of inhibitory synapses in brain maturation [11,41,42,50]. In this study, no attempt was made to compensate for hidden spines [19]. *Number of branch points* (i.e., total, and 1st-through 7th-order dendrites; these constitute 8 of the 13 measures) have importance in defining the sphere within which a neuron receives sensory inputs. As with other parameters, this is one that has previously been shown to be altered by differential experience [13,33] and development [54,66]. *Form factor* ($4 \cdot \pi \cdot \text{area} / \text{perimeter squared}$) is an index of soma sphericity; a perfectly spherical soma = 1, a flat, lima bean shaped soma = 0.2. This factor was chosen for reasons of internal validity with regard to the cell body shape of stellate neurons in our sample.

Brain and group identities were decoded only after the entire sample of neurons had been digitized. Treatment effects were evaluated with analyses of variance using Statistica, version 5.0, (StatSoft, 1995, Tulsa, OK). In a 3-way MANOVA dealing with environmental effects, rearing condition (In vs. Out) \times litter (1–6) effects were treated as between-group variables with brain area (AC, SSC, VC) as a within-group variable. Neurons were held as random factors. In a 3-way MANOVA dealing with developmental patterns, the In and Out rearing groups were collapsed to form an 8 week group where treatment (Week 8 vs. Day 3) \times litter (1–6) effects were tested as between-group variables with brain area (AC, SSC, VC) as the within-group variable. Neurons were again treated as random factors. Planned comparisons were employed to investigate the

Table 2
Means (\pm S.E.M.) of dependent measures

	Layer IV stellates		Layer IV stellates	
	In	Out	Day 3	Week 8
<i>Auditory</i>				
Membrane surface area (μm^2)	2511.0 \pm 217.2	2632.9 \pm 147.4	3465.3 \pm 209.7	2598.6 \pm 130.4
Length (μm)	449.2 \pm 29.2	525.9 \pm 29.5	698.9 \pm 41.0	493.8 \pm 21.0
Soma area (μm^2)	186.9 \pm 6.8	187.3 \pm 8.6	163.3 \pm 5.3	188.0 \pm 5.5
Spine density (spines/ μm)	0.110 \pm 0.010	0.141 \pm 0.010	0.117 \pm 0.007	0.124 \pm 0.007
Form factor	0.89 \pm 0.006	0.88 \pm 0.008	0.87 \pm 0.007	0.89 \pm 0.005
<i>Somatosensory</i>				
Membrane surface area (μm^2)	2777.5 \pm 150.6	3226.8 \pm 173.1	3122.0 \pm 211.6	2977.5 \pm 114.9
Length (μm)	579.0 \pm 30.4	656.6 \pm 37.0	650.4 \pm 43.2	613.6 \pm 24.0
Soma area (μm^2)	179.5 \pm 6.8	192.6 \pm 7.9	162.6 \pm 5.3	185.1 \pm 5.2
Spine density (spines/ μm)	0.099 \pm 0.012	0.104 \pm 0.012	0.121 \pm 0.006	0.104 \pm 0.008
Form factor	0.89 \pm 0.006	0.90 \pm 0.005	0.89 \pm 0.006	0.90 \pm 0.004
<i>Visual</i>				
Membrane surface area (μm^2)	3229.9 \pm 273.6	3044.8 \pm 169.4	2568.5 \pm 172.6	3072.7 \pm 157.2
Length (μm)	680.9 \pm 60.0	654.5 \pm 40.5	553.7 \pm 36.1	653.2 \pm 35.5
Soma area (μm^2)	164.1 \pm 7.9	158.7 \pm 5.6	138.3 \pm 6.3	158.9 \pm 4.7
Spine density (spines/ μm)	0.106 \pm 0.011	0.102 \pm 0.010	0.121 \pm 0.008	0.104 \pm 0.007
Form factor	0.89 \pm 0.007	0.88 \pm 0.006	0.89 \pm 0.005	0.88 \pm 0.005

magnitude of any differences in the dependent measures. Significance was reached with the nominal alpha value of 0.05.

