Transcriptome Response to Infraorbital Nerve Transection in the Gonadally Intact Male Rat Barrel Cortex: RNA-Seq

John J. Orczyk,[1]* Rishabh Sethia,[1] Dominique Doster,[1] and Preston E. Garraghty[1,2]

[1]Department of Psychological and Brain Sciences, Indiana University, Bloomington, Indiana 47045
[2]Program in Neuroscience, Indiana University, Bloomington, Indiana 47045

ABSTRACT

The effects of infraorbital nerve (ION) transection on gene expression in the adult male rat barrel cortex were investigated using RNA sequencing. After a 24-hour survival duration, 98 genes were differentially regulated by ION transection. Differentially expressed genes suggest changes in neuronal activity, excitability, and morphology. The production of mRNA for neurotrophins, including brain-derived neurotrophin factor (BNDF), was decreased following ION transection. Several potassium channels showed decreased mRNA production, whereas a sodium channel ($Na_V\beta4$) associated with burst firing showed increased mRNA production. The results may have important implications for phantom-limb pain and complex regional pain syndrome. Future experiments should determine the extent to which changes in RNA result in changes in protein expression, in addition to utilizing laser capture microdissection techniques to differentiate between neuronal and glial cells. J. Comp. Neurol. 524:152–159, 2016.

© 2015 Wiley Periodicals, Inc.

INDEXING TERMS: plasticity; injury; cerebral cortex; Sprague–Dawley rats (RRID:RGD_70508); CLC Main Workbench (RRID:OMICS_01813); Ingenuity Pathway Analysis (RRID:nif-0000–33144)

The rat posteromedial barrel subfield (PMBSF; or barrel cortex) is a well-suited model in which to study the effects of sensory deprivation in the central nervous system. Mystacial whiskers map to "barrels" (cellular aggregates) in the barrel cortex in a one-to-one anatomically defined relationship (Woosley and Van Der Loos, 1970). Transecting the infraorbital nerve (ION) deprives the barrel cortex of its principle driving input. In primates, depriving the hand representation of input through peripheral nerve transection leads to a reorganization of the somatosensory cortex as deprived neurons come to respond to novel receptive fields (Merzenich, 1983a; Garraghty and Kaas, 1991; Schroeder et al., 1997). In rats, transecting the sciatic nerve results in an enlargement of the saphenous representation of the hind limb (Wall and Cusick, 1984). However, changes in cortical organization following ION transection are more limited in scope, as only small expansions of responses from the lower jaw and digits are found, leaving roughly 75% of the barrel cortex unresponsive to stimuli 60 days after ION transection (Waite, 1984).

Our laboratory previously studied the effects of input deprivation in the squirrel monkey hand representation using autoradiography and immunohistochemistry. Transection of the median and ulnar nerves decreased the number of γ-aminobutyric acid (GABA)-positive cells by almost 75% in deprived cortex in comparison with adjacent or ipsilateral nondeprived cortex (Garraghty et al., 1991). Receptor autoradiographic studies found that at 2–5 hours after nerve transection, a decrease in both $GABA_A$ and $GABA_B$ receptors was present throughout all layers of the cortex. However, a decrease in $GABA_A$ in layer IV of deprived somatosensory cortex was greater than for other layers of the cortex (Wellman et al., 2002). Reductions in $GABA_A$ receptor binding in layer IV are still present 1–2 months after nerve transection. The percent reduction of $GABA_B$ receptor binding in layer IV almost doubles over 1–2 months in comparison with reductions observed 1–2 days after nerve transection (Garraghty et al., 2006). In rats, transection of the sciatic nerve

© 2015 Wiley Periodicals, Inc.

*CORRESPONDENCE TO: John Orczyk, Department of Psychological and Brain Sciences, Indiana University, 1101 E 10th Street, Bloomington, IN 47405. E-mail: jorczyk@indiana.edu

Received April 8, 2015; Revised June 12, 2015;
Accepted June 16, 2015.
DOI 10.1002/cne.23831
Published online July 16, 2015 in Wiley Online Library (wileyonlinelibrary.com)

followed by a 2-week survival period was accompanied by a 16% decrease in the number of detectable glutamic acid decarboxylase (GAD)-positive cells staining in layer IV only, with other layers maintaining concentrations of GAD-positive cells similar to those found in intact rats. These results suggest that expression of GAD in layer IV varies inversely with the degree of sensory input (Warren et al., 1989). Excitatory drive is also increased in the somatosensory cortex in response to input deprivation. Receptor autoradiography reveals an increase in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in layer IV of deprived cortex 1–2 months following median and ulnar nerve transection in adult squirrel monkeys, but not 0–3 days after transection. In contrast, the number of N-methyl-D-aspartate (NMDA) receptors did not significantly change during either 0–3 days or 1–2 months following nerve transection (Garraghty et al., 2006).

