Individual Differences in Cortical Connections of Somatosensory Cortex Are Associated With Parental Rearing Style in Prairie Voles (*Microtus ochrogaster*)

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ABSTRACT

Early-life sensory experiences have a profound effect on brain organization, connectivity, and subsequent behavior. In most mammals, the earliest sensory inputs are delivered to the developing brain through tactile contact with the parents, especially the mother. Prairie voles (*Microtus ochrogaster*) are monogamous and, like humans, are biparental. Within the normal prairie vole population, both the type and the amount of interactions, particularly tactile contact, that parents have with their offspring vary. The question is whether these early and pervasive differences in tactile stimulation and social experience between parent and offspring are manifest in differences in cortical organization and connectivity. To address this question, we examined the cortical and callosal connections of the primary somatosensory area (S1) in high-contact (HC) and low-contact (LC) offspring using neuroanatomical tracing techniques. Injection sites within S1 were matched so that direct comparisons between these two groups could be made. We observed several important differences between these groups. The first was that HC offspring had a greater density of intrinsic connections within S1 compared with LC offspring. Additionally, HC offspring had a more restricted pattern of ipsilateral connections, whereas LC offspring had dense connections with areas of parietal and frontal cortex that were more widespread. Finally, LC offspring had a broader distribution of callosal connections than HC offspring and a significantly higher percentage of labeled callosal neurons. This study is the first to examine individual differences in cortical connections and suggests that individual differences in cortical connections may be related to natural differences in parental rearing styles associated with tactile contact. J. Comp. Neurol. 000:000–000, 2015.

INDEXING TERMS: development; epigenetics; primary somatosensory cortex; S1; individual differences

Everything we know about the world is relayed through our sensory receptor arrays. Early experiences mediated through our sensory systems, to a large extent, govern how we will process future sensory information and, ultimately, how we will behave in a complex and dynamic physical and social environment. In most mammals, the earliest and most pervasive sensory inputs are delivered to the developing brain through contact with the parents, especially the mother.

The significance of this relationship was confirmed decades ago by Harlow and Zimmermann (1959), who demonstrated in macaque monkeys that the amount of time spent in contact with the mother has an enormous impact on offspring outcomes, with abnormalities in this relationship resulting in a variety of behavioral anomalies ranging from depression to psychosis. What is it about mother/infant interactions that shape offspring development and subsequent behavior? Given that early life represents a critical period for neural development, it is likely that the tactile, thermal, and olfactory experiences accompanying maternal (and

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paternal) interactions with the infant play a pivotal role in shaping the brain. Studies with rats have supported this supposition by demonstrating that tactile and social stimulation such as licking and grooming by the mother are linked to changes in subsequent behavior of the offspring such as spatial learning (Liu et al., 2000), the expression of oxytocin (OT) receptors (Francis et al., 2000), dendritic structure and function in both the neocortex and the hippocampus (Pinkernelle et al., 2009; Smit-Rigter et al., 2009; Takatsuru et al., 2009), and changes in the expression of neurotrophic factors (Liu et al., 2000; Macri et al., 2010). Finally, differential amounts of maternal licking and grooming determine levels of glucocorticoid receptor expression in the offspring's hippocampus that remain stable in adulthood and regulate stress responsivity (for review see Hackman et al., 2010). These effects are not genetically mediated but are instead regulated through epigenetic mechanisms (Champagne, 2008; Champagne and Curley, 2009).

Nonetheless, this important work represents only one piece of the puzzle. It is well established that the basic pattern of cortical organization and connectivity is produced prenatally through a series of genetic cascades (e.g., Bishop et al., 2002; Hamasaki et al., 2004; for review see O'Leary and Sahara, 2008). However, it is still not known how individual differences in these basic patterns of connections arise, whether early social experiences can alter neural circuitry, or how these anatomical changes are related to subsequent differences in social behavior.

To address this question, we use a unique animal model, the prairie vole (*Microtus ochrogaster*), which is one of only a small proportion of mammals that are monogamous and pair bonded and that rear their young biparentally (Getz et al., 1981; Perkeybile et al., 2013; Thomas and Birney, 1979; Williams et al., 1992). Similar to other small rodents, prairie voles have a gestational period of 21 days, experience eye opening at about postnatal day (P) 10, and begin eating solid food at about P15

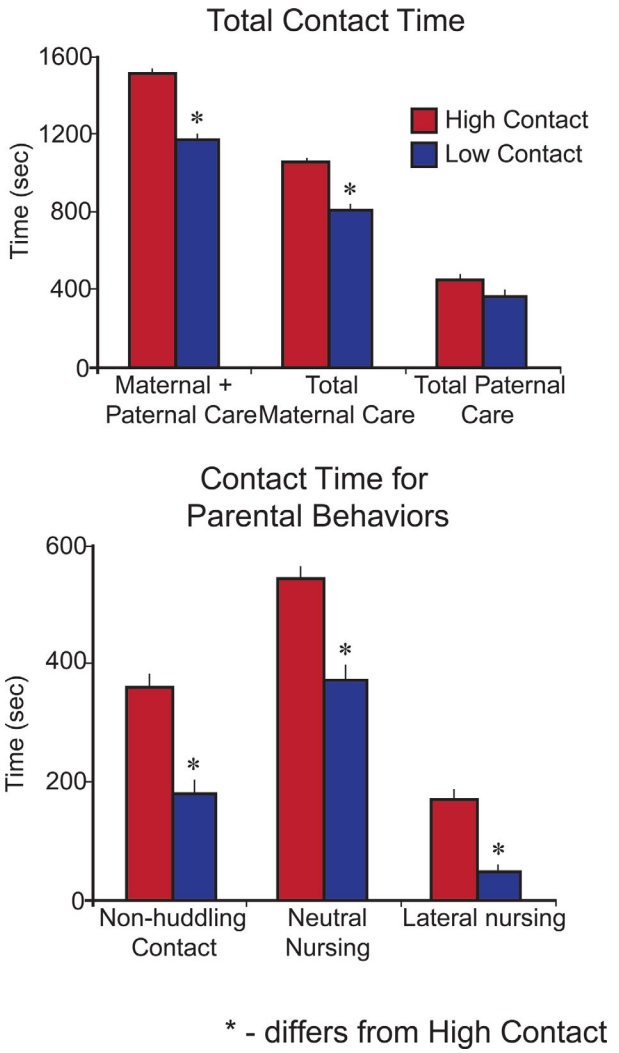


Figure 1. Behavioral assessment of HC and LC voles. **A:** Total amount of time HC (red) and LC (blue) parents spent in contact with their pups. LC animals differed from HC animals on measures of maternal + paternal care (left) and total maternal care (center), but they did not differ on a measure of total paternal care (right). **B:** Amount of time HC and LC parents spent in specific pup-oriented behaviors. During nonhuddling contact, the fathers were quiescent and in contact with the pups. Lateral nursing involved the mother lying on her side with the pups latched to her ventrum. Neutral nursing involved standing over the pups in a relaxed position without locomotion. HC parents (red) spent significantly more time than LC parents (blue) engaging in each of these behaviors. Mean ± SE. Asterisks indicate significant difference from HC. Adapted from Perkeybile et al. (2013).

