Early experiences can alter the size of cortical fields in prairie voles (Microtus ochrogaster)

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Abstract

The neocortex of the prairie vole is composed of three well-defined sensory areas and one motor area: primary somatosensory, visual, auditory areas, and the primary motor area, respectively. The boundaries of these cortical areas are identifiable very early in development, and have been thought to resist alteration by all but the most extreme physical or genetic manipulations. Here we assessed the extent to which the boundaries of sensory/motor cortical areas can be altered by exposing young prairie voles (Microtus ochrogaster) to a chronic stimulus, high or low levels of parental contact, or an acute stimulus, a single dose of saline, oxytocin (OT), or oxytocin antagonist on the day of birth. When animals reached adulthood, their brains were removed, the cortex was flattened, cut parallel to the pial surface, and stained for myelin to identify the architectonic boundaries of sensory and motor areas. We measured the overall proportion of cortex that was myelinated, as well as the proportion of cortex devoted to the sensory and motor areas. Both the chronic and acute manipulations were linked to significant alterations in areal boundaries of cortical fields, but the areas affected differed with different conditions. Thus, differences in parental care and early exposure to OT can both change cortical organization, but their effects are not identical. Furthermore, the effects of both manipulations were sexually dimorphic, with a greater number of statistically significant differences in females than in males. These results indicate that early environmental experience, both through exposure to exogenous neuropeptides and parental contact can alter the size of cortical fields.

Key words: prairie vole; neocortex; development; parental behavior; oxytocin

Introduction

The mammalian brain is characterized by the presence of a six-layered neocortex, which is involved in processing sensory inputs and generating motor output. Sensory cortex is segregated by modality and within each modality distinct cortical fields that form a topographic representation of the sensory receptor array are observed, as well as a motor cortical area in which roughly topographic maps of body part movements are found. These functional representations correspond with a unique architectonic appearance as well a specific pattern of connections. In adults, the boundaries of the primary cortical areas can be visualized using a myelin stain; primary sensory
and motor areas stain more darkly for myelin than other areas of the neocortex (e.g. [1]).

The boundaries of primary sensory and motor cortex are identifiable as early as 5 days after birth in rats [2]. Under normal developmental circumstances these borders are stable across the lifespan, although the internal representations of the sensory arrays may change with alterations in the use or morphology of the sensory effector organ [2–5]. There have been a few instances where the size of primary sensory areas has been altered, but these have employed extreme experimental interventions at early developmental stages such as bilateral enucleation or limb amputation [3, 6, 7]. Likewise, alterations in gene expression patterns in the developing cortex can change the size of primary sensory and motor areas [8–10].

Although variations in gene expression have been demonstrated to exert macroscopic influences on cortical organization, the environmental and epigenetic factors driving cortical arealization have been less thoroughly investigated. It is well documented that within any given population there exists variation in the size, shape, and location of primary sensory and motor regions [11, 12]. Furthermore, the existence of variation between individuals is one of the cornerstones upon which the theory of evolution via natural selection depends [11, 13]. However, how early experience might contribute to individual differences in aspects of cortical organization, such as relative size of cortical fields, is currently unknown.

To address this question we used the prairie vole (Microtus ochrogaster), a monogamous and biparental rodent that is native to the grasslands of Illinois and Indiana in the central USA [14–17]. They exhibit a wide range of well-defined social behaviors in which the underlying neuroendocrine mechanisms are well understood [18–20]. Critically, they also exhibit natural variation in the size and amount of parental care expressed towards their pups [17], which directly translates into a variation in the amount and type of early sensory experience. Differences in parenting style in voles have been linked to differences in offspring behavior [17], neuroanatomical connections between cortical regions [21], stress reactivity [22, 23], and oxytocin (OT) receptor binding [24].

The neuropeptide OT (see Table 1 for abbreviations) appears to be a common factor underlying differences in social behavior, parental behavior, and stress reactivity. Vole parents with high OT receptor density exhibit high levels of parental contact, which in turn yields offspring that exhibit high OT receptor density and high levels of alloparenting [24]. The converse is true for vole parents that exhibit low levels of OT receptor binding [24]. Humans also show alterations in OT levels in response to parental contact and social experience [25]. Furthermore, OT has been implicated in the plasticity of the developing neocortex [26].