The first phase of cortical reorganization is the unmasking of latent inputs following sensory deprivation. This phase appears to begin immediately and occurs simultaneously in the cortex as well as in subcortical structures. Recordings were simultaneously obtained from adult rat brainstem trigeminal spinal tract nucleus (SpV), thalamic ventral posteromedial nucleus (VPM), and somatosensory cortex (SI) before and after some of the whiskers were anesthetized by an injection of lidocaine. After only 3–5 minutes following lidocaine injection, neurons that previously responded to stimulation of whiskers affected by lidocaine began to respond to the stimulation of neighboring whiskers. No sequence of changes in neuronal receptive fields could be determined, as neurons from SpV, VPM, and SI all responded to novel receptive fields simultaneously. The authors hypothesize that the unmasking of alternate receptive fields reflects changes in the balance between excitation and inhibition that occur both cortically and subcortically immediately following the loss of sensory input (Faggin et al., 1997).

In the present study, we examined changes in gene expression in rat barrel cortex 24 hours after ION transection in intact male rats. Given the immediacy of the unmasking observed by Faggin et al. (1997), we expected to see a large number of genes differentially expressed in the sensory-deprived barrel cortex of gonadally intact male rats.

MATERIALS AND METHODS

Six gonadally intact male Sprague–Dawley rats (RRID:RGD_70508) were obtained from Harlan Laboratories (Indianapolis, IN) and housed at the Indiana University Psychology core facility under a 12-hour light/dark cycle (lights on at 7:30 am). Gondally intact males were used, as this study is the first of a series investigating the effects of hormonal status and ION transection on plasticity in the barrel cortex. Rats were provided free access to food and water. All animals were treated humanely, and all procedures involving the use of animals were approved by the Bloomington Institutional Animal Care and Use Committee.

For ION transection surgeries, animals were anesthetized with a mixture of 2–4% isoflurane and oxygen via inhalation. The absence of a withdrawal reflex upon pinching the foot was used as a measure of anesthetic depth. Ophthalmic ointment was applied to the eyes to prevent desiccation. Meloxicam was administered subcutaneously at a dose of 2 mg/kg. The left snout was shaved and disinfected with three alternating scrubbings of betadine and alcohol. All surgeries were performed under aseptic conditions in a surgical suite. Rats were adults, between 3 and 6 months of age at the time surgery was performed.

A 1-cm incision was made over the left ION foramen. The ION was located by blunt dissection using hemostats, elevated with forceps, and transected with a scalpel blade. Usually, two to three transections were necessary to ensure the entire nerve was transected, as the ION branches rapidly upon exiting the foramen. After entire transection of the nerve was ensured, the wound was sutured and the animal was closely monitored during recovery from anesthesia.

After a 24-hour survival duration, the animals were again anesthetized with 2–4% isoflurane and oxygen. After the appropriate anesthetic depth was ensured, animals were decapitated using a guillotine, and a blood sample was collected in a heparin-containing vial. The animal's scalp was removed using a scalpel, and the skull was removed with rongeurs. The brain was removed, and the PMBSF was located on each side of the brain using the middle cerebral artery on the cerebral surface, which has previously been shown to correspond to the location of the PMBSF (Cox et al., 1993). A 5-mm-diameter section of cortex was extracted by using a modified hypodermic needle to isolate the tissue and forceps for extraction. Cortical tissue samples were immediately placed in RNAlater (Qiagen, Valencia, CA) to preserve RNA for extraction and sequencing. The surgical site was then examined to ensure that the ION had been completely transected. Tissue samples in RNAlater were stored at room temperature for 24 hours to allow penetration of the RNAlater solution. Samples were then moved to a −20°C freezer until RNA extractions were performed.

Cortical tissue was homogenized in 750 μL Qiazol (Qiagen) by using a rotor stator homogenizer. The lysate was then transferred to a QIAshredder spin column (Qiagen) and centrifuged according to the manufacturer's

directions. RNA was extracted by standard trizol/chloroform extraction. The resulting RNA was purified using an RNeasy Mini Kit (Qiagen) according to the manufacturer's directions. RNA samples were then checked for purity and concentration using the Take3 microplate and Epoch microplate spectrophotometer (BioTek, Winooski, VT), utilizing the ratio of absorbance intensity at 260/280 nm. Each piece of tissue was handled individually, allowing comparisons to be made within individual animals.

Polyadenylated messenger RNA was separated from total RNA and used to construct a cDNA library with a kit according to the manufacturer's instructions (Illumina, San Diego, CA) using the low sample protocol. Libraries were sequenced on an Illumina HiSeq2000 next-generation sequencer according to the manufacturer's instructions.

