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Hematopoietic Origin of Pathological Grooming in *Hoxb8* Mutant Mice

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SUMMARY

Mouse *Hoxb8* mutants show unexpected behavior manifested by compulsive grooming and hair removal, similar to behavior in humans with the obsessive-compulsive disorder spectrum disorder trichotillomania. As *Hox* gene disruption often has pleiotropic effects, the root cause of this behavioral deficit was unclear. Here we report that, in the brain, *Hoxb8* cell lineage exclusively labels bone marrow-derived microglia. Furthermore, transplantation of wild-type bone marrow into *Hoxb8* mutant mice rescues their pathological phenotype. It has been suggested that the grooming dysfunction results from a nociceptive defect, also exhibited by *Hoxb8* mutant mice. However, bone marrow transplant experiments and cell type-specific disruption of *Hoxb8* reveal that these two phenotypes are separable, with the grooming phenotype derived from the hematopoietic lineage and the sensory defect derived from the spinal cord cells. Immunological dysfunctions have been associated with neuropsychiatric disorders, but the causative relationships are unclear. In this mouse, a distinct compulsive behavioral disorder is associated with mutant microglia.

INTRODUCTION

Grooming in mammals is an innate, stereotypic behavior with a well defined syntax (Berridge et al., 1987). The head is invariably groomed first, followed by body regions, and finally the anogenital region and tail. This cephalocaudal progression of grooming is defined as the "syntactic groom chain." Previous studies have shown that multiple regions of the rodent brain, notably the brainstem, striatum, and cortex are used to implement the syntactic groom chain (Aldridge et al., 1993; Berridge, 1989; Berridge and Whishaw, 1992).

Mice homozygous for a loss of function mutation in *Hoxb8* show excessive grooming. The syntax of grooming appears normal, but the number of incidences per unit time and the duration of grooming bouts are increased (Greer and Capecchi, 2002). However, the behavior is pathological, for it leads to hair removal and self-inflicted open skin lesions at the over groomed sites. This behavior is very similar to that described for humans with the obsessive-compulsive disorder (OCD) spectrum disorder trichotillomania, where compulsive removal of hair is also a hallmark. This disorder is quite common in humans with an occurrence ranging from 1.9 to 2.5 per 100 in seven separate international communities (Horwath and Weissman, 2000). Curiously, these mutant mice also excessively groom their wild-type cage mates. This aspect of the phenotype suggested that the peripheral nervous system is not likely responsible for the excessive grooming behavior (Greer and Capecchi, 2002).

Hoxb8 mutant mice also show altered response to nociceptive and thermal stimuli, which have been attributed to deficiencies in the formation and organization of interneurons in the dorsal spinal cord laminae I and II that receive the majority of nociceptive inputs (Holstege et al., 2008). Holstege et al. (2008) further suggested that the excessive and pathological grooming defects previously described in *Hoxb8* mutants result from to these sensory spinal cord defects.

It was quite unexpected that disruption of a *Hox* gene should result in a distinct behavioral deficit such as excessive and pathological grooming (Greer and Capecchi, 2002). *Hox* genes are normally involved in establishing body plans by providing positional values along the major axes of the embryo (Capecchi, 1997). However, *Hox* genes also have direct roles in the formation of multiple tissues and organs, including the formation of the hematopoietic system, and, with respect to *Hoxb8,* maintenance and differentiation of myeloid progenitor cells, one of the two known sources of microglia (Kawasaki and Taira, 2004; Krishnaraju et al., 1997; Perkins and Cory, 1993).

Since implementation of grooming in rodents is rooted within the brain, we anticipated that *Hoxb8* would be expressed in a neural circuit that modulates grooming behavior. Instead, as we report here, we have surprisingly found that, in the brain, the site that generates and implements grooming behavior, the only detectable cells derived from *Hoxb8* cell lineage are microglia. Second, we demonstrate that normal bone marrow transplantation into lethally irradiated *Hoxb8* mutant mice rescues the excessive pathological grooming behavior, without correcting the spinal cord defects. Third, conditional restriction of *Hoxb8* deletion to the hematopoietic system results in mice

with the excessive grooming and hair removal behavioral defects, without induction of the nociceptive/spinal cord defects. Finally and conversely, conditional deletion of *Hoxb8* in the spinal cord generates mice with the spinal cord sensory defects, but with normal grooming behavior.

The above experiments strongly support the hypothesis that the excessive pathological grooming behavior observed in *Hoxb8* mutant mice originates from defective microglia, thus directly connecting hematopoietic function to mouse behavior. The extensive role of microglia, as the brain's monitor and responder of immune activity, in the normal function of our brain is becoming increasingly apparent. As examples, immunological dysfunctions have been widely linked to many psychiatric disorders including OCD, major depression, bipolar disorder, autism, schizophrenia, and Alzheimer's disease (Ashwood et al., 2006; da Rocha et al., 2008; Kronfol and Remick, 2000; Lang et al., 2007; Leonard and Myint, 2009; Strous and Shoenfeld, 2006). In addition, results from genome-wide association studies suggest that genes whose dysfunction have been implicated in immune dysfunction and/or signaling contribute to increased susceptibility to the above mentioned mental disorders (Hounie et al., 2008; Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009).