In these experiments, we exposed voles pups to exogenous OT. OT in the form of Pitocin is commonly used in American hospitals to induce and enhance labor [27]. We have previously administered OT within 24 hours of birth to simulate neonatal OT exposure. A single dose of exogenous OT can affect pair-bond formation in both males and females [28, 29], as well as aggression [20], sexual behavior [30], and the vasopressin receptor system [18].

We asked two questions. First, are variations in early sensory experience (mediated by early parenting) associated with differences in the size of cortical fields? Second, is it possible to experimentally alter the relative size of cortical fields through the early administration of OT?

**Results**

Staining the neocortex for myelin clearly revealed the borders of cortical fields, including the primary sensory areas (Fig. 1A and B).

| Table 1. Table of abbreviations |
|-------------------------------|
| **List of abbreviations**      |
| AC auditory cortex            |
| FM frontal myelinated area    |
| HC high contact               |
| LC low contact                |
| M1 primary motor cortex       |
| OT Oxytocin                   |
| OTA Oxytocin antagonist       |
| OTR oxytocin receptor         |
| PV parietal ventral area      |
| S1 primary somatosensory cortex |
| S2 second somatosensory cortex |
| V1 primary visual cortex      |
| V2 second visual cortex       |

The myelination patterns of the prairie vole neocortex have been described previously in [21, 31], and the cortical morphology observed is like that described in the previous studies. Briefly, V1 is located on the caudal pole of the neocortex and it stains darkly for myelin, whereas V2 is located immediately lateral to V1 and it stains less darkly for myelin. AC is a round structure that is found lateral to V1 and V2 and stains darkly for myelin. S1 is found rostral to V1, V2, and AC, and stains darkly, but non-uniformly, for myelin. In other mammalian species, S1 of prairie voles includes a somatotopic representation of the body, with the hind limb represented most medially, followed by the forelimb, vibrissae, nose, and snout represented laterally [21, 31]. The heterogeneous staining within S1 is indicative of these different body part representations, with the most obvious being the barrel field. S2/PV is located adjacent and rostral to the lateral edge of S1, and stains uniformly darkly for myelin. M1 is found immediately rostral to S1 and stains moderately for myelin. Frontal myelinated (FM) is found rostral and lateral to M1, medial to the rhinal sulcus, and stains darkly for myelin.

**Alterations in Cortical Field Size**

In the following sections, we will discuss the effects of both the chronic (parental care) and acute (OT/ oxytocin antagonist (OTA)/saline) manipulations on the boundaries of specific cortical areas. See Table 2 for means.

| % Myelin |
|---------|
| After determining the architectonic boundaries of different cortical regions, we calculated the proportion of the cortical sheet that was densely myelinated, (% Myelin = the sum of S1, S2/PV, M1, V1, V2, AC, and FM divided by the size of the entire cortical sheet; Figs 2 and 3). The areas of the neocortex that stain most darkly for myelin are the primary sensory and motor areas (with the exception of the secondary visual area). In animals exposed to different amounts of parental care, there was a significant main effect of sex (F = 9.504, P = 0.0067), a trend towards a significant effect of condition (F = 4.023, P = 0.0611), and a significant sex by condition interaction (F = 5.609, P = 0.0300). Post-hoc tests revealed that the % Myelin was significantly lower in LC females than in HC females, HC males, and LC males (t = 2.110, P < 0.0137), which Cohen’s d indicated to be a large effect (d > 1.785) (Fig. 3). In animals treated with OT/OTA/saline, there were no significant main effects of sex (F = 0.4763, P = 0.4944), condition (F = 0.0158, P = 0.9844), or a significant sex by condition interaction (F = 1.0490, P = 0.3605). |
%S1 and %S2/PV