Bioinformatics were performed on the resulting raw sequence data. Adaptor trimming and quality filtering with a minimum quality score of 20 were performed using Trimmomatic ver.0.32 (Bolger et al., 2014). Reads were required to have a minimum of 35 bases. Over 95% of the sequenced reads passed the quality filters. These filtered reads were then mapped to the assembled rat genome Rnor_5.0 (GCA_000001895.3), annotated with Ensembl system ver 5.0.75, using TOPHAT ver 2.0.10. This process results in the number of read counts per mapped gene.

Differential expression analysis was accomplished using CLC Main Bench software (Qiagen; RRID:OMICS_01813). For comparing data between left and right hemispheres, a paired-subject analysis was used with a common estimate of dispersion. Genes were declared to be differentially expressed (DE) if the false discovery rate (FDR) was 0.05 or less. Data were analyzed through the use of Qiagen's Ingenuity® Pathway Analysis (IPA®, Qiagen, Redwood City, CA; www.qiagen.com/ingenuity; RRID:nif-0000–33144).

Fold change (FC) values were calculated by CLC Main Bench software, comparing the deafferented barrel cortex with the opposite barrel cortex with its input still intact. Bilateral changes in gene expression, such as those resulting from stress or anesthesia, are accounted for by using the opposite hemisphere as a control. Using a within-subjects design also permitted the use of a paired-subject statistical test. Individual differences would likely obscure changes due to peripheral deafferentation if comparisons were made with animals that had undergone a sham operation. However, it is possible that bilateral changes in gene expression in the same direction were undetected, due to the comparison with the opposite hemisphere.

RESULTS

Transection of the ION results in changes in expression of a number of genes within 24 hours. RNA sequencing revealed that 98 genes are differentially expressed as a function of ION transection in gonadally intact males. Of these, 85 displayed a decrease in mRNA production and 13 displayed an increase

DE genes after peripheral nerve injury in gonadally intact males were fit into known "canonical" pathways developed with a curated gene database using right-tailed Fisher's exact test (IPA analysis software). A number of pathways were overrepresented following peripheral nerve transection in intact male rats (Fig. 1). Some of the most overrepresented pathways include "$G\alpha_s$ signaling" (-log(*P* value) = 3.91), "cAMP-mediated signaling" (-log(*P* value) = 2.54), "axonal guidance signaling" (-log(*P* value) = 2.51, and "neurotrophin/0TRK signaling" (-log(*P* value) = 2.48.

The relative expression levels of selected genes from differentially regulated pathways are presented in Figure 2. Neurotrophin-3 (NTF3) mRNA showed a 7-fold decrease after ION transection. Production of brain-derived neurotrophic factor (BDNF) mRNA was halved after ION transection. The production of $G\alpha_s$-coupled receptor mRNA was mostly decreased, except for adenosine receptor A2a (ADORA2A) mRNA, which showed a 2.5-fold increase in mRNA concentration. The production of mRNA for many of the ephrin receptors was decreased by ION transection, including ephrin A8, (EPHA8) and ephrin B3 (EPHB3), which were decreased by 3-fold and 2.5-fold, respectively. The production of mRNA for potassium channel subunits forming delayed rectifiers was decreased in all three comparisons, but mRNA coding for a sodium channel beta-subunit associated with resurgent sodium currents was increased by over 2-fold after nerve injury.

A functional analysis of predicted downstream effects was also conducted using IPA software based on prior knowledge stored in IPA's gene database. The associated gene products of DE genes after ION transection in these animals predict decreases in synaptogenesis, activation of neurons, migration of cells, and formation of plasma membrane (Table 1).

DISCUSSION

In the present study, we investigated changes in gene expression associated with peripheral deafferentation of the rat barrel cortex. Changes in the expression of genes of the neurotrophin and $G\alpha_s$ signaling pathways suggest a decrease in neural activity. Changes in neurotrophin and axonal guidance signaling are consistent with the beginning of morphological changes leading to a reduction in the dendritic branching of input-deprived barrel cortex pyramidal neurons.