3. Results

3.1. General results

All of the animals used in this study were healthy over the course of the experiment, and the brains displayed no signs of gross neural pathology. Measures of neuronal form factor (mean \pm S.E.M.) revealed no differences between groups (In = 0.89 ± 0.006 , Out = 0.88 ± 0.006 , Day 3 = 0.88 ± 0.006), thus suggesting that similar populations of stellate cells were sampled with regard to cell body shape. Furthermore, numerous significant findings both between and within primary cortical primary areas (see below) substantiated the sensitivity of the Golgi–Cox protocol to detect subtle differences in brain anatomy found in pigs reared in separate environmental conditions. Table 2 contains the means for all dependent variables with the exception of dendritic branching. A large degree of variation existed between litters indicating the importance of controlling genetics in studies such as this one.

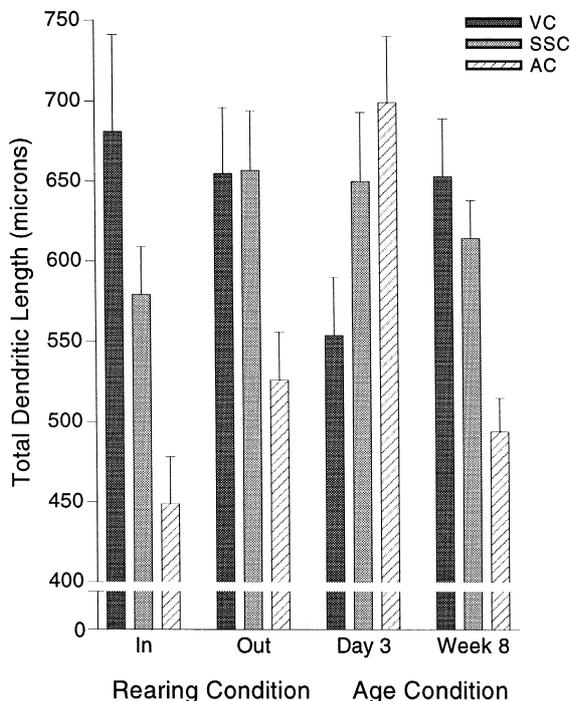


Fig. 4. This graph illustrates the difference between indoor (In) and outdoor (Out) bred pigs and between Day 3 and Week 8 pigs on the measure of total length in the 3 sensory areas sampled from. (A) The indoor/outdoor analysis demonstrated that auditory cortex neurons were, on average, shorter than either somatosensory or visual cortical neurons. (B) In the Day 3/Week 8 analysis, Week 8 auditory neurons were shorter than Week 8 visual, Week 8 somatosensory, and Day 3 auditory cortical neurons.

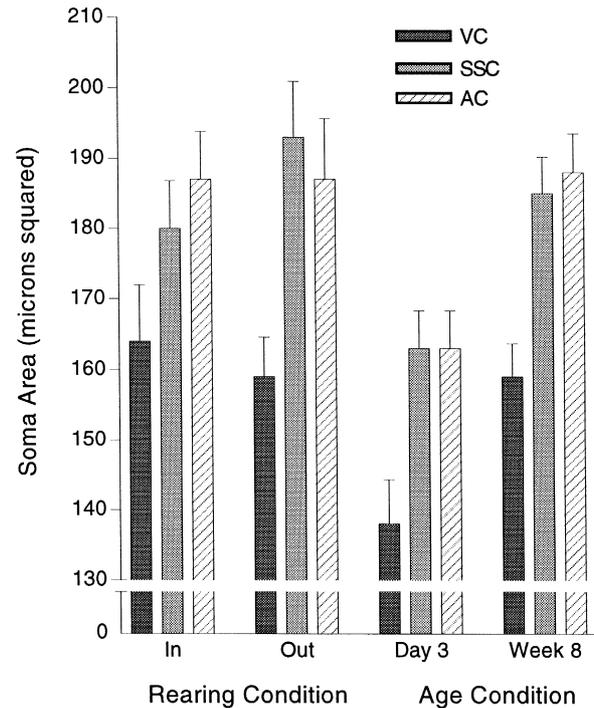


Fig. 5. This graph represents differences between indoor (In) and outdoor (Out) reared pigs and between Day 3 and Week 8 pigs on the measure of soma area. (A) Indoor/outdoor analysis indicated that neuron soma size in both auditory (AC) and somatosensory (SSC) cortices were significantly larger than those of the visual cortex (VC). (B) Day 3/Week 8 analysis showed that average soma size in the visual cortex was smaller than in the other sensory cortices sampled at Day 3 and Week 8. These data seemed to indicate that soma size grew at a linear rate from 3 days to 8 weeks of age.