We next compared the proportion of the cortical sheet occupied by %S1 in each group (Fig. 4). We found no significant main effect of sex ($F = 0.0044, P = 0.9477$) or condition ($F = 0.1736, P = 0.6821$), and no significant sex by condition interaction ($F = 0.0008, P = 0.9777$) between the %S1 in HC females, LC females, HC males, and LC males. In animals treated with OT/OTA/saline, there were no significant main effects of sex ($F = 1.4716, P = 0.2328$), condition ($F = 1.3104, p = 0.4331$), or a significant sex by condition interaction ($F = 2.0745, P = 0.1400$). However, a pre-planned comparison revealed that %S1 in females treated with OT was significantly lower than in females treated with saline ($P = 0.0437$) or OTA ($P = 0.0313$), which Cohen’s $d$ indicated to be a large effect ($d > 1.502$).

We examined the proportion of the cortical sheet devoted to S2/PV (%S2/PV). In voles exposed to different amounts of parental care the %S1 showed a significant effect of sexual differentiation ($F = 1.642, P = 0.0074$) (Fig. 6). In animals treated with OT/OTA/saline, there were no significant main effects of sex ($F = 0.3933, P = 0.6033$), and no sex by condition interaction ($F = 0.0494$) or OTA ($P = 0.0404$). Additionally, we found that females treated with OT had a significantly higher %V1 than males ($F = 1.9758, P = 0.1779$) (Fig. 7). In all of these cases, Cohen’s $d$ indicated that the effect size was large ($d > 1.374$).

%M1

We then examined the proportion of the cortical sheet occupied by %M1 (%M1) in HC and LC males and females (Fig. 5). There were no significant main effect of sex ($F = 1.7187, P = 0.2073$) or condition ($F = 1.3851, P = 0.2555$), but there was a significant sex by condition interaction ($F = 5.4782, P = 0.0317$). A post-hoc comparison revealed that the %M1 in LC females was significantly smaller than both LC males ($P = 0.0265$) and HC females ($P = 0.0410$), which Cohen’s $d$ indicated to be a large effect ($d > 1.642$). In animals treated with OT/OTA/saline, there were no significant main effects of sex ($F = 0.0349, P = 0.8529$), condition ($F = 0.8150, P = 0.4504$), or a significant sex by condition interaction ($F = 0.8379, P = 0.4407$).

%V1, %V2, and %AC

We also compared the proportion of the cortical sheet occupied by V1, V2, and AC (Figs 6 and 7). In voles exposed to different amounts of parental care the %V1 showed a significant effect of sex ($F = 5.3618, P = 0.0333$), with females showing a significantly lower %V1 than males. There was no significant main effect of condition ($F = 0.983, P = 0.3093$), and no sex by condition interaction ($F = 0.0770, P = 0.7848$) (Fig. 6). In animals treated with OT/OTA/saline, there were no significant main effects of sex ($F = 1.3531, P = 0.2522$), condition ($F = 2.1307, P = 0.1331$), or a significant sex by condition interaction ($F = 1.6126, P = 0.2131$). A pre-planned comparison revealed that %V1 in females treated with OT was significantly higher than in females treated with saline ($P = 0.0494$) or OTA ($P = 0.0404$). Additionally, we found that females treated with OT had a significantly higher %V1 than males treated with OT ($t_{12} = 2.110, P = 0.0283$). In all of these cases, Cohen’s $d$ indicated that the effect size was large ($d > 1.374$).

The %V2 in voles exposed to different levels of parental care did not show any significant main effects of sex ($F = 0.2645, P = 0.6136$), condition ($F = 2.7988, P = 0.1126$), or a significant sex by condition interaction ($F = 0.9758, P = 0.3179$) (Fig. 7). In voles treated with OT/OTA/saline, there was no significant main effect of sex ($F = 0.4577, P = 0.5029$) or condition ($F = 1.7986, P = 0.1797$). There was a significant sex by condition interaction ($F = 3.9497, P = 0.0279$), with females treated with OT having significantly smaller %V2 than females treated with saline ($P = 0.0074$) and males treated with OT ($P = 0.0325$) which Cohen’s $d$ indicated to be a large effect ($d > 1.080$).
The %AC in voles exposed to different levels of parental care did not show any significant main effects of sex ($F = 0.0744$, $P = 0.7884$), condition ($F = 1.3464$, $P = 0.2620$), or a significant sex by condition interaction ($F = 0.0003$, $P = 0.9876$). Similarly, in voles treated with OT/OTA/saline there were no significant main effects of sex ($F = 0.2653$, $P = 0.6096$), condition ($F = 0.6573$, $P = 0.5242$), or a significant sex by condition interaction ($F = 0.0240$, $P = 0.9763$).