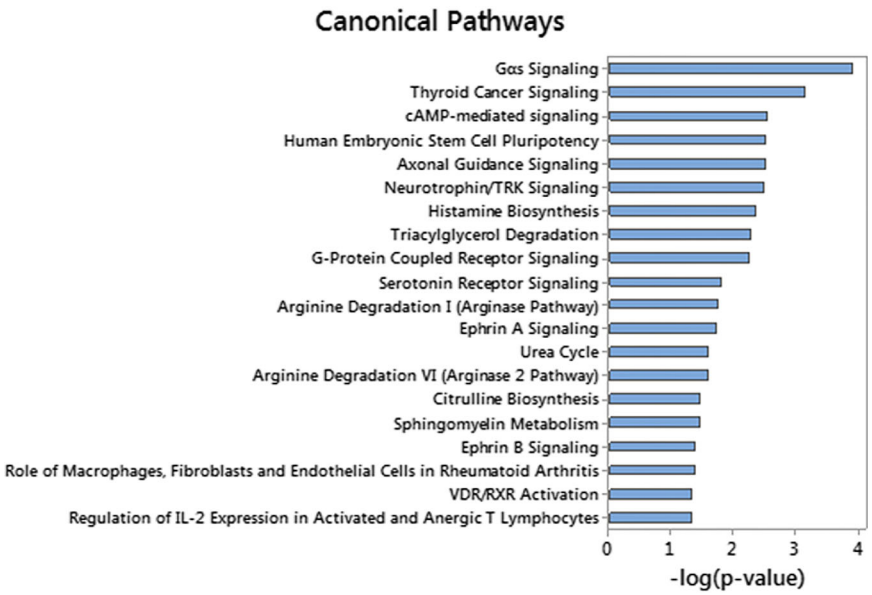

Figure 1. Canonical pathways differentially regulated by infraorbital nerve (ION) transection in sensory deprived barrel cortex of intact rats. Significantly differentially regulated genes were fit to canonical pathways developed with a curated gene database using Fisher's exact test by Ingenuity Pathway Analysis©. Gαs- and cAMP-mediated signaling appear near the top along with axonal guidance and neurotrophin signaling. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

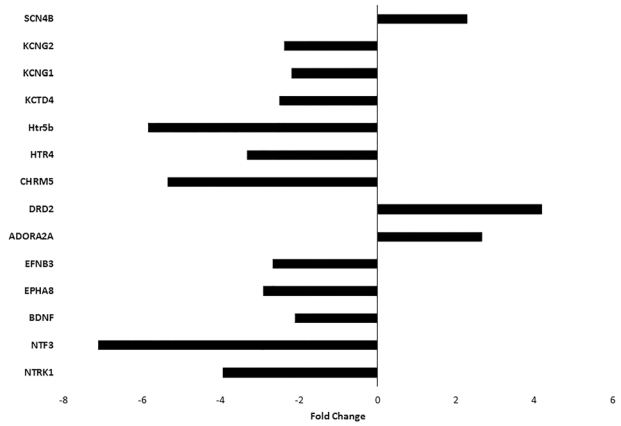

Figure 2. Relative expression levels for genes from overrepresented pathways. Genes overrepresented from the neurotrophic signaling pathway: neurotrophic tyrosine kinase, receptor type 1 (NTRK1); neurotrophin 3 (NTF3); brain-derived neurotrophic factor (BDNF). Genes overrepresented from the ephrin signaling pathway: ephrin receptor A8 (EPHA8), ephrin B3 (EFNB3). Genes overrepresented from the $G\alpha_s$ signaling pathway: adenosine receptor A2a (ADORA2A); dopamine receptor 2 (DRD2); cholinergic receptor, muscarinic 5 (CHRM5); serotonin receptor 4 (HTR4); serotonin receptor 5b (Htr5b). Differentially regulated ion channel subunits: potassium channel tetramerization domain containing 4 (KCTD4); potassium voltage-gated channel, subfamily G member 1 (KCNG1); potassium voltage-gated channel, subfamily G, member 2 (KCNG2); sodium channel, voltage-gated, type IV, beta subunit (SCN4B).

Implications for neural activity

We have shown previously that ION transection ultimately results in a reduction in staining intensity for the metabolic enzyme cytochrome oxidase (CO) in the deprived whisker barrels (Mowery et al., 2009). This change almost certainly reflects a reduction in activity of neurons in those deprived barrels. The expression of neurotrophins is activity dependent. Tetanic stimulation of the CA1 stratum radiatum induces long-term potentiation (LTP), and also an increase in both BNDF and NT-3 mRNA, as measured in hippocampal slices using in situ hybridization. Grain counting analysis reveals that the average level of BDNF mRA in potentiated CA1 slices is 2.2 times greater than in control slices. The average level of NT-3 mRNA expression was 1.5 times greater in potentiated slices than in control slices (Patterson et al., 1992). We observed a downregulation of both BDNF and NT-3 with ION transection. Because tetanic stimulation promotes expression of neurotrophins, a reduction of neural stimulation would be expected to reduce neurotrophin expression.

Further support for the activity-dependent regulation of neurotrophins comes from the observation that BDNF expression is calcium dependent. Promoter sequences of exon III of the BDNF gene confer over 80% of its calcium responsiveness. This exon contains two calcium-responsive sequences. One is a calcium or cyclic adenosine monophosphate response element (CaRE/CRE), and one is a

TABLE 1.

