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LETTER TO THE EDITOR

# Establishing brain functional laterality in adult mice through unilateral gene manipulation in the embryonic cortex

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**Dear editor,**

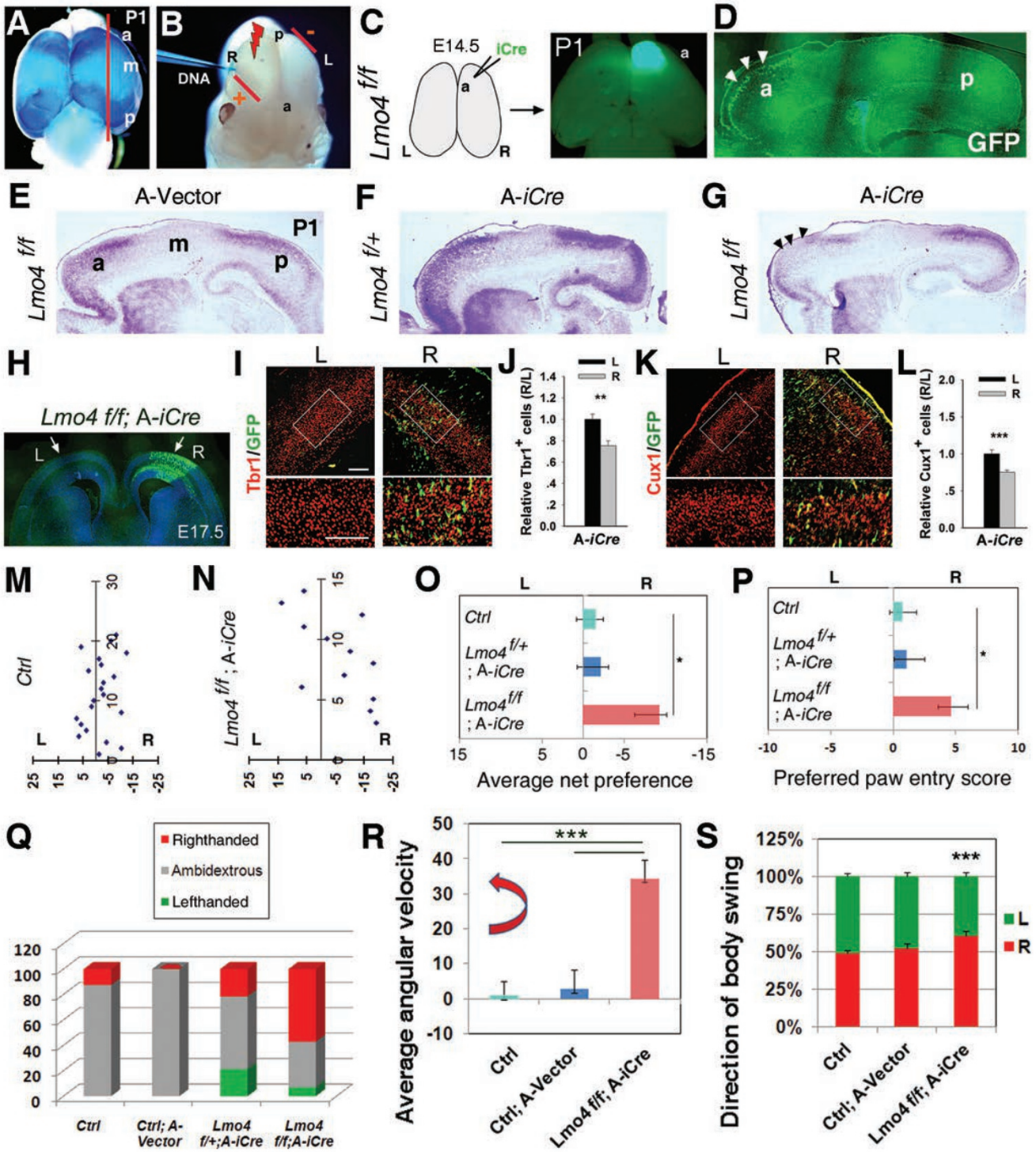
Brain functional laterality appears either in individuals or in a population in vertebrates, though the degree of asymmetry varies [1, 2]. Behavioral asymmetry such as paw preference, a food reaching skill, is observed in individual mice to a certain degree in some strains, but not on a larger population level and not in all strains [3]. The hemisphere with the larger motor cortex tends to be dominant in controlling manual skills, leading to a stronger preference of using the contralateral front paw [4, 5]. However, the mechanism underlying the establishment of the dominant functional areas in the left or right hemispheres is poorly understood [6, 7].