**Discussion**

Primary sensory and motor areas of the neocortex are defined by their functional organization, neuroanatomical connections, and architectonic boundaries [32]. Both during development and in adults, enhancing or reducing sensory input to these areas can alter both the functional and neuroanatomical organization of the primary sensory areas [3, 5, 7, 33–35], but the architectonic boundaries which define the size of cortical fields, are set very early in development, around postnatal day (P)5 in rodents [3]. After this critical period these architectonic boundaries do not change, even following extreme peripheral manipulation, such as limb deafferentation or amputation, [3]. Even before the closure of the critical period; however, altering
architectonic boundaries was only accomplished using the highly invasive peripheral manipulations, including nerve deafferentation or enucleation [3, 7], or by altering patterns of gene expression during embryogenesis [10, 36].

In this experiment, we tested two environmental manipulations that involved sensory mediated social experience, which should engage the OT system; as well as pharmacological manipulation of OT itself. We exposed young prairie voles to one of two experimental conditions: chronic exposure to differential levels of parental care, and acute exposure to a single dose of OT on the day of birth. Both of these treatments were linked to changes in the size of the sensory and motor areas of the neocortex. In both cases, exposure to the stimulus occurred before the closure of the critical period for the formation of architectonic boundaries around P5. Interestingly, these two interventions resulted in qualitatively different alterations to the boundaries. Voles exposed to differential levels of parental care exhibited changes in the total proportion of cortex that was myelinated, as well as the boundaries of M1. In contrast, voles that were given a single dose of OT on the day of birth exhibited changes in the boundaries of S1, V1, and V2. These differences could be explained by the intensity of the manipulation. The bolus dose of OT that was given exceeded normal physiological levels, but was only present in the body for a short period of time. In contrast, different parenting styles, which in rats are linked to alterations in both maternal and offspring OT levels [37, 38], may have resulted in longer-term exposure to a lower dose of OT.

Furthermore, the age at which the exposure occurred may have influenced the cortical regions that were altered. Cortical arealization depends on a number of factors, including cell cycle regulation [39, 40] and gene expression and epigenetic effects [8, 41, 42], as well as extrinsic factors such as sensory stimulation [7, 43, 44]. Sensory experience, in turn, regulates the synthesis and expression of OT within the cortex, which increases cross-modal plasticity [26]. Thus, increases in the amount of OT, whether through environmental stimulation or pharmacological administration, could induce cortical plasticity, thereby altering selected cortical borders.

Another interesting feature of the data is that administration of the OT antagonist did not produce a result opposite to that of OT administration. In every case, the OTA treatment did not differ from saline. This suggests that while OT receptors may be involved in the development of sensory and motor cortex, they are not strictly necessary. It is most probable that arginine vasopressin, a peptide that has cross-reactivity with OT, is also involved in this process; or even that OT itself is acting through vasopressin receptors [45–48]. One interesting line of research would be to study the sensory and motor cortex from animals in which the gene for OT receptors or vasopressin V1a receptors have been knocked out.

In this study, we found that both manipulations had sexually dimorphic effects, leading to significant alterations in cortical areas in females, but not in males. These results are not uncommon when dealing with neuropeptides like OT. In rats, high levels of maternal licking and grooming are linked to increased OTR binding in the amygdala and bed nucleus of the stria terminalis in females but not in males [37]. In prairie voles, exposure to OT results in sexually dimorphic effects, including increased aggression towards strangers [20], pair-bonding [49, 50], alloparental care [29], as well as the distribution of vasopressin V1a receptors [18] and estrogen receptors [51]. Males and females often differ by the direction of effects or the dosage required.