Cellular and Organismal Functions Associated With Differentially Expressed Genes 24 Hours After Infraorbital Nerve (ION) Transection in Intact Male Rats With Activation z-Scores > |2.0| [1]

Disease or function annotation	*P* value	Predicted activation state	Activation z-score
Organismal death	8.99E-04	Increased	3.499
Neonatal death	7.82E-04	Increased	2.959
Cyanosis	3.32E-03	Increased	2
Lymphocyte migration	3.98E-04	Decreased	−2.005
Cell movement of lymphocytes	2.27E-04	Decreased	−2.006
Development of gap junctions	3.95E-06	Decreased	−2.178
Synaptogenesis	2.16E-05	Decreased	−2.178
Activation of neurons	3.37E-05	Decreased	−2.195
Retraction of neurites	9.45E-06	Decreased	−2.213
Cell movement of leukocytes	3.60E-04	Decreased	−2.261
Formation of plasma membrane	2.36E-07	Decreased	−2.272
Assembly of intercellular junctions	1.54E-06	Decreased	−2.272
Migration of cells	4.07E-03	Decreased	−2.387
Chemotaxis of cells	1.84E-03	Decreased	−2.394
Cell movement of mononuclear leukocytes	3.68E-05	Decreased	−2.435
Cell movement	1.21E-03	Decreased	−2.515
Leukocyte migration	4.18E-04	Decreased	−2.607
Quantity of neurons	2.67E-06	Decreased	−2.745
Cell movement of blood cells	1.25E-04	Decreased	−2.766
Quantity of cells	1.54E-03	Decreased	−2.919
Survival of organism	1.44E-03	Decreased	−3.13

[1]All z-scores with a magnitude greater than 2.0 are considered significant.

novel calcium response element. Mutations of the CaRE/CRE partially attenuate calcium responsiveness in embryonic cultures and completely inhibit it in postnatal cultures. Antibodies to the transcription factor CREB bind to an oligonucleotide containing the promoter sequence from BDNF exon III, suggesting that CREB binds to the CaRE/CRE promoter sequence. Finally, the expression of a constitutively active form of calcium/calmodulin-dependent protein kinase type IV (CaM kinase IV) leads to the transcription of the BDNF promoter in the absence of stimulus, and even occludes calcium-dependent transactivation of the promoter, suggesting its involvement in calcium signaling leading to BDNF expression (Shieh and Ghosh, 1999). The decreased production of BDNF mRNA observed in sensory-deprived barrel cortex suggests that intracellular calcium levels are lower in deprived cortex, and lower intracellular calcium levels clearly imply reduced neural activity.

Changes in G-coupled protein signaling have mixed effects on the production of cAMP in sensory-deprived barrel cortex. Transcription is enhanced for the gene ADORA2A by ION transection. This gene codes for the adenosine 2A receptor, which increases cAMP production when activated (Olah, 1997). However, DRD2, which codes for dopamine receptor 2 (D2), also shows enhanced expression by ION transection. Activation of the D2 receptor decreases the production of cAMP (Missale et al., 1998). The production of mRNA for some serotonin receptors is also decreased by ION transection. The production of mRNA for the HTR4 gene, which codes for the 5-HT 4 receptor, is decreased. 5-HT 4 receptors are coupled to Gs proteins and increase the production of cAMP (Claeysen et al., 2002).

Taken together, these results suggest that neural activity is reduced after ION transection. Expression of both BDNF and NT-3 is reduced after ION transection, implying reduced activity and reduced calcium entry into neurons. Notwithstanding upregulation of ADORA2A, changes in G-coupled protein signaling mainly favor a reduction in the production of cAMP. A reduction in cAMP would reduce the amount of neurotransmitter released.

Implications for mechanisms related to intrinsic excitability

Our results contain evidence for changes in the composition of voltage-gated potassium and sodium ion channels in favor of greater excitability. The gene KCNG1 codes for the potassium channel subunit Kv6.1, whereas KCNG2 codes for Kv6.2. Kv6.1 and Kv6.2 are unable to form functional channels as homotetramers but instead heterotetramerize with Kv2 family members to form conductive channels that function as delayed rectifiers. These channels help to reestablish the resting membrane potential after an action potential has been fired. Kv2.1–Kv6.1 hetromultimeric channels have a decreased rate of inactivation in comparison with Kv2.1 homomeric channels. Allowing more potassium into the cell biases the cell's membrane potential toward the potassium equilibrium potential, enhancing afterhyperpolarization. In turn, the refractory period between

action potentials is prolonged (Post et al., 1996). A decrease in mRNA production for Kv6.1 and Kv6.2 would be expected to shorten the refractory period, enabling cells to fire action potentials at higher rates, assuming that decreased transcription also resulted in decreased protein expression