(Perkeybile et al., 2013). Pair-bonded parents show remarkable variability in rearing styles, particularly in behaviors requiring close physical contact, such as nursing, huddling, and nonhuddling contact, all of which profoundly shape tactile experience (Perkeybile et al., 2013; Fig. 1) as well as olfactory experience. Additionally, because of extensive research of their behavioral, hormonal, and anatomical organization (e.g., Bales and Carter,

Abbreviations

AC	auditory cortex.
Cing	cingulate cortex.
CT	caudotemporal area.
FM	frontal myelin field.
HC	high contact.
LC	low contact.
M1	primary motor cortex.
MM	multimodal cortex.
PC	pyriform cortex.
PR	perirhinal cortex.
PV	parietal ventral area.
S1	primary somatosensory cortex.
S2	secondary somatosensory cortex.
V1	primary visual cortex.
V2	secondary visual cortex

2003; Bales et al., 2007, 2013; Campi et al., 2007, 2010; Getz et al., 1981; Kenkel et al., 2014; Perkeybile et al., 2013), prairie voles are an excellent model for studying the neuroanatomical and neuroendocrine bases of social behavior and its consequences.

MATERIALS AND METHODS

Subjects

In total, 26 injections of neuroanatomical tracers were given in 13 adult prairie voles (*M. ochrogaster*) weighing 34–60 g. To make precise comparisons between high-contact (HC) and low-contact (LC) animals, voles in each group had to be matched for sex and for placement and size of the injection. For example, small injections in the forelimb representation in primary somatosensory area (S1) in an LC female could be directly compared only with an injection of a similar size in a similar location in S1 in an HC female (rather than a large injection in the face representation of an HC female or male). Thus, among the 13 voles (and 26 injections), six injections (three in HC animals and three in LC animals) were used for this study. No animals included in this study were littermates. Animals were born and housed in the UC Davis Psychology Department vivarium (see Table 1 for details about individual subjects). These animals were descendants of a wild stock originally caught near Champaign, Illinois. The animals were pair housed in small laboratory cages (27 × 16 × 13 cm) in which food and water were available ad libitum. All animals were maintained on a 14:10-hour light/dark cycle, with the lights on at 6 AM. All experiments were performed under National Institutes of Health guidelines for the care of animals in research and were approved by the Institutional Animal Care and Use Committee of the University of California, Davis (protocol No. 18000). All surgeries were performed with animals under isoflurane anesthesia, and all attempts were made to minimize suffering.

Behavioral assessment

Subjects were the offspring of animals that had been previously assessed for parenting style. The behavioral assessments involved in this determination have been described previously (Perkeybile et al., 2013, 2015). Briefly, breeder pairs were observed interacting with their offspring during P1–3. Maternal and paternal behavior toward the pups was identified and categorized into behavioral types, including huddling, licking and grooming, retrieval, nest building, and nursing. The amount of time spent in each of these behaviors was quantified and summed to generate the total amount of time each breeding pair spent in contact with the pups (Fig. 1). The breeding pairs in the top and bottom quartiles of total contact time were assigned to the HC and LC groups, respectively. In all cases, parental behavior toward a second litter was also assessed to ensure that parenting style was consistent across litters.

TABLE 1.
Subjects

Case No.	Sex	Weight (g)	Condition
11–188	F	34	LC
11–207	F	44	HC
12–24	F	37	LC
12–140	M	51	LC
12–144	F	37	HC
13–81	M	60	HC

Surgery

Surgeries were performed according to standard sterile surgical procedures. On the day of surgery, subjects were weighed and anesthetized with isoflurane (1–2%). Temperature was maintained, and respiratory rate was monitored throughout the experiment. A longitudinal incision was made along the midline of the scalp, a small hole was drilled over the perioral/face representation within S1, and 0.2 μl Fluoro-ruby or Fluoro-emerald (Molecular Probes, Eugene, OR) was injected into the cortex with either a picospritzer (four animals; General Valve Corp., Fairfield, NJ) or a calibrated 1.0-μl syringe (two animals; Hamilton Co., Reno, NV). After the injection, the hole was closed with Gelfoam (Pfizer, New York, NY) and cyanoacrylate adhesive (Gluture; Abbott Laboratories, Abbott Park, IL). The scalp was then sutured and secured with cyanoacrylate adhesive. The animal was given postsurgical analgesic (buprenorphine, 0.03 mg/kg) and allowed to recover for 6–7 days to allow for transport of the tracer.

Histology

After completion of the experiment, animals were killed with an overdose of sodium pentobarbital (250 mg/kg, IP) and transcardially perfused with 15 ml of 0.9% saline, followed by 15 ml of 4% paraformaldehyde in phosphate buffer and then 15 ml of 4% paraformaldehyde with 10% sucrose. After perfusion, the brain was extracted and the cortex removed from the subcortical structures. In all cases, the neocortex was flattened between two glass slides and left to soak overnight in phosphate buffer. The flattened tissue was sectioned at 20 μm with a freezing microtome. Alternating cortical sections were stained for myelin and mounted for fluorescent microscopy.

Data analysis

All reconstructions were performed blind to the condition of the animal. In each case, we made camera lucida reconstructions of individual myelin sections using a stereomicroscope (Zeiss Stemi SV6; Carl Zeiss Microimaging, Thornwood, NY). As described previously (e.g., Seelke et al., 2012), because individual sections can contain many partial anatomical boundaries, the entire series of sections was examined and combined into a single comprehensive reconstruction to determine the full extent of cortical field boundaries (Fig. 2). Each reconstruction contained the outline of the section, blood vessels, tissue artifacts, probes, and architectonic borders. Sections were aligned according to these landmarks and compiled into one composite image.

Injection sites and retrogradely labeled cell bodies (Figs. 3, 4) were plotted with an X/Y stage encoding system (MD Plot; Minnesota Datametrics, St. Paul, MN; HTSeqTools, RRID:OMICS_01233) that was mounted to a fluorescence microscope and connected to a computer. Blood vessels and tissue artifacts from these sections were then aligned with histologically processed tissue, and all the data were compiled into one comprehensive reconstruction in which architectonic boundaries of the neocortex were related to patterns of connections. These methods have been described previously (e.g., Campi et al., 2010; Dooley et al., 2013). For both the ipsilateral and the contralateral hemispheres, labeled cells in each architectonically defined cortical field were counted (see below).

As noted above, subjects from HC and LC groups were matched by sex, injection size, and injection location. Previous work in our laboratory has demonstrated that that the functional boundaries of S1 are coextensive with architectonic boundaries determined with myelin stains, so we could accurately estimate the major body part representation in which our injections were located (e.g., face vs. forelimb; Fig. 2). Injection sizes and locations in HC and LC groups were matched by aligning the rostral border of S1 and comparing the relative placement of injection sites (Fig. 5). To be considered matching, injection sizes had to be in a comparable location and of a comparable size. After matching HC and LC cases were identified, the proportion of labeled cells was calculated by counting the total number of cells in each hemisphere and dividing the