The normal distribution of parental care in prairie voles may be analogous to a well-studied form of parental care in another species: maternal licking and grooming (LG) in rats [17]. There are several similarities between the two behaviors. First, rat dams exhibit variation in the amount of LG that they perform [52], much like prairie vole parents exhibit variation in the amount of time spent in contact with their offspring [17]. Furthermore, differences in the amount of LG received as pups is linked to variations in adult behavior [53], as is the amount of parental care received by young prairie voles [23]. The amount of time spent performing LG behaviors is mediated by maternal OT levels in rats [54], and maternal behaviors in prairie voles are mediated by OT levels [55]. The culmination of the LG literature in rats was the discovery that the mediation of the long-term consequences of early behavioral experiences occurred through epigenetic processes [56, 57]. Although this has not yet been explicitly demonstrated in prairie voles, there are suggestions that the transmission of social behavior through parental care in voles may also be epigenetically regulated [24].
The administration of a single bolus dose of OT on the day of birth is designed to mirror the administration of OT as a labor-induction technique. Worldwide, the administration of OT, either alone or in combination with other techniques, is the most common method of labor induction [58]. In the USA in 2010, 23.8% of labors were induced [59]. Additionally, OT is routinely administered to postpartum mothers in order to aid in the contraction of the uterus and reduce the risk of maternal hemorrhage [60]. Despite the commonality of this practice, little is known about the long-term effects of perinatal OT administration. Recently, studies have indicated a relationship between OT-induced labor and attention deficit hyperactivity disorder diagnoses [61]. It has also been suggested that perinatal OT is linked to later diagnosis of autism spectrum disorders [62–64], although several studies have found no support for that link [59, 63]. Further, many clinical trials investigating OT administration as a treatment for social and behavioral symptoms of ASD are underway or have been completed (i.e. [65]).

Little to nothing is known about the anatomical or physiological effects of early OT administration in humans. The recent findings in animals that OT may be involved in cortical plasticity [26], and that differences in parental care can alter behavior [17, 24] as well as corticocortical connections [21] indicate that early alterations in OT levels may have major long-term consequences. In light of these recent findings, as well as the data presented here, we would urge caution when administering OT to patients whose brains are still developing.

Finally, perhaps the most intriguing aspect of these data is what they imply about the generation of phenotypic diversity within a population. In order to reduce the variability within studies, inbred strains of laboratory animals have been developed, which are phenotypically, if not genotypically, distinct from their wild counterparts [66, 67]. Although we have long recognized phenotypic variability in wild organisms, the same cannot be said for laboratory animals. This is problematic, because phenotypic variability is one of the principles through which evolution by natural selection operates, and while we are beginning to understand how variation across species occurs, little is known about the origins of individual variation.

In humans, individual variation has been a focus of research, particularly in an attempt to understand the origins of specific behaviors. Characteristics such as the accuracy of introspection [68], musical ability [69], depression [70], working memory and attention [71], and language impairments [72] have all been linked to individual differences in brain anatomy. However, the origins of these differences remain unclear.

Although we appreciate that genetic variability obviously plays a large role in inducing individual differences within a population, here we demonstrate two methods by which individual variation can be achieved without alterations in gene sequence: differences in early parental care and administration of OT shortly after birth. Intermediary molecular mechanisms, such as gene expression and epigenetic markers such as methylation or histone acetylation, would be the logical next steps in exploring this variation.

Methods

Subjects

A total of 64 prairie voles were included in this study. A subset of subjects was used in other anatomical experiments, including functional mapping and neuroanatomical tracer experiments. Animals were born and housed in the UC Davis Psychology Department vivarium. 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 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.

Behavioral Assessments: (HC vs LC)

Breeder pairs were observed to characterize the type and amount of parental behavior directed towards their offspring during the first several postnatal days. The observations performed are described in detail elsewhere [17]. Briefly, breeder pairs were observed four times during the P1–3, twice in the morning and twice in the afternoon. Observations lasted for 20 minutes and included maternal and paternal huddling, pseudohuddling, non-huddling contact, licking/grooming, anogenital licking/grooming, retrievals, hunching, nest building, autogrooming, and, in the mother, lateral, active, and neutral nursing. Behaviors were recorded using behavioral software (www.behaviortracker.com).