Our previous work has identified a transcription factor Lim domain only 4 (LMO4), which shows an asymmetric expression pattern in human fetal brains and in the developing mouse cortices in the tested strain [8]. In postnatal day 1 (P1) mouse brains, *Lmo4* is strongly expressed in the anterior and posterior regions but not in between (Figure 1A). To test whether unilateral manipulation of Lmo4 expression in the anterior cortical region, where the motor cortex resides, could cause brain functional laterality in adult mice, we generated a mouse model in which Lmo4 expression was only altered in the right hemisphere by *in utero* electroporation at E14.5 (Figure 1B). In the floxed *Lmo4* transgenic mice (*Lmo4*$^{f/f}$), electroporation of constructs expressing increased *Cre* (*iCre*) together with enhanced *green fluorescent protein* (*eGFP*) into the anterior cortex of the right hemisphere should conditionally and unilaterally knockdown *Lmo4*, generating *Lmo4*$^{f/f}$;A-*iCre* mice (Figure 1C and 1D) [9]. Indeed, when examining the P1 brains that received electroporation at E14.5, we found that while Lmo4 expression was not affected by the vector (A-Vector) electroporation (Figure 1E), it was greatly reduced in the anterior region, where *iCre* construct was electroporated, in *Lmo4*$^{f/f}$ mice (Figure 1G), but not in *Lmo4*$^{f/+}$ mice (Figure 1F). Therefore, we can manipulate Lmo4 expression in specific cortical regions within one hemisphere (right) by utilizing *in utero* electroporation in *Lmo4*$^{f/f}$ mice.

We next tested whether unilateral knockdown of *Lmo4* alters the organization of cortical functional areas by examining the expression pattern of several cortical regional markers. Compared with the left hemisphere with no electroporation, *Cdh8* anterior expression was greatly reduced, and the anterior expression of *Auts2* and *Rorβ* shifted rostrally in the right hemisphere of *Lmo4*$^{f/f}$;A-*iCre* mice (Supplementary information, Figure S1). The numbers of both early-born (Tbr1$^+$ and Ctip2$^+$) and late-born neurons (Cux1$^+$) were greatly reduced in the coronal sections of E17.5 and P0 right hemispheres that received *iCre* (visible by GFP staining) electroporation at E14.5, compared with those in the left hemisphere sections (Figure 1H-1L and Supplementary information, Figures S2-S4). However, perturbed neurogenesis was recovered in adult brains (Supplementary information, Figure S5). Furthermore, axonal projections from the right hemisphere were significantly thinner than those from the left, as determined by neural cell adhesion molecule L1 labeling and by DiI tracing in P0 and P16 *Lmo4*$^{f/f}$;A-*iCre* mice, respectively (Supplementary information, Figure S6). Consistent with the role of Lmo4 in specifying neuronal subtypes in the motor cortex [10], our results indicate that unilateral depletion of Lmo4 expression in embryonic cortices is sufficient to suppress early neurogenesis in one hemisphere, and results in asymmetric functional area formation, neuronal production and axonal projection.

To test the behavioral consequence of unilateral alteration of Lmo4 expression in the right hemisphere at E14.5, P1 pups were selected by GFP expression and allowed to develop until 12 weeks old.

In the paw preference test, wild-type control (*Ctrl*, C57BL/6 × 129 background), *Lmo4*$^{f/+}$ and *Lmo4*$^{f/f}$ mice used the left and right front paw equally in a total of 50 paw entries (Figure 1M and data not shown). Sham-electroporated *Ctrl* mice, and *Ctrl* and *Lmo4*$^{f/+}$ mice




electroporated with the empty vector and *iCre*, respectively, also did not show paw preference (Supplementary information, Figure S7). These results indicate that the electroporation procedure and *iCre* itself do not affect lateralized behaviors in wild-type and *Lmo4*$^{f/+}$ mice. The net paw preference and the preferred paw entry score analyses revealed that more *Lmo4*$^{f/f}$;A-*iCre* mice preferred to use the right front paw and showed a stronger right preference (Figure 1N-1P). Moreover, while the *Ctrl* mice were mostly detected as ambidextrous (more than 80%), over 50% of the tested *Lmo4*$^{f/f}$;A-*iCre* mice were grouped as right handed (Figure 1Q).

