

Rere-dependent Retinoic Acid signaling controls brain asymmetry and handedness

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Title: Rere-dependent Retinoic Acid signaling controls brain asymmetry and handedness

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While the vertebrate brain appears largely bilaterally symmetrical in humans, it
presents local morphological Left-Right (LR) asymmetries as, for instance, in the petalia. Moreover, higher functions such as speech or handedness are asymmetrically localized in the cortex. How these brain asymmetries are generated remains unknown. Here, we reveal a striking parallel between the control of bilateral symmetry in the brain and in the precursors of vertebrae called somites, where a "default" asymmetry is buffered by Retinoic Acid (RA) signaling. This mechanism is evident in zebrafish and mouse and, when perturbed in both species, it translates in the brain into lateralized alterations of patterning, neuronal differentiation and behavior. We demonstrate that altering levels of the mouse RA coactivator Rere results in subtle cortex asymmetry and profoundly altered handedness, linking patterning and function in the motor cortex. Together our data uncover a novel patterning and function in the motor cortex. Together our data uncover a novel mechanism that could underlie the establishment of brain asymmetries and handedness in vertebrates.

T もけれる embryonic organization, but how developmental programs are coordinated to ϵ
that the left side develops as a mirror image of the right side is mostly unknowr
formation of somites provides an excellent illustration of t that the left side develops as a mirror image of the right side is mostly unknown. The
formation of somites provides an excellent illustration of the LR coordination of
patterning processes in the vertebrate embryo. Somit formation