Unfortunately, animal models that directly associate distinct behavioral deficits with defective microglia have been lacking. Here we provide such a model, which should allow interrogation at the molecular genetic and cellular levels, the roles of microglia in promoting normal behavior, and how perturbation of microglia leads to pathological behavior.

RESULTS

Automated Analysis of Excessive Grooming in the *Hoxb8* Mutant Mice

Hoxb8 mutant behavior is characterized by excessive pathological grooming. Previously, we determined the number and duration of grooming bouts from continuous video recording of mouse activity (Greer and Capecchi, 2002). This procedure was robust but very labor intensive. More recently, we have been using technology developed by B.V. Metris based on the use of very sensitive vibration detectors (Laboras platforms). Each activity such as drinking, eating, rearing, climbing, locomotion, immobility, grooming, and scratching is associated with characteristic patterns of vibration, which are continuously recorded. A computer algorithm then interprets specific vibration patterns as individual behaviors. The advantage of this approach is that behavior is classified automatically and collected unobtrusively over any chosen period of time. We typically monitor activity over 24 hr periods. We have evaluated the Laboras platforms for their assessment of time spent grooming by co-monitoring mouse activity using our video camera system. The Laboras platforms are remarkably accurate, comparable to human classification and far less labor intensive. Figure S1A (available online) shows the average time spent grooming for 25 *Hoxb8* mutant mice and 22 controls over 24 hr periods as measured by the Laboras platforms. These results compare very well with those previously obtained by analyzing continuous video recordings, illustrating that on average *Hoxb8* mutant mice spend approximately twice as much time grooming as their wild-type littermates (Greer and Capecchi, 2002). The penetrance for excessive grooming in *Hoxb8* mutant mice is 100%.

Hoxb8 Cell Lineage Gives Rise to Brain Microglia

The expression pattern of *Hoxb8* in the adult brain is broad (Greer and Capecchi, 2002). However, the expression level is very low and dispersed in the brain, making it difficult to identify the cell type(s) expressing *Hoxb8*. To identify the *Hoxb8* cell lineage in the mouse brain, we generated a *Hoxb8-IRES-Cre* (*Hoxb8-ICre* onwards) driver that could be used to activate Cre-dependent *LacZ* or *YFP* reporter genes targeted to the ubiquitously expressed *ROSA26* locus (Figure S1B; Soriano, 1999). In mice carrying both the *Hoxb8-ICre* driver and *ROSA26-YFP* reporter alleles, activation of *Hoxb8* expression also triggers YFP production. Brains of such mice were collected at pre- and postnatal stages and examined by immunohistochemistry. In adult brains, YFP-positive cells can be found throughout the brain, but consistent with previous results, predominantly in the cerebral cortex, striatum, olfactory bulb, and brainstem (data not shown). These cells appear morphologically to be microglia and indeed coexpress the general microglia marker CD11b and Iba1, a marker of activated microglia (Figures 1A–1C and data not shown), indicating that *Hoxb8* is expressed in microglia or their progenitor cells. Notably, not all microglia in the brain are YFP positive, suggesting that *Hoxb8* expression was present only in a subpopulation of microglia or their progenitors (~40% of total, see Figure 7C).

In newborn mice, very few *Hoxb8*-labeled cells are observed in the brain and these cells are found predominantly in the choroid plexus (Figure 1D), meninges, and the ventricular lining, with their numbers declining with increased distance from the ventricular zone. This gradient suggests migration of YFP-positive cells from the ventricular zone into the forebrain areas. Between P2 and P14, YFP-positive cell count in the mouse brain increases dramatically and is then maintained at this high level (Figures 1E and 1F and data not shown).

Although the origin of microglia is still debated, there is general agreement that at least one subpopulation is of bone marrow origin, (i.e., derived from circulating monocytes; Kaur et al., 2001; Ransohoff and Perry, 2009). The time of first appearance and the site of entry of *Hoxb8*-labeled microglia is consistent with this subpopulation being of hematopoietic, bone marrow-derived origin.

To assess if the *Hoxb8* mutation affects the number of microglia present in the adult brain, comparable sections of the brain were evaluated for the presence of Iba1-positive cells in six *Hoxb8* mutants and six control mice. We consistently observe an approximate 15% reduction of total number of microglia present in *Hoxb8* mutant versus control mice (Figure S2). Currently, we cannot specifically label *Hoxb8* mutant (i.e., $Hoxb8^{-/-}$) microglial lineage because our *Hoxb8-ICre* driver is a "knockin" *IRES-Cre* driver, which does not itself affect *Hoxb8* function. Therefore, the above count for reduction of microglia in *Hoxb8* mutant mice may represent an underestimate of the actual reduction of the hematopoietic bone marrow-derived microglia subpopulation, should such loss be partially compensated by an increase in the resident non-*Hoxb8*-expressing microglial subpopulation.