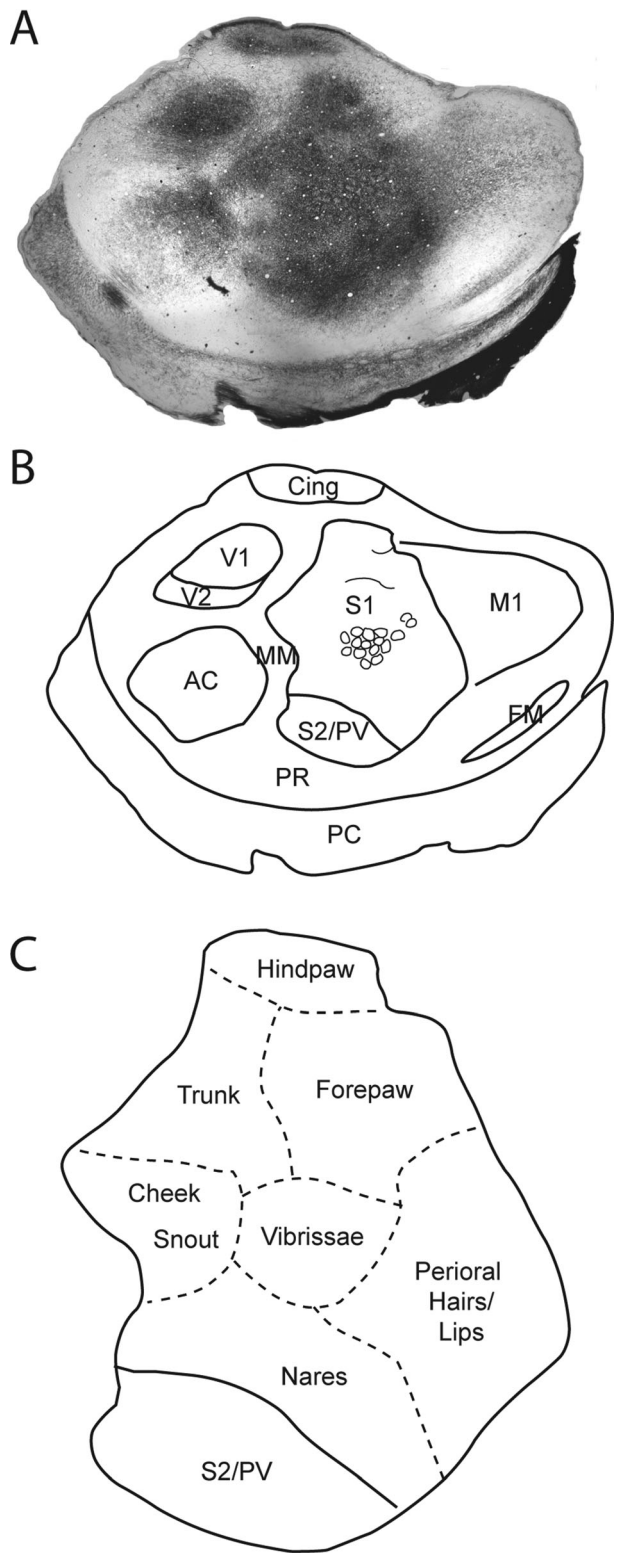


Figure 2. Reconstruction of the flattened vole cortex. **A:** A tangential section of cortical tissue stained for myelin. Dark-stained fields correspond to S1 and S2/PV. Note that S1 is not homogeneous but is broken into myelin light and dark regions that separate major body part representations. **B:** By using an entire series of myelin sections, we were able to identify the borders of the sensory areas and divisions within S1. **C:** Representations of different body parts within S1. Adapted from Campi et al., 2010. See list for abbreviations. Conventions as in Figure 1.

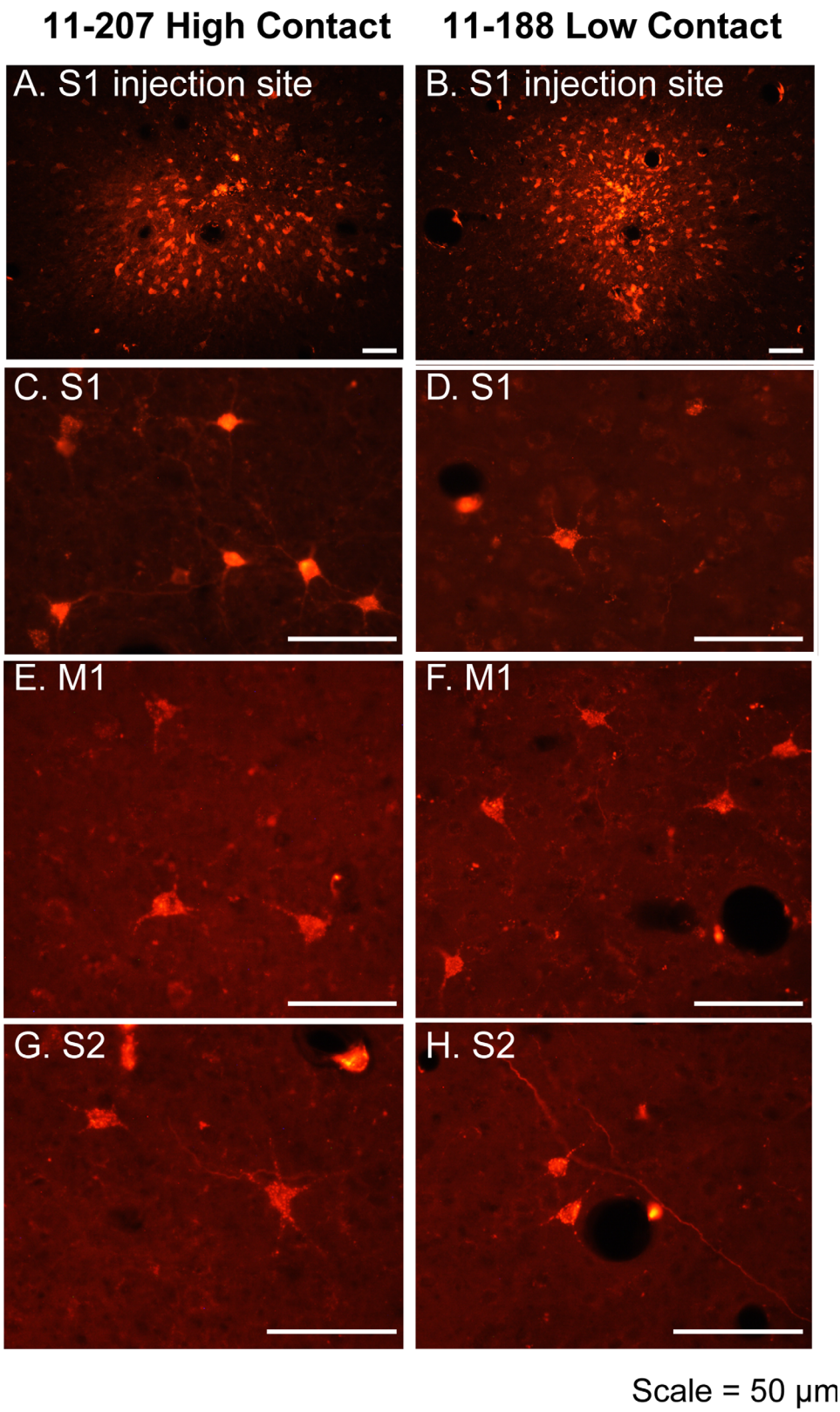


Figure 3. Examples of injections and cells retrogradely labeled with Fluoro-ruby. Digital images show the injection site in S1 of cases 11–207 (**A**) and 11–188 (**B**). In both cases, the injection site is small and localized to S1. Labeled cells resulting injections at these sites are clearly visible. In HC (**C**) animals, S1 contains a higher proportion of labeled cells than in LC (**D**) animals. In contrast, M1 (**E,F**) and S2 (**G,H**) contain a lower proportion of labeled cells in HC than in LC animals. For all cases, the number and percentage of labeled cells were quantified. Scale bars = 50 µm.