We also found that the production of mRNA for a voltage-gated sodium channel subunit Navβ4 (SCN4B) was increased by ION transection. This subunit antagonizes the deactivation of sodium channels by interfering with the gating mechanism. Excess sodium current leads to repetitive or burst firing (Bant and Raman, 2010). Sensory deprivation of the barrel cortex achieved through whisker trimming is associated with an increase in layer V pyramidal neurons that display burst firing (Breton and Stuart, 2009). Our results suggest that this increase could be accomplished through an upregulation of Navβ4, if the increase in transcription positively correlates with subunit expression. Changes in neuronal conductance caused by activity deprivation have been previously reported in stomatogastric ganglion neurons from the spiny lobster, *Panulirus interruptus* (Turrigiano et al., 1994). These neurons usually fire in bursts of action potentials when released from inhibition. When isolated in primary cell culture, neurons fire tonically when depolarized or released from hyperpolarization. However, after 3–4 days of isolation in culture, these neurons fired in bursts when released from hyperpolarization, yet, if these neurons are supplied with an exogenous rhythmic hyperpolarizing current, mimicking in vivo conditions, they lose their ability to endogenously produce burst firing and revert back to tonic firing when the rhythmic stimulation is removed. These experiments show that neurons change their intrinsic conductance in response to changing inputs.

Taken together, changes in ion channel expression after ION transection favor increased neuronal excitability. Voltage-gated potassium channel subunits that prolong the time between the firing of action potentials showed a reduction in mRNA production. In addition, a voltage-gated sodium channel subunit that promotes burst firing shows increased transcription.

Implications for cellular morphology

Our results show a decrease in mRNA production for both neurotrophins and ephrins. Axonal branching and synaptogenesis are differentially regulated by neurotrophins and ephrins. In cultured mice hippocampal slices, BDNF treatment leads to an increase in synaptic density. This increase is significantly reduced with coincubation with EphA7-FC, which activates ephrinA receptors (Marler et al., 2008). Ephrins play an important role in axonal guidance, mainly acting to repel axons and migrating cells. Ephrins also play a role in dendritic spine morphology. In the rodent barrel cortex, ephrins guide specific thalamocortical axons to their precise targets within the barrel field through a gradient in ephrin expression (Dufour et al., 2003). A number of ephrin receptors showed decreased mRNA production as a result of ION transection (Fig. 2). This result may be interpreted as a relaxation of axonal repulsion. However, neurotrophin receptor NtrkA, and the neurortrophins NTF-3 and BDNF, also show decreased transcription after ION transection. The TrkB receptor, which is activated by BDNF, is essential for maintaining dendritic structure and cell morphologies of pyramidal neurons in the neocortex. Pyramidal neurons in conditional $TrkB^{-/-}$ knockout mice have reduced dendritic branching, thinner dendrites, and a rounded soma. In these mice, many $TrkB^{-/-}$ neurons do not survive from 4 to 10 weeks after birth, resulting in a compressed neocortex (Xu et al., 2000).

A reduction in the transcription of neurotrophins is consistent with previous results, assuming that reduced transcription results in reduced protein expression. ION transection does not lead to large-scale somatotopic reorganization of the barrel cortex. Instead, most of the barrel cortex is unresponsive to peripheral stimulation 60 days after ION transection (Waite, 1984). More directly, as referenced above, our laboratory previously reported decreases in CO staining in the barrel cortex after ION transection. In the same paper, we also reported decreases in the sizes of deprived cortical barrels (Mowery et al., 2009). A decrease in neurotrophin expression might be the cause of decreasing barrel diameters. Barrels consist of a cell-dense wall and cell-sparse hollow, with a predominance of dendrites directed toward the hollow. A reduction of dendritic branching might compress the barrels if sensory-deprived barrels contain thinner dendrites and less elaborate dendritic trees.

This line of reasoning has interesting implications for the cortical genetic response to a loss of input due to peripheral nerve damage in general. As discussed earlier, ION transection in adult rats results in little topographic reorganization within the deprived whisker barrel cortex, even after 60 days (Waite, 1984). In marked contrast, transection of the median nerve or both the median and ulnar nerves in adult monkeys is followed by apparently complete topographic reorganization throughout the deprived region of cortex within weeks (Merzenich et al., 1983a,b; Garraghty and Kaas, 1991). Thus, although one might characterize the cortical area normally activated by the transected nerves in either case as deprived, the consequences of the deprivation are markedly different. Given the fundamentally different outcomes, one would expect that genetic

responses and their consequences would differ markedly after ION transection in rats versus median or median and ulnar nerve transections in monkeys. Although there are no data from monkeys that one can compare with the present results, there are data on neuronal morphology in deprived cortex after peripheral nerve injury in monkeys. In marked contrast to the predictions made here that one would expect to find regressive changes in cellular morphology in deprived whisker barrels, Churchill et al. (2004) reported evidence of progressive changes in dendritic morphology of neurons in primate somatosensory cortex after peripheral nerve injury. This serves as a further reminder (Garraghty et al., 1994; Sengelaub et al., 1997) that all sensory deprivations of the cerebral cortex are not created equally, even if the method of producing the deprivation (here, peripheral nerve transections) is held constant. Therefore, caution must be used when comparing data from different sensory deprivation experiments.