Rankings were calculated by summing the total amount of time the parents spent in contact with the pups to generate a parental behavior score. Scores were then ranked into quartiles, with the highest quartile becoming the high contact (HC) group, the lowest quartile becoming the low contact (LC) group, and the middle 50% of animals were excluded from analysis [17]. Twenty-one animals (5 HC females, 6 HC males, 3 LC females, and 7 LC males) were included in this experimental group. Because these subjects were exposed to different amounts of parental care over a long period of time (the first P20), and because differences in parental care are linked to differences in OT receptor levels [24], subjects in these groups were exposed to chronic differences in OT levels.

Pharmacological Treatments: (Saline/OT/OTA)

Forty-three animals were included in this experimental group. Within 24 hours of birth, experimental subjects were briefly removed from the cage, sexed, weighed, and toe-clipped for identification. All pups, both male and female, were randomly assigned to treatment groups, receiving an intraperitoneal injection of isotonic saline (eight males, eight females), OT (11 males received 3 μg and 3 females received 6 μg), or OTA ([d(CH$_2$)$_3$]-Tyr(Me)$_2$, Orn$_4$; vasotocin; five females and eight males each received 0.3 μg). OT doses were chosen based on previous studies, as the ones that would maximize facilitation of pair bonding in adult animals [28, 29]. All injections were 25.0 μl in volume and administered via a 250 μl gas-tight Hamilton syringe. In contrast to the subjects exposed to HC or LC parenting styles, these subjects were all the offspring of medium contact parents and experienced a single acute dose of OT, OTA, or saline.

Histology

Animals were euthanized with an overdose of sodium pentobarbital (250 mg/kg, IP) and transcendially 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 was removed from the subcortical structures. The neocortex
was flattened and postfixed in 30% sucrose overnight. The flattened tissue was sectioned at 20 μm using a freezing microtome, and the resulting sections were stained for myelin [73].

**Analysis**

Borders were drawn identifying the boundaries of sensory regions within the neocortex. As described previously in [2], while individual sections of tissue can contain many partial anatomical boundaries, complete boundaries were obtained by combining the entire series of sections into a single comprehensive reconstruction. This was accomplished by taking photomicrographs of individual sections that were stained for myelin. All the photomicrographs for an individual case were imported into Adobe Illustrator (Adobe Systems, San Jose, CA), and sections were aligned using landmarks, including blood vessels, tissue artifacts, and the outline of the section (Fig. 1). In all cases, the largest section in the series was used to define the outline of the cortical hemisphere, and the person drawing the boundaries was blind to the condition of the subject.

Once the consolidated architectonic boundaries were determined, we found the area in mm² of the following regions: the corticocortical sheet (excluding the olfactory bulb, piriform cortex, and entorhinal cortex), primary somatosensory cortex (S1), secondary somatosensory cortex/parietal ventral area (S2/PV), primary motor cortex (M1), primary visual cortex (V1), second visual cortex (V2), auditory cortex (AC), and FM area. We then calculated the proportion of the cortical sheet that was comprised of each of those regions, as well as the total proportion of myelinated cortical regions (Fig. 2). Due to differences in the method of flattening, in some cases the cingulate cortex was visible and increased the area of the cortical sheet. In those cases, we subtracted the area of the cingulate cortex from the area of the cortical sheet and used that value as the denominator when calculating the proportion of the cortical sheet that was comprised of each region. In these cases, the resulting area of the cortical sheet was comparable to that of cases where the cingulate cortex was not visible.

In cases where both hemispheres were available, the values were averaged between hemispheres to generate a single set of data for the animal. Values were then averaged within groups. Animals that were exposed to HC and LC parenting styles were analysed separately from animals treated with OT/OTA/Saline. Differences within each group were compared using two-way analysis of variance (ANOVA; JMP, SAS, Cary, NC). Individual differences between specific groups were determined using Student’s t-tests. Effect size was calculated using Cohen’s d. For all tests, \( p < 0.05 \).

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**Conflict of interest statement.** None declared.

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