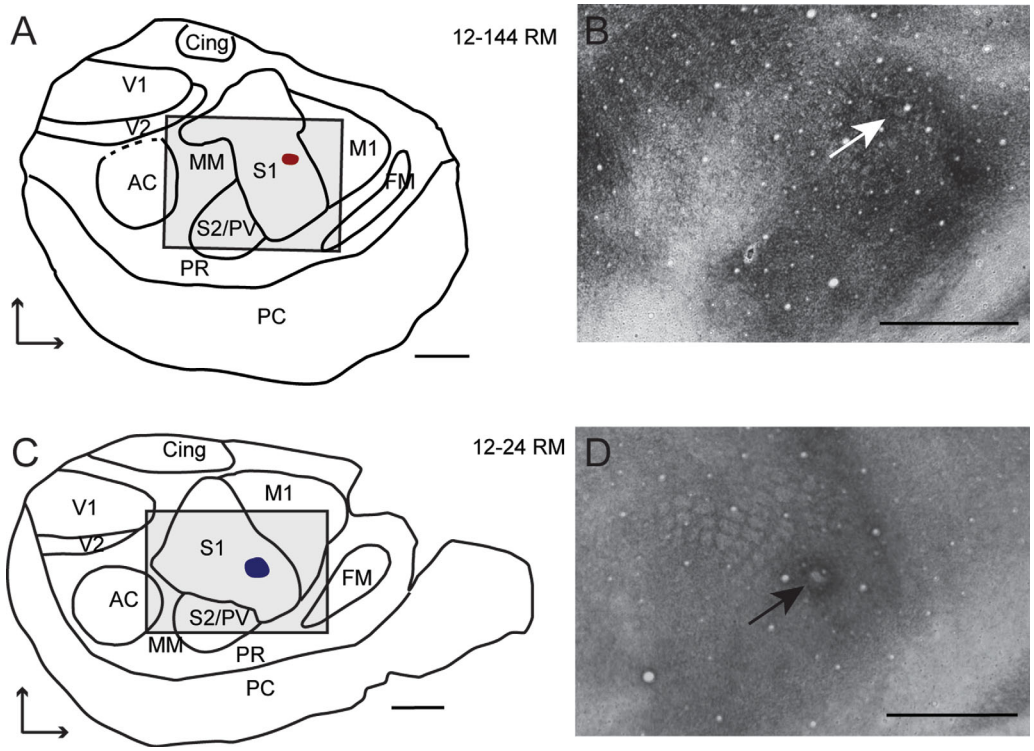


Figure 4. Locations of matched injection sites. Sites are shown in an HC animal (**A**) and an LC animal (**C**) relative to architectonic boundaries in tissue stained for myelin (**B,D**). The boxes in A and C delineate the extent of the area shown in the myelin-stained tissue in B and D. The boundaries drawn in A and C were determined from the entire series of myelin-stained sections. Arrows indicate the locations of the injection sites. Medial is upward, rostral is rightward. See list for abbreviations. Conventions as in previous figures. Scale bars = 1 mm.

number of labeled cells in each cortical field by the total number of cells in each hemisphere. Although we strictly matched both the size and the location of injections, there may still have been natural variation of neuronal uptake of the tracer molecule. This variation can lead to differences in the total number of labeled cells, which can bias any reporting of the number of labeled cells per unit area. For this reason, we express neuronal density as a proportion of total labeled cells. This approach eliminates this potential confound and allows us to standardize the numbers across all the animals used in the study (for details see Cooke et al., 2012)

We next examined the difference between the proportion of labeled cells in each cortical field in HC and LC cases. The proportion of labeled cells in each cortical field was averaged across HC cases and across LC cases, and the average of the LC cases was subtracted from the average of the HC cases.

The proportion of labeled cells in the contralateral hemisphere of HC and LC cases was compared as well. For each case, the total number of labeled cells in the contralateral hemisphere was divided by the total number of cells in both the ipsilateral and the contralateral hemispheres. These values were first compared between matched HC and LC cases, and then the values for all HC cases and all LC cases were averaged. The mean HC value was compared with the mean LC value by a *t*-test. For all statistical tests, $\alpha = 0.05$. Figures were created in Adobe Illustrator (Adobe Systems, San Jose, CA; RRID:nlx_157287), and statistical analyses were performed in JMP statistical analysis software (SAS, Cary, NC; RRID:nif-0000–31484).

RESULTS

Our previous study characterized the differences between HC and LC parenting styles (Perkeybile et al., 2013). To reiterate, HC pups received significantly more total parental contact than LC pups ($n = 304$; $F_{1,228} = 3.69$, adjusted $P = 0.05$; Fig. 1), and this effect was driven by an increase in the amount of maternal contact ($F_{1,228} = 9.51$, adjusted $P = 0.002$; Fig. 1). In particular, HC parents spent significantly more time engaging in nonhuddling contact ($F_{1,228} = 30.24$, adjusted $P = 0.0004$; Fig. 1), neutral nursing ($F_{1,228} = 32.06$, adjusted $P = 0.0006$; Fig. 1), and lateral

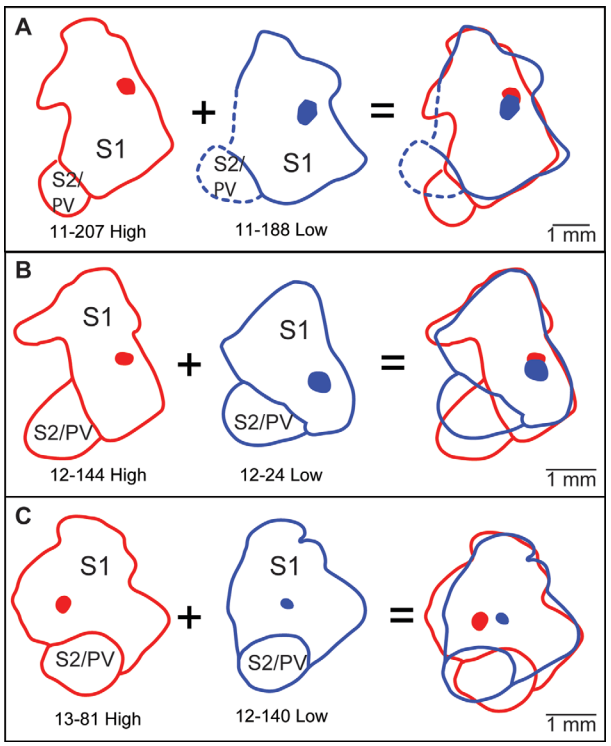


Figure 5. Matching injection sites. **A–C:** Schematic illustrates how injection locations and sizes were matched for analysis in HC and LC animals. The boundaries of the S1 are shown as thick red (HC) and blue (LC) lines. The extents of the injection sites are indicated by red (HC) and blue (LC) ovoids. This includes the injection site and the halo surrounding the site. Injection site locations and sizes were matched by aligning the rostral and lateral boundaries of S1. Injection sites were considered to be matched when they were of similar size and located in close proximity to each other (400 μm). Conventions as in previous figures. Scale bars = 1 mm.

nursing ($F_{1,228} = 29.17$, adjusted $P = 0.0006$; Fig. 1) than LC parents. See Perkeybile et al. (2013) for a complete description of these data.

Although it is likely that a number of cortical and subcortical structures may vary because of differences in the amount of early sensory stimulation, we focused this study on the connections of the S1 because much is known about its functional organization and connectivity. In addition, this field is likely to be one of the structures impacted by differences in early tactile contact. For all animals, injection sites and reconstructed labeled cells were directly related to architectonically defined cortical field boundaries previously described by Campi et al. (2007; Fig. 4).

Staining the neocortex for myelin clearly revealed the borders of distinct cortical fields, including the primary sensory processing areas (Fig. 2A,B). The myelination patterns found within the prairie vole neocortex have been described in detail in previous articles by Campi et al. (2007, 2010), and the results obtained here do not differ from these studies. Briefly, the primary visual area (V1) is located on the caudal pole of the neocortex, and it stains darkly for myelin, whereas the second visual area (V2) is located immediately lateral to V1 and stains less darkly for myelin. The auditory cortex (AC) is located lateral to V1 and V2 and is a round structure that stains moderately for myelin. S1 is immediately rostral to V1 and V2, and the secondary somatosensory area (S2)/parietal ventral area (PV) is located just rostral to and adjacent to the lateral edge of S1. S1 and S2/PV both stain darkly for myelin; however, the internal organization revealed by the stain is quite different for these areas. S2/PV is much smaller than S1 and stains relatively uniformly. In contrast, S1 is heterogenous in appearance, revealing the outlines of various body part representations. The most obvious of these is the barrel field, which corresponds with the functional representation of the vibrissae. As is the case with most mammals, the hind limb is represented medially, followed by the forelimb, vibrissae, and then the nose and snout laterally (Fig. 2C). The primary motor area, M1, is located immediately rostral to S1. These areas stain moderately for myelin. The frontal myelinated region (FM), which stains darkly for myelin, is found lateral to M1 and medial to the rhinal sulcus. Finally, the cingulate cortex (Cing) is located on the medial wall of the neocortex but can be revealed during the flattening process. Cing stains very darkly for myelin.