**Figure 1** Generation of a mouse model with functional laterality by unilateral alteration of Lmo4 expression in the developing cortices in *Lmo4*$^{f/f}$ mice using *in utero* electroporation. **(A)** Lmo4 expression is detected in the anterior (a) and posterior (p) but not medial (m) regions in the P1 mouse cortex by whole-mount *in situ* hybridization. **(B)** *In utero* electroporation in the anterior region in the right (R) but not left (L) hemisphere of a mouse embryo. **(C**, **D)** Knockdown of *Lmo4* in the anterior cortical regions by electroporating *iCre* (GFP$^+$, arrowheads) into the right hemisphere of E14.5 *Lmo4*$^{f/f}$ mice. The expression of Lmo4 was test at P1. **(E)** Electroporation of empty vector (A-Vector) into the anterior cortex at E14.5 did not change Lmo4 expression in P1 cortices (a section collected at the red line in **A**). **(F**, **G)** Lmo4 expression in the anterior cortices was depleted by *iCre* (A-*iCre*, arrowheads) electroporation in *Lmo4*$^{f/f}$ mice **(G)**, but not in *Lmo4*$^{f/+}$ mice **(F)**. **(H)** Electroporated sites (GFP$^+$) in the anterior right hemisphere (arrows) in coronal sections of E17.5 *Lmo4*$^{f/f}$;A-*iCre* brains. **(I-L)** Immunohistochemistry and quantification of Tbr1$^+$ **(I**, **J)** and Cux1$^+$ **(K**, **L)** neurons in the left and right hemispheres. $n > 6$; ** $P < 0.002$; *** $P < 0.0003$. Scale bars: 100 μm. **(M**, **N)** Distribution of net preference of using the left or right front paw in wild-type control (*Ctrl*) and *Lmo4*$^{f/f}$;A-*iCre* mice. **(O**, **P)** Average net preference and preferred paw entry score towards the right in the indicated mice. *Ctrl*: $n = 21$, *Lmo4*$^{f/+}$: $n = 14$, *Lmo4*$^{f/f}$: $n = 14$; * $P < 0.04$. **(Q)** Handedness grouping using the number of the right-paw entry score. **(R)** The free swimming test. Arrow indicates counter-clockwise turning. *Ctrl*: $n = 17$, *Ctrl*;A-Vector: $n = 20$, *Lmo4*$^{f/f}$;A-*iCre*: $n = 10$, *** $P < 0.0002$. **(S)** The tail-suspension test. *** $P < 0.0004$.

Furthermore, we performed a free swimming test by measuring angular velocity, which reflects clockwise or counter-clockwise turning while the mouse swims. While the *Ctrl* mice displayed similar frequencies of turning towards either direction, *Lmo4*$^{f/f}$;A-*iCre* mice preferred to make counter-clockwise turns, indicating biased use of the right front and hind paws (Figure 1R and Supplementary information, Figure S8). In a tail-suspension test, while the control group displayed an equal tendency to swing their bodies towards either the left or right, *Lmo4*$^{f/f}$;A-*iCre* mice showed a higher tendency (60%) to swing their bodies towards the right, indicating preferred use of the right front and hind paws (Figure 1S). Moreover, in an adhesive-removal test, the left front paw of *Lmo4*$^{f/f}$;A-*iCre* mice showed better sensory perception and no detectable movement defects, suggesting that the right paw preference is not due to the left paw impairment (Supplementary information, Figure S9).

To further validate that functional laterality is indeed caused by altered Lmo4 expression, *Lmo4* was knocked down in the left anterior hemisphere at E14.5. Interestingly, these mice showed reversed laterality by showing preference to use the left front paw (Supplementary information, Figure S10). These results indicate that unilateral alteration of Lmo4 expression in embryonic cortices in mice that normally do not exhibit brain functional asymmetry results in lateralized manual performance.

By unilateral manipulation of Lmo4 expression in the cortex, here we have generated mouse models that show not only consistent functional asymmetry but also a high degree of laterality. Our data illustrate a mechanism whereby asymmetric architectural assembly of neuronal production and axonal connections results in different sizes of functional representative areas between two hemispheres. Our results further suggest that brain functional asymmetry is likely an outcome of establishing the dominant functional representative area between two hemispheres during the early development.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)