CONCLUSIONS

In conclusion, our results indicate that depriving the barrel cortex of sensory input leads to a use-dependent decrease in mRNA production for several neurotrophins and ephrins, changes in the mRNA concentrations of G-protein–coupled receptors affecting the production of cAMP, and changes in the mRNA expression of membrane ion channels. These changes have implications for neuronal activity, excitability, and morphology.

Phantom-limb pain and complex regional pain syndrome (CRPS) are two neuropathic pain syndromes associated with maladaptive somatosensory reorganization (Flor et al., 1995, 2006; Maihofner et al., 2003). Recovery from CRPS is associated with a reversal of cortical reorganization during therapy (Maihofner et al., 2004). Pharmacological inventions that target neuroplastic changes, including NMDA receptor antagonists or GABA agonists, have been suggested as options to treat phantom pain (Flor et al., 2006). Our results reveal that changes in neuronal excitability following sensory deprivation are due to changes in ion channel subunit composition. Future research could investigate ion channel subunit–specific expression in neuropathic pain models. Moreover, cortical reorganization fails to occur in the rat barrel cortex following peripheral deafferentation. DE genes in our study suggest that downregulation of neurotrophins may be partially responsible for the lack of cortical reorganization. Future studies should compare neurotrophin expression in deafferented rat barrel cortex with expression in deafferented somatosensory cortex of nonhuman primates, as cortical reorganization occurs readily with median nerve transection in monkeys (Merzenich, 1983a; Garraghty and Kaas, 1991; Schroeder et al., 1997). A tantalizing possibility is that neurotrophins are upregulated in reorganizing cortex, suggesting that Trk receptor antagonists may block cortical reorganization, and could be administered immediately after trauma to prevent the development of CRPS or phantom-pain syndromes. In support of this possibility is the observation that neurotrophins and their receptors are upregulated in as little as 3 days following binocular retinal lesions (Obata et al., 1999).

A major limitation of the current study is that only mRNA was measured. Protein expression does not necessarily correlate with transcription. Future studies are needed to confirm that the reduction of mRNA for specific proteins results in changes in protein expression. Future studies would also benefit from the preselection of individual cells before tissue processing. It might then be possible to determine whether the differential genetic expression reported here relate to specific cell types. Such studies targeting specific cell types could employ laser captured microdissection techniques, and compare, for example, pyramidal cells with inhibitory interneurons. Such studies could also determine whether there is a laminar profile to the changes reported here. Additional post-transection survival durations could also be investigated, as the present results reflect a stop-action picture of the cortex along what is most likely a temporal cascade of genetic responses to the loss of sensory input.

ACKNOWLEDGMENTS

We thank our school's clinical veterinarian, Randalyn Shepherd, for providing invaluable training on surgical techniques. We also thank the Indiana University Harlan Family Scholars Program for their generous support of our research. Finally, we thank the Indiana University Center for Genomics and Bioinformatics for assistance with RNA-seq.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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: PG. Acquisition of data: RS, DD, JO, and PG. Analysis and interpretation of data: JO, PG, RS, and DD, Drafting of the manuscript: PG and JO.

RESOURCES CITED

CLC Main Workbench (RRID:OMICS_01813)
Ingenuity Pathway Analysis (RRID:nif-0000–33144)
Sprague–Dawley Rats (RRID:RGD_70508)

LITERATURE CITED

Bant JS, Raman, IM. 2010. Control of transient, resurgent, and persistent current by open-channel block by Na channel β4 in cultured cerebellar granule neurons. Proc Natl Acad Sci U S A 107:12357–12362.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics btu170.

Breton JD, Stuart GJ. 2009. Loss of sensory input increases the intrinsic excitability of layer 5 pyramidal neurons in rat barrel cortex. J Physiol 587:5107–5119.

Churchill JD, Tharp JA, Wellman CL, Sengelaub DR, Garraghty PE 2004. Morphological correlates of injury-induced reorganization in primate somatosensory cortex. BMC Neurosci 5:43.

Claeysen S, Joubert L, Sebben M, Bockaert J, Dumuis A. 2003. A single mutation in the 5-HT4 receptor (5-HT4-R D100 (3.32) A) generates a Gs-coupled receptor activated exclusively by synthetic ligands (RASSL). J Biol Chem 278: 699–702.

Cox SB, Woolsey TA, Rovainen CM. 1993. Localized dynamic changes in cortical blood flow with whisker stimulation corresponds to matched vascular and neuronal architecture of rat barrels. J Cereb Blood Flow Metab13:899–913.

Dufour A, Seibt J, Passante L, Depaepe V, Ciossek T, Frisén J, Kullander K, Flanagan JG, Polleux F, Vanderhaeghen P. 2003. Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. Neuron 39:453–465.

Faggin BM, Nguyen KT, Nicolelis MA. 1997. Immediate and simultaneous sensory reorganization at cortical and subcortical levels of the somatosensory system. Proc Natl Acad Sci U S A 94:9428–9433.

Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumer N, Larbig W, Taub E. 1995. Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. Nature 375:482–484.

Flor H, Nikolajsen L, Jensen TS. 2006. Phantom limb pain: a case of maladaptive CNS plasticity? Nat Rev Neurosci 7: 873–881.

Garraghty PE, Kaas JH. 1991. Large-scale functional reorganization in adult monkey cortex after peripheral nerve injury. Proc Natl Acad Sci U S A 88:6976–6980.

Garraghty PE, Lachica EA, Kaas JH. 1991. Injury-induced reorganization of somatosensory cortex is accompanied by reductions in GABA staining. Somatosens Mot Res 8: 347–354.

Garraghty PE, Hanes DP, Florence SL, Kaas, JH. 1994. Pattern of peripheral deafferentation predicts reorganizational limits in adult primate somatosensory cortex. Somatosens Mot Res 11:109–117.

Garraghty PE, Arnold LL, Wellman CL, Mowery TM. 2006. Receptor autoradiographic correlates of deafferentation-induced reorganization in adult primate somatosensory cortex. J Comp Neurol 497:636–645.

Maihöfner C, Handwerker HO, Neundörfer B, Birklein F. 2003. Patterns of cortical reorganization in complex regional pain syndrome. Neurology 61:1707–1715.

Maihöfner C, Handwerker HO, Neundörfer B, Birklein F. 2004. Cortical reorganization during recovery from complex regional pain syndrome. Neurology 63:693–701.

Marler KJ, Becker-Barroso E, Martínez A, Llovera M, Wentzel C, Poopalasundaram S, Hindges R, Soriano E, Comella J, Drescher U. 2008. A TrkB/EphrinA interaction controls retinal axon branching and synaptogenesis. J Neurosci 28:12700–12712.

Merzenich MM, Kaas JH, Wall J, Nelson RJ, Sur M, Felleman DJ. 1983a. Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience 8:33–55.

Merzenich MM, Kaas JH, Wall JT, Sur M, Nelson R ., Felleman D J. 1983b. Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. Neuroscience 10:639–665.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. 1998. Dopamine receptors: from structure to function. Physiol Rev 78:189–225.

Mowery TM, Elliott KS, Garraghty PE. 2009. The cortical response to sensory deprivation in adult rats is affected by gonadectomy. Exp Brain Res 195:27–33.

Obata S, Obata J, Das A, Gilbert CD. 1999. Molecular correlates of topographic reorganization in primary visual cortex following retinal lesions. Cereb Cortex 9:238–248.

Olah ME. 1997. Identification of A2a adenosine receptor domains involved in selective coupling to GS analysis of chimeric A1/A2a adenosine receptor. J Biol Chem 272:337–344.

Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M. 1992. Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 9:1081–1088.

Post MA, Kirsch GE, Brown AM. 1996. Kv2. 1 and electrically silent Kv6. 1 potassium channel subunits combine and express a novel current. FEBS Lett 399:177–182.

Schroeder CE, Seto S, Garraghty PE. 1997 Emergence of radial nerve dominance in "median nerve cortex" after median nerve transection in an adult squirrel monkey. J Neurophysiol 77:522–526.

Sengelaub DR, Muja N, Mills A, Myers WA, Churchill JD, Garraghty PE. 1997. Denervation-induced sprouting of intact peripheral afferents into the cuneate nucleus of adult rats. Brain Res 769:256–262.

Shieh PB, Ghosh A. 1999. Molecular mechanisms underlying activity-dependent regulation of BDNF expression. J Neurobiol 41:127–134.

Turrigiano G, Abbott LF, Marder E. 1994. Activity-dependent changes in the intrinsic properties of cultured neurons. Science 264:974–976.

Waite PM. 1984. Rearrangement of neuronal responses in the trigeminal system of the rat following peripheral nerve section. J Physiol 352:425–445.

Wall JT, Cusick CG. 1984. Cutaneous responsiveness in primary somatosensory (SI) hindpaw cortex before and after partial hindpaw deafferentation in adult rats. J Neurosci 4:1499–1515.

Warren R, Tremblay N, Dykes RW. 1989. Quantitative study of glutamic acid decarboxylase-immunoreactive neurons and cytochrome oxidase activity in normal and partially deafferented rat hindlimb somatosensory cortex. J Comp Neurol 288:583–592.

Wellman CL, Arnold LL, Garman EE, Garraghty PE. 2002. Acute reductions in GABAA receptor binding in layer IV of adult primate somatosensory cortex after peripheral nerve injury. Brain Res 954:68–72.

Woolsey TA, Van der Loos H. 1970. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17:205–242.

Xu B, Zang K, Ruff NL, Zhang YA. McConnell SK, Stryker MP, Reichardt LF. 2000. Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron 26: 233–245.