When the tracer injections were matched for location and size, we saw similar overall patterns of connections in both LC and HC groups, but the density of those connections and the distribution of projection cells varied between groups. (Figs. 6, 7). Patterns of connectivity that were observed for both groups included intrinsic connections with other portions of S1 as well as ipsilateral connections with areas M1, FM, multimodal cortex, S2/PV, and perirhinal cortex (PR). In both HC and LC groups, most labeled cells in the ipsilateral hemisphere were intrinsic to S1 (Fig. 8).

Although S1 contained the majority of labeled cells in both groups, there were distinct differences between the distribution of labeled cells in the ipsilateral cortex of HC and LC groups. To quantify these differences, we calculated the average proportion of labeled cells in each cortical field for HC and LC groups (for values see Tables 2, 3). We then subtracted the mean proportion of labeled cells in each cortical area in LC animals from that in HC animals (Fig. 8A,B). This revealed that HC voles contained a much higher proportion of labeled cells in S1 than LC voles (81.1% vs. 68.3%, respectively). In contrast, areas M1 and S2/PV contained a

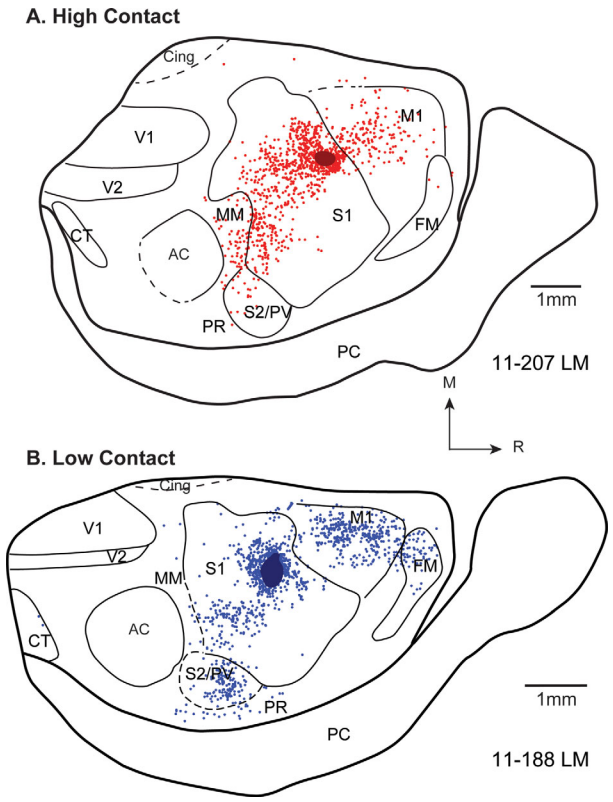


Figure 6. Patterns of ipsilateral connections. Comparison of patterns of ipsilateral cortical connections resulting from size- and location-matched S1 injections in HC (**A**) and LC (**B**) voles. Red and blue dots denote individual neurons labeled by the neuroanatomical tracer in HC and LC animals, respectively. In the HC animal (11–207; A), most ipsilateral labeling is intrinsic to S1; moderate labeling is seen in M1; and weak labeling is seen in S2/PV, MM, and FM. In the LC animal (11–188; B), most ipsilateral labeling is intrinsic to S1; moderate labeling is seen in M1 and S2/PV; and weak labeling is seen in FM, MM, and PR. Note the difference in the distribution of labeled cells, particularly in M1, FM, and S2/PV. See Figure 8 for the quantified differences in the distribution of labeled cells. See list for abbreviations. Conventions as in previous figures. Scale bars = 1 mm

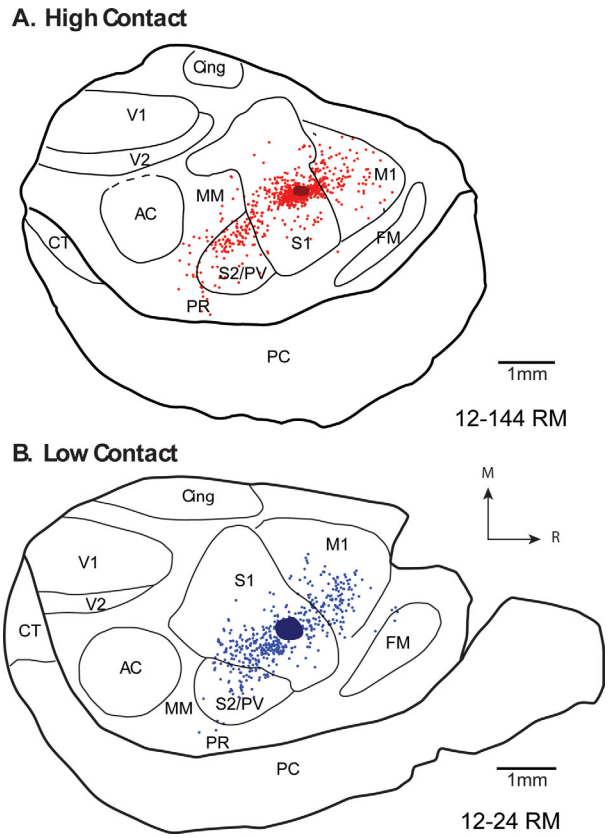


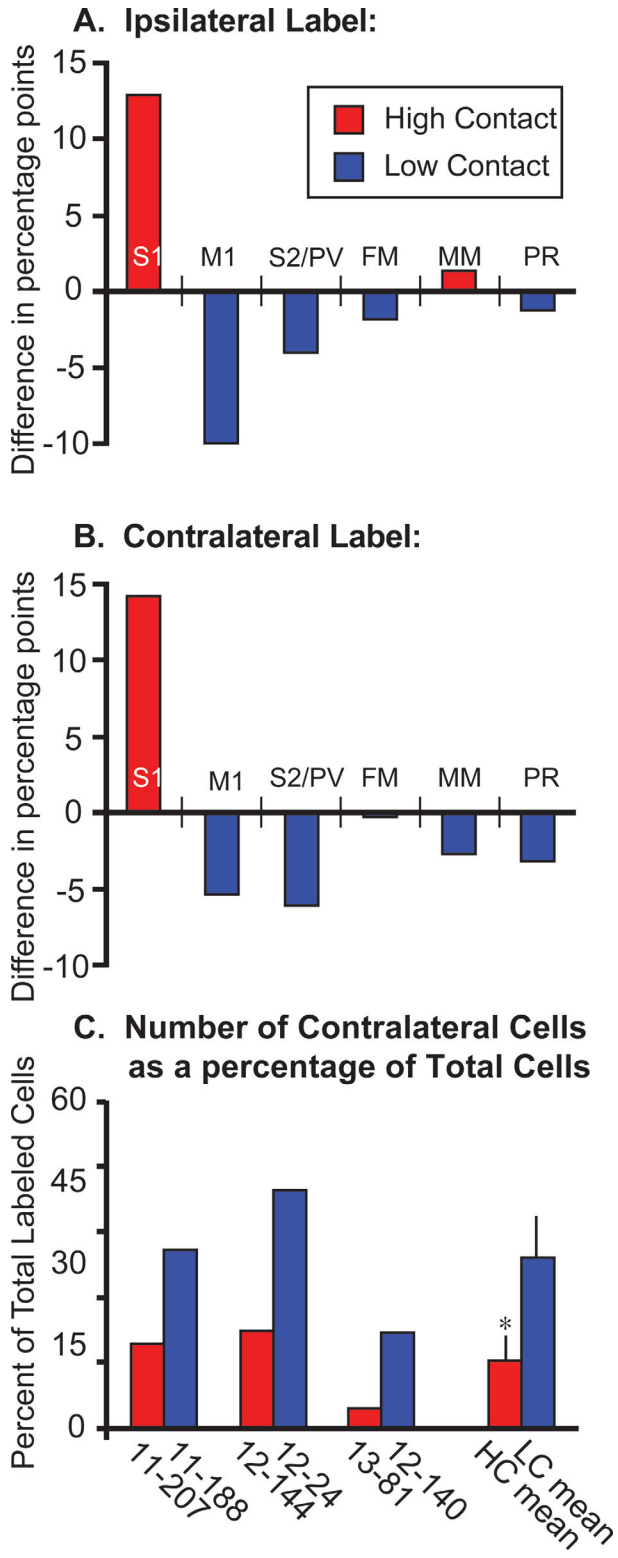
Figure 7. Patterns of ipsilateral connections. Comparison of patterns of ipsilateral cortical connections resulting from size- and location-matched S1 injections in HC (**A**) and LC (**B**) voles. Red and blue dots indicate individual neurons labeled by the neuroanatomical tracer in HC and LC animals, respectively. Patterns of labeling in S1, M1, and S2/PV are similar to those observed for HC and LC animals shown in Figure 6, but with differences in density. Rostral is to the right and medial is upward. See list for abbreviations. Conventions as in previous figures. Scale bars = 1 mm.