3.2. In vs. Out groups

MANOVA revealed a main effect of environmental condition (Rao's $R = 2.11$, $p < 0.03$). This difference existed in 5th-order dendrites ($F(1, 67) = 4.89$, $p = 0.03$). There was also a main effect of brain area and these differences existed on the measures of total length ($F(2, 134) = 7.30$, $p < 0.001$) (see Fig. 4), total segments ($F(2, 134) = 6.34$, $p = 0.002$), soma area ($F(2, 134) = 10.37$, $p < 0.001$) (see Fig. 5), 1st- ($F(2, 134) = 3.24$, $p = 0.04$), 2nd- ($F(2, 134) = 4.63$, $p = 0.01$), 3rd- ($F(2, 134) = 4.34$, $p = 0.01$), 4th- ($F(2, 134) = 3.02$, $p = 0.05$), and 6th- ($F(2, 134) = 3.12$, $p = 0.05$) order dendrites. See Table 3 for means of dendritic branches.

There was a significant interaction of brain area by rearing treatment with differences (see Fig. 6) existing in the AC in the number of primary dendrites ($F(1, 67) = 8.42$, $p = 0.005$) and spine density ($F(1, 67) = 3.84$, $p = 0.05$) with a strong tendency toward significance in total length ($F(1, 67) = 3.73$, $p = 0.06$) as well. These differences were all greater in outdoor compared to indoor pigs. The other significant difference was found in VC 7th-order dendrites ($F(1, 67) = 3.95$, $p = 0.05$) with indoor

Table 3
Mean (\pm S.E.M.) number of dendritic branches per neuron

Order	Layer IV stellates		Layer IV stellates	
	In	Out	Day 3	Week 8
<i>Auditory</i>				
1	4.22 \pm 0.14	4.82 \pm 0.14	4.48 \pm 0.15	4.54 \pm 0.10
2	5.87 \pm 0.35	6.23 \pm 0.36	7.46 \pm 0.36	6.13 \pm 0.25
3	4.00 \pm 0.42	4.42 \pm 0.36	5.80 \pm 0.43	4.25 \pm 0.28
4	1.62 \pm 0.31	1.76 \pm 0.33	3.08 \pm 0.36	1.74 \pm 0.23
5	0.71 \pm 0.18	0.42 \pm 0.14	1.32 \pm 0.27	0.57 \pm 0.11
6	0.22 \pm 0.10	0.21 \pm 0.13	0.34 \pm 0.11	0.22 \pm 0.08
7	00 \pm 00	0.17 \pm 0.13	0.06 \pm 0.03	0.08 \pm 0.06
Total	16.6 \pm 1.09	18.0 \pm 0.96	22.7 \pm 1.08	17.5 \pm 0.73
<i>Somatosensory</i>				
1	5.19 \pm 0.17	4.69 \pm 0.15	4.67 \pm 0.15	4.93 \pm 0.11
2	6.97 \pm 0.32	7.24 \pm 0.30	7.39 \pm 0.31	7.06 \pm 0.22
3	4.26 \pm 0.41	4.85 \pm 0.37	6.29 \pm 0.41	4.60 \pm 0.28
4	1.80 \pm 0.34	2.51 \pm 0.33	3.43 \pm 0.37	2.16 \pm 0.24
5	0.82 \pm 0.22	0.26 \pm 0.19	1.08 \pm 0.19	0.50 \pm 0.15
6	00 \pm 00	00 \pm 00	0.41 \pm 0.11	00 \pm 00
7	00 \pm 00	00 \pm 00	0.10 \pm 0.04	00 \pm 00
Total	19.0 \pm 0.98	19.7 \pm 0.97	23.4 \pm 1.05	19.3 \pm 0.69
<i>Visual</i>				
1	4.84 \pm 0.15	4.81 \pm 0.19	4.68 \pm 0.15	4.84 \pm 0.12
2	6.88 \pm 0.31	7.67 \pm 0.41	7.16 \pm 0.36	7.23 \pm 0.26
3	5.16 \pm 0.55	6.18 \pm 0.44	6.38 \pm 0.53	5.62 \pm 0.35
4	2.62 \pm 0.39	2.91 \pm 0.35	2.58 \pm 0.37	2.67 \pm 0.26
5	1.19 \pm 0.30	0.92 \pm 0.21	0.81 \pm 0.23	0.97 \pm 0.18
6	0.19 \pm 0.09	0.39 \pm 0.11	0.11 \pm 0.09	0.28 \pm 0.07
7	0.19 \pm 0.08	00 \pm 00	00 \pm 00	0.09 \pm 0.04
Total	21.1 \pm 1.30	22.9 \pm 1.15	21.6 \pm 1.29	21.7 \pm 0.86

reared pigs having a greater number than outdoor reared pigs.¹ No other significant differences were found between indoor and outdoor reared pigs.

3.3. Week 8 vs. Day 3 groups

Multiple morphological differences were found within sensory cortex across age as well as between cortical areas across age. There was a main effect of age (Rao's $R(13, 104) = 4.35$, $p < 0.001$): differences were found in total segments ($F(1, 116) = 15.22$, $p < 0.001$), soma area ($F(1,$

¹ Since the three cortical areas evaluated were not independent of overall differences in the brains resulting from global uncontrolled factors (for example, metabolic patterns, the genetic differences mentioned above, or differential shrinkage during fixation and storage would affect the whole brain), a 3-way MANOVA treating brain area (AC, SSC, VC) as a within-group variable was used to minimize the variance resulting from systematic differences between brains that were unrelated to the independent variable of environment. The alternative strategy of performing a 2-way MANOVA separately on each brain area sacrifices some statistical power, and hence could be considered a more conservative statistical analysis. For comparative purposes, we checked the comparisons we report with 2-way MANOVAs as well. When the present analyses were rerun using this latter strategy, all variables retained their initial significance, with the exception that the difference in the number of 7th order dendrites was no longer significant ($p = 0.13$).

116) = 17.67, $p < 0.001$), 2nd- ($F(1, 116) = 6.79$, $p = 0.01$), 3rd- ($F(1, 116) = 13.89$, $p < 0.001$), 4th- ($F(1, 116) = 7.51$, $p = 0.007$), and 5th-order dendrites ($F(1, 116) = 3.77$, $p = 0.05$) (see Table 2). Refer to Table 3 for means of dendritic branching.

There was also a main effect of brain area (Rao's $R(26, 91) = 3.10$, $p < 0.001$). Differences were found in soma area ($F(2, 232) = 13.43$, $p < 0.001$) (see Fig. 5), total segments ($F(2, 232) = 3.22$, $p = 0.04$), 1st- ($F(2, 232) = 3.00$, $p = 0.05$), and 3rd-order dendrites ($F(2, 232) = 4.05$, $p = 0.02$).

A significant brain area by age interaction was found (Rao's $R(26, 91) = 1.87$, $p = 0.02$). Differences existed in the AC on the measures of soma area ($F(1, 116) = 8.31$, $p = 0.005$) with the Week 8 pigs having larger neuronal somata than Day 3 piglets but possessing shorter total length ($F(1, 116) = 25.80$, $p < 0.001$) (see Fig. 4), less membrane surface area ($F(1, 116) = 13.11$, $p < 0.01$), and fewer total segments ($F(1, 116) = 14.38$, $p < 0.01$) (see Fig. 7), 2nd- ($F(1, 116) = 7.80$, $p = 0.006$), 3rd- ($F(1, 116) = 8.15$, $p = 0.005$), 4th- ($F(1, 116) = 9.55$, $p = 0.002$), and 5th- ($F(1, 116) = 8.33$, $p = 0.005$) order dendrites. In the SSC, Day 3 piglets had smaller somata than Week 8 pigs ($F(1, 116) = 5.96$, $p = 0.02$), but Week 8 pigs had a greater number of total segments ($F(1, 116) =$

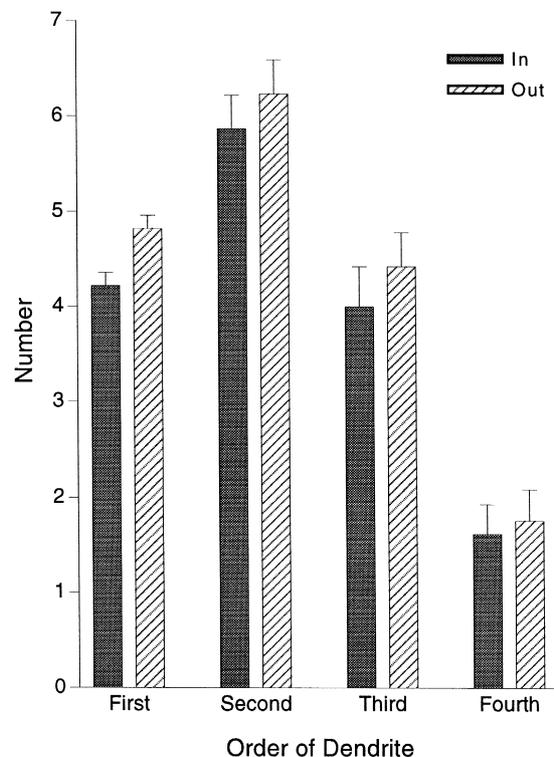


Fig. 6. All of the proximal dendrites of the AC are represented in this graph comparing indoor (In) and outdoor (Out) pigs. Significant findings included more numerous primary dendrites in favor of the outdoor pigs although there was a trend in this same direction for 2nd and 3rd order proximal dendrites as well.

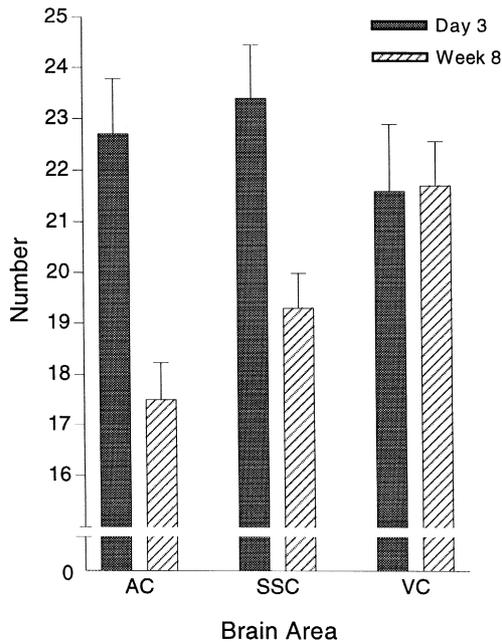


Fig. 7. Differences existed in the number of total segments in the Day 3/Week 8 comparison. The auditory cortex in the Week 8 pigs had fewer total segments than Week 8 visual, Day 3 auditory, and Day 3 somatosensory cortices.

9.63, $p = 0.002$), 3rd- ($F(1, 116) = 10.05$, $p = 0.002$), 4th- ($F(1, 116) = 7.61$, $p = 0.007$), 5th- ($F(1, 116) = 4.64$, $p = 0.03$), 6th- ($F(1, 116) = 24.75$, $p < 0.001$), and 7th- ($F(1, 116) = 4.65$, $p = 0.03$) order dendrites. The only difference found in the VC was on the measure of soma area ($F(1, 116) = 6.72$, $p = 0.01$) with the Week 8 pigs having larger cell bodies than the Day 3 piglets.

4. Discussion

The major findings in this study fall into two categories. First, pigs reared under different developmental conditions, that is in the indoor and outdoor environments, from birth to eight weeks of age displayed small but reliable differences in stellate cell dendritic morphology—particularly in the auditory cortex. Second, different patterns of dramatic morphological changes observed in primary auditory, somatosensory and visual cortex indicate that the different sensory areas mature at different rates.

In terms of plasticity of dendritic morphology, the pattern of changes observed in the auditory cortex was the most extensive. Outdoor-raised pigs had significantly more 1st-order dendrites (Out = 4.86 vs. In = 4.24) and an increase in spine density (Out = 0.135 spines/ μm vs. In = 0.113 spines/ μm) in stellate neurons in layer IV of the auditory cortex. Furthermore, these neurons in AC of the outdoor-raised pigs also exhibited a nearly significant ($p = 0.06$) trend towards greater overall dendritic length (Out = 526 μm , In = 449 μm), as well as similar trends in the

length of the 2nd- (Out = 6.23, In = 5.87) and 3rd-order (Out = 4.42, In = 4.00), or proximal, dendritic branches.

Because the present analyses represented an initial examination of the impact of conventional developmental environments on brain morphology, it was impractical to anticipate what cortical regions might be affected—or in what way—or to predict which environmental variables might prove to be most influential. Since the effects on dendritic organization in the AC were not specifically foreseen, full characterizations of the acoustic properties of the two environments were not undertaken. Post-hoc sound analyses using a sound level data logger (Extech Instruments, model #407762) collecting data every 22 s over a 24 h period, indicated that the indoor group environment was nearly 10 decibels louder (65.3 dB vs. 55.7 dB) on average than the outdoor group. (This determination is probably an underestimate of the difference, because it was made at time when the indoor barn was only 60% full, whereas the barn was fully occupied during the experiment.) Unfortunately, descriptions of acoustic frequency spectra (which might bear simpler relationships to receptive fields or tuning curves of neurons in the primary AC), ranges, temporal distributions, presence of sounds which might mask pig vocalizations, were not available. The differences in average instantaneous loudness measures, however, are suggestive. It is conceivable that the observed differences in AC reflect demands of varying stringencies on the auditory systems of the two groups. For example, if the indoor environment had louder background noise in the frequency bands of pig vocalizations, the primary cortices of the indoor animals might have been challenged with more difficult discriminative tasks than the corresponding regions of the outdoor pigs. In this regard, it also may be relevant that the subjects had fewer behavioral strategies to effectively compensate for, or regulate, their acoustic environments—as opposed to their somatosensory or visual environments—and thus, that environmental effects on brain morphology were more prominent in the auditory system.

Two other, more restricted or focal effects also differentiated the pigs raised in the different developmental situations. Compared to the outdoor-reared pigs, the indoor-reared animals had significantly more 5th-order dendrites across all three primary sensory areas as well as significantly more 7th-order dendrites in the VC. As is in the case of the auditory cortex differences, it is difficult to correlate these changes with particular environmental features with any certainty. The effect on 5th-order dendrites across the three sensory modalities might conceivably be related to some general aspect of physiology which would not be selective for any sensory modality (e.g., thermogenesis or metabolism). The effect on 7th-order dendrites in VC might be correlated with the different hours of day light the two groups experienced, the different distances to the perceptual horizon inside and out, or the less variable illumination inside as compared to outside.

The extremely restricted or focal nature of these effects (e.g., 5th- and 7th- were, whereas 1st- through 4th- as well as 6th-order dendrites were not, significantly affected) and the modest size of the differences, however, suggest caution in speculating extensively on the results until they are replicated in an experiment that carefully measures and specifies potentially pertinent environmental correlates.²

Several possibilities exist that may help explain why significant results were modest. First, the production strategies employed in the present experiment, although representative of extremes in farm environments, may not have been different enough in either the quantity or quality of sensory stimuli to cause the widespread and larger brain changes commonly seen in animals raised in deprived environments such as prolonged dark-rearing. Second, impoverished vs. enriched environments involving laboratory rodents have traditionally meant the animal was isolated without toys, while the enriched environment had these items replaced with novel stimuli every day [34,57]. In our study, there was neither a clearly impoverished nor enriched condition. Each farrowing and nursery environment offered certain fixed characteristics, none of which were manipulated on a day-to-day basis. Socialization with conspecifics occurred in both our indoor and outdoor groups. Our conditions, thus, were closer to a social vs. a social plus environmentally enriched group, and this comparison has rarely brought about significant differences in any animal model [60].

Nonetheless, the overall pattern of the present results—including the several complementary differences

observed in auditory cortex stellate neurons as well as the more restricted effects in 5th- (all 3 sensory areas) and 7th- (VC) order dendrites, taken together with apparent effects in somatosensory cortex layer II pyramidal cells Grandin observed [32], suggests that the organization of primary sensory cortex of the pig can be affected by relatively modest differences in developmental environments presently used in commercial production.

Although detailed developmental morphological analyses have not previously been performed on pig sensory cortex, gross anatomical data suggest that swine brain weight doubles from roughly 5 days post partum to 9 weeks of age [4,16,17,22]. Dickerson and Dobbing [16] and Dickerson et al. [17] have concluded, based on these estimates, that the brain grows most rapidly from 6 weeks before birth to 5 weeks thereafter. Protein to DNA ratios, generally thought to reflect changes in cell size, increase at a moderate rate from 3 weeks of age to 8 weeks of age and then level off until 11 weeks when another moderate increase continues beyond 29 weeks [22]. Likewise, it appears that the active myelination phase of miniature swine brain development concludes between 8 weeks [16,17] and 10 weeks [20]. Also, GABA levels in the pig brain have been shown to be at adult level at 5 days of age [4]. It has been suggested that low levels of GABA at birth indicate a limited degree of functional activity in the CNS while higher levels imply a greater amount of functional or structural integration [2]. This evidence suggests that Day 3 piglet brain morphology may also be near adult levels of complexity, at least in the primary sensory areas.

In our study, a comparison between Day 3 and Week 8 pigs demonstrated that many morphological differences exist. In the SSC, the only difference was in soma size. Perhaps, the SSC was not fully developed at 3 days of age with snout connections still being made. Increases in soma size may be either reflections of the animal getting larger or a reflection of the metabolic need of the neuron. In the VC, the differences were found not only in the soma, but also in length and membrane surface area. These findings are in agreement with many other developmental studies [44,45] and demonstrate a similar trend among pigs, primates and rabbits in neuronal growth patterns.

The AC was the site of most of the developmental differences (a situation potentially responsible for our having observed the most consistent plastic effects in auditory stellate neurons). Soma sizes changed in a direction consistent with those of the other sensory brain areas explored, but the dendrites were longer and more branched in the Day 3, compared to the Week 8 group. This is exactly the opposite of what we found in the SSC and VC.

Our study offers evidence that the sensory areas in the pig may be developing at different rates. Week 8 pigs had dendrites that were much longer (VC), similar (SSC), or much shorter (AC), in length than corresponding Day 3 piglet dendrites. In general, speculations on the importance of these findings, however, should be postponed until

² In the context of the present results, it may be possible to gauge the importance of a potential source of sampling bias in the current experiment. Since the present experiment employed a section thickness of 170 μm , neurons with dendritic arbors spanning more than 170 μm in diameter would not have been sampled. Our analyses of environmental effects should most accurately be qualified as based on the subset of relatively spiny stellate neurons that occupy dendritic envelopes with diameters of 170 μm or less. Practically, however, this is unlikely to have produced a major bias in the present sample for several reasons. First, as described by Lund [43] (see particularly the figures), the diameters of the arbors of spiny stellate neurons are about 150 μm . Secondly, the present data are consistent with this size estimate. Specifically, the total dendritic length of the stellate neurons meeting the staining and other criteria in our study ranged from 449 to 699 μm , but this dendritic extent was distributed among, on average, nearly 5 independent primary dendrites. These measures suggest that the total length of any one dendrite might have been about 100 μm . Since primary dendrites do not continue perfectly orthogonal to a soma and since branch points generate higher order processes that course side by side, covering radial distances roughly equal to the combined length of the daughter processes divided by the number of those processes, this total dendritic length of 100 μm is unlikely to have typically covered an 85 μm radius around the soma. Thirdly, the fact that we detected significant differences among 5th and 7th order dendrites, as just discussed, underscores that we did not have a sample of truncated neurons without higher order dendrites. Finally, there is no a priori reason to expect that the patterns of changes observed for the great majority of stellate neurons would not extrapolate to any exceptionally large stellates.

studies are completed on anatomical and behavioral effects with multiple age groups.

The overall trend in the AC was for dendrites to shorten with maturation, the effects of the two environmental conditions on stellate neurons must have been to retard to different degrees this process of dendritic reduction. Such an argument reinforces the point that developmental profiles for the relevant cortical neurons, combined with a battery of auditory, proprioceptive, and visual tests specifying the important differences between environments, would allow a more complete interpretation of findings to be made.

The results of this experiment suggest that even commonly found developmental environments can alter brain morphology in developing pigs during the interval of ontogeny in which primary cortical neurons are still undergoing maturational changes.

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