higher proportion of labeled cells in LC animals than in HC animals (M1: 9.3% in HC voles, 19.1% in LC voles; S2/PV: 4.8% in HC voles, 8.6% in LC voles).

There was only one similarity in contralateral connections between HC and LC animals; specifically, both groups had dense projections from S1 of the opposite hemisphere (Fig. 9). Under both conditions, the majority of labeled cells in S1 were in a location homotopic to the injection site in the opposite hemisphere. However, as was seen in the ipsilateral hemisphere, the contralateral cortex of HC voles contained a higher proportion of labeled cells in S1 than did that of LC voles (89.7% vs. 75.4%, respectively). In LC voles, the contralateral M1 had only a few labeled cells compared with M1 in HC voles. It is noteworthy that there were some contralateral projections to S1 in LC voles that were not present in HC voles, including projections from S2/PV and PR.

To determine whether the distribution of labeled cells in the contralateral cortex differed between HC and LC groups, we calculated the average proportion of labeled cells in each cortical field and then subtracted the mean proportion of labeled cells in each cortical area in LC animals from those in HC animals. As in the ipsilateral cortex, the contralateral cortex of HC voles contained a much higher proportion of labeled cells in S1 than did that of LC voles (Figs. 8, 9). In contrast, areas M1 and S2/PV contained a higher proportion of labeled cells in LC animals than those in HC animals (M1: 9.6% in HC voles, 14.6% in LC voles; S2/PV: 0% in HC voles, 6.1% in LC voles). These results are summarized in Figure 10.

Another critical difference between the two groups involves the proportion of labeled cells in the contralateral hemisphere overall (Fig. 8C). For each case, the number of labeled cells in the contralateral cortex was divided by the total number of labeled cells in both cortical hemispheres. Voles in the LC group exhibited almost threefold more labeled cells in the contralateral hemisphere than voles in the HC group (26.1% vs. 10.3%). A paired, two-tailed *t*-test confirmed that the LC group contained a significantly larger percentage of labeled cells in the contralateral hemisphere than the HC group ($t_3 = 3.70$, $P < 0.05$).



DISCUSSION

Prairie voles are unusual among mammalian species in that they are both socially monogamous and biparental (Getz et al., 1981; Perkeybile et al., 2013; Thomas and Birney, 1979; Williams et al., 1992). These attributes make them an ideal model for examining the endocrine and neuroanatomical underpinnings of many social behaviors, including variations in parental care. We previously categorized the rearing style of pair-bonded voles by quantifying the amount of time parents spend in contact with pups; voles that score in the top quartile are termed HC, whereas those that score in the bottom quartile are termed LC (Perkeybile et al., 2013). The natural variability in parental contact with offspring displayed by prairie voles is remarkably similar to the variability in licking and grooming behavior seen in rat dams (Champagne et al., 2003; Francis et al., 1999; Perkeybile et al., 2013). Furthermore, these variations result in differences in subsequent social behavior. For example, the offspring of HC voles spend more time sniffing a novel animal and less time autogrooming compared with the offspring of LC voles, and both LC

Figure 8. Distribution of labeled cells in HC and LC animals. Differences in the distribution of labeled cells in HC and LC animals in both the ipsilateral (**A**) and the contralateral (**B**) hemispheres. The proportion of labeled cells in each cortical field was averaged across animals within a condition (n = 3). For the ipsilateral hemisphere, the mean percentages for LC animals were subtracted from the mean percentages for HC animals. The same calculations were performed for the contralateral hemisphere (B). Positive values indicate a higher proportion of labeled cells in a given cortical field in the HC animals, whereas negative values indicate a higher proportion of labeled cells in the LC animals. Thus, in both the ipsilateral and the contralateral hemispheres, HC animals had a higher proportion of labeled cells in S1 (12.8% more ipsilaterally, 14.2% more contralaterally) than LC animals, whereas LC animals had a higher proportion of labeled cells in M1 (9.8% more ipsilaterally, 5.1% more contralaterally), S2/PV (3.9% more ipsilaterally, 6.1% more contralaterally), and PR (1.2% more ipsilaterally and 3.2% more contralaterally) than HC animals. **C:** The proportion of labeled cells in the contralateral hemisphere from among the total number of labeled cells in both hemispheres was also determined for both HC (red) and LC (blue) voles. In matched pairs and means across each group, HC voles had proportionally fewer contralaterally labeled cells than LC voles. Asterisk indicates significantly different from LC.

TABLE 2.
Ipsilateral Connections[1]

Case No.	Condition	S1	M1	S2/PV	FM	MM	PR
11–207	HC	77.57	14.92	1.88	0.36	5.27	0
12–144	HC	70.82	13.50	12.01	0	2.40	1.26
13–81	HC	98.87	0	0.47	0	0.66	0
11–188	LC	59.03	28.04	9.97	0.23	0.39	2.34
12–24	LC	60.82	24.46	11.90	0.43	0	2.38
12–140	LC	88.07	6.88	4.65	0.13	0	0.20

[1]Values indicate the percentage of labeled neurons found within a given cortical area.

TABLE 3.
Callosal Connections[1]

Case No.	Condition	S1	M1	S2/PV	FM	MM	PR
11–207	HC	77.71	21.08	0	0.60	0.60	0
12–144	HC	92.16	7.85	0	0	0	0
13–81	HC	100	0	0	0	0	0
11–188	LC	68.19	19.13	3.74	0.62	0.21	8.11
12–24	LC	79.55	18.94	0	0.38	0	1.14
12–140	LC	78.93	5.75	14.56	0	0	0.38

[1]Values indicate the percentage of labeled neurons found within a given cortical area.

and HC voles perform like their parents on an alloparental care test, thus perpetuating these two distinct phenotypes (Perkeybile et al., 2013).

Cross-fostering of offspring indicates that at least some of these differences in behavior are a result of early experience rather than heredity (Perkeybile et al., 2015). Specifically, the behavior of cross-fostered offspring mimics that of the adoptive parents rather than their biological parents. This demonstrates that experience alone can generate differences in subsequent social and parental behaviors. The question is whether these behavioral differences are associated with measurable differences in brain organization and connectivity. A relationship among differential sensory experience, parental rearing styles, and cortical connectivity of the brain has never been established. In fact, the development of differences in connectivity within a population has itself never been examined.

Here we compare the distribution and density of ipsilateral and contralateral connections of the S1 in prairie voles reared by HC and LC parents. We chose to target the perioral region for two reasons. First, prairie voles are born with very immature visual and auditory systems, and during early development they depend primarily upon somatosensory and olfactory stimulation for their interactions with the environment and with their parents. Second, the perioral structures are disproportionately represented within the primary somatosensory cortex (Seelke et al., 2012; Fig. 2). Thus, most sensory information (both olfactory and somatosensory) that these very young animals receive comes through the snout and perioral region. By targeting this area we were able to examine both the largest body part representation within S1 as well as the area that received the greatest amount of sensory stimulation during early development.

Our data indicate that there are quantifiable differences in the connectivity of the primary somatosensory area in LC and HC offspring. Individuals that received more tactile contact have less broadly distributed connection profiles and more intrinsic connectivity within S1. Individuals that have experienced less tactile contact have more broadly distributed connections both ipsilaterally and contralaterally and less intrinsic connectivity within S1. Although it is difficult to postulate what these differences in connections mean for sensory processing, these patterns of connectivity suggest that the offspring of LC parents have a greater potential for rapid (monosynaptic) multimodal sensory integration than offspring of HC parents. These results are both novel and intriguing, but we caution that they are also preliminary. More work, including cross-fostering studies, examination of expression of genes associated with the formation of cortical and subcortical connections, and OT receptor binding analyses will elucidate the mechanisms underlying these different patterns of connections and uncover a causal relationship between parental rearing style and alterations in brain organization and connectivity.

Although phenotypic variety is the cornerstone of evolution by natural selection, naturally occurring individual differences in cortical organization and connectivity

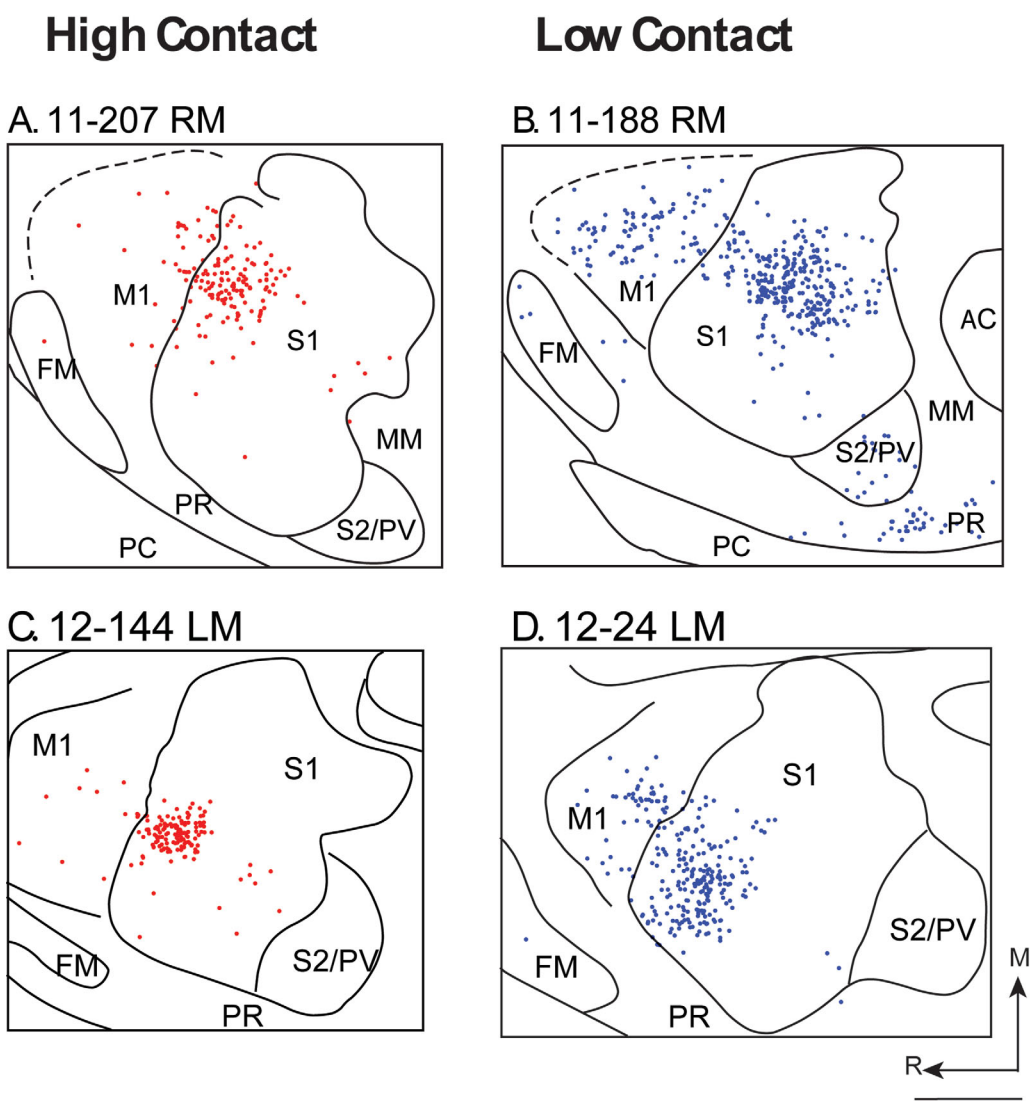


Figure 9. Patterns of contralateral connections. Comparison of patterns of contralateral cortical connections resulting from size- and location-matched S1 injections in HC (**A,C**) and LC (**B,D**) voles. Red and blue dots indicate individual cells labeled by the neuroanatomical tracer in HC and LC animals, respectively. HC cases are characterized by dense homotopic connections within S1, sparse or no label within M1, and a lack of labeled cells within S2/PV. In contrast, although LC cases also show dense homotopic connections within S1, they have moderate numbers of labeled cells within M1. Labeled cells were also found within S2/PV, MM, FM, and PR. Rostral is to the left and medial is upward. See list for abbreviations. Conventions as in previous figures. Scale bar = 1 mm.

within a population have rarely been studied. In fact, most experiments endeavor to minimize individual differences, going so far as to examine biological processes within only one sex of a few select species (for review see Krubitzer and Seelke, 2012). However, when examined, individual variability within a population has been observed. For example, the functional organization of primary sensory and motor areas in the neocortex subtly, and sometimes dramatically, differs among individuals of the same species (Adams and Horton, 2003; Jain et al., 1998, 2001; Seelke et al., 2011; Tennant et al., 2011; for review see Krubitzer and Seelke, 2012). Likewise, the architectonic boundaries of cortical areas vary among individuals (Karlen and Krubitzer, 2006), as does the distribution of vasopressin receptors (Hammock and Young, 2002). Given the importance of understanding how individual differences within a population emerge, it is somewhat surprising that so little research has been carried out by examining how these differences are expressed at multiple levels of organization (i.e., behavior, anatomy, and genome) and the factors that contribute to these differences.

Although only a few studies have examined individual differences of some aspect of cortical organization within a population, numerous studies have examined the role of early sensory experience in establishing

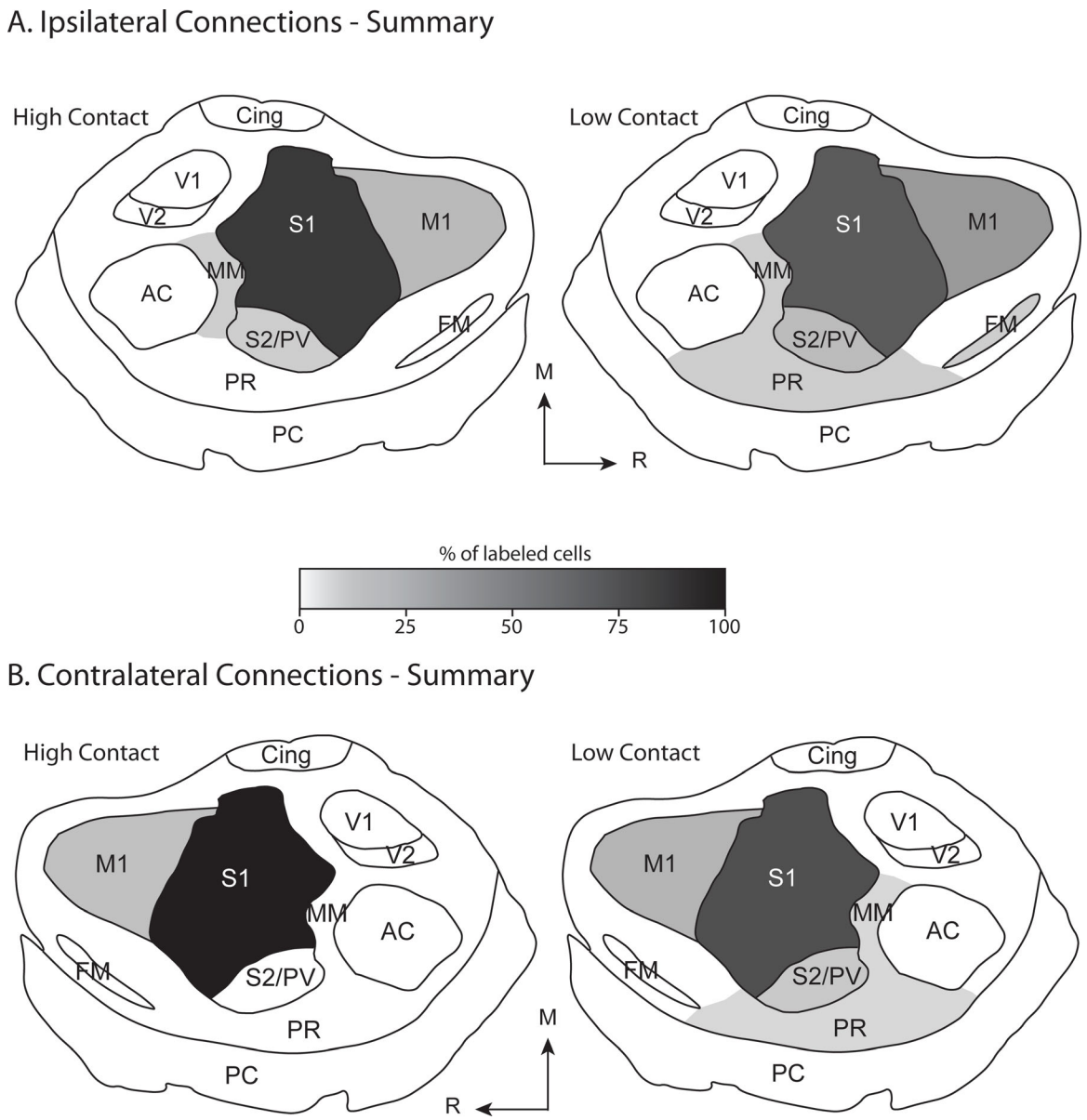


Figure 10. Summary of HC and LC connections. Schematic represents the distribution of labeled cells in the ipsilateral and contralateral cortex of HC and LC voles following the injection of neuroanatomical tracers into S1. The mean proportion of labeled cells found within a given cortical area is represented by a gradient ranging from black (100% of labeled cells) to white (0% of labeled cells). Within the ipsilateral hemisphere of HC voles (**A**, left), S1 contained 81% of labeled cells, S2/PV contained 5% of labeled cells, M1 contained 9% of labeled cells, and MM contained 3% of labeled cells. Within the ipsilateral hemisphere of LC voles (A, right), S1 contained 68% of labeled cells, S2/PV contained 9% of labeled cells, M1 contained 19% of labeled cells, and FM, MM, and PR contained 2%, 1%, and 2% of labeled cells, respectively. Within the contralateral hemisphere of HC voles (**B**, left), S1 contained 90% of labeled cells and M1 contained 10% of labeled cells. Within the contralateral hemisphere of LC voles (B, right), S1 contained 75% of labeled cells, S2/PV contained 6% of labeled cells, M1 contained 15% of labeled cells, and MM and PR each contained 3% of labeled cells. Areas containing less than 1% of labeled cells were not included in this analysis. Conventions as in previous figures.

aspects of cortical organization and thalamocortical connectivity. For example, in rats, exposure to continuous auditory stimuli during early development results in a loss of tone-evoked responsiveness over a large area of the primary AC (Chang and Merzenich, 2003). Similarly, removal of vibrissae in rats results in an increased functional representation and size of vibrissae "barrels" of adjacent vibrissae representations and a decrease or loss of barrels associated with the removed vibrissae (Fox, 1994; Shepherd et al., 2003). Finally, the lack of visual experience during critical periods, whether because of eyelid suturing or a blockade of activity

(e.g., with tetrodotoxin), results in alterations to the normal formation of ocular dominance columns in the primary visual cortex and eye-specific layers in the lateral geniculate nucleus (Chapman, 2000; Chapman et al., 1986; Tagawa et al., 2005). These relatively dramatic changes in cortical organization have all been generated through experimental manipulation of early sensory experience. However, no studies have taken advantage of the natural variations in sensory experience generated by different parenting styles.

What are the underlying mechanisms that generate these differences in cortical connectivity within a population? Of course, genes play an important role in how the neocortex differentiates into distinct areas with specific patterns of connectivity (Krubitzer and Dooley, 2013; O'Leary and Sahara, 2008; Rash and Grove, 2006). However, cross-fostering work in rats (Francis et al., 1999) and voles (Perkeybile et al., 2015) indicates that subsequent social behavior and the type of parenting style that the offspring ultimately adapt are based on how they were reared (LC vs. HC) rather than the biological relationship to the parent. Thus, we propose that variations in parent/infant tactile contact might drive some features of cortical connectivity by invoking epigenetic modifications in gene expression during development, modifications that remain stable into adulthood.

The word *epigenetics* is used to describe stable changes in gene expression without an underlying change in gene sequence (Goldberg et al., 2007). Given the tight temporal and spatial control of gene transcription that unfolds as development proceeds, it is not surprising that epigenetic mechanisms are involved in neuronal differentiation, migration, maturation, and circuit formation (Chittka, 2010; Cho et al., 2011; Fuentes et al., 2012; Golshani et al., 2005; Nott et al., 2013). The fact that epigenetic mechanisms also mediate environmental effects on the brain sheds light on why cortical development is particularly sensitive to environmental cues, such as the amount of tactile contact during critical periods. This sensitivity to external fluctuations allows brain development to be highly dynamic and subsequent behavior to be contextually appropriate.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no identified conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: LAK, KLB, AMHS. Acquisition of data: AMHS, AMP, RG. Analysis and interpretation of data: AMHS, AMP, KLB, LAK. Drafting of the manuscript: AMHS, LAK. Critical revision of the article for important intellectual content: AMHS, AMP, RG, KLB, LAK. Statistical analysis: AMHS, KLB, AMP, LAK. Obtained funding: LAK, KLB. Administrative, technical, and material support: RG. Study supervision: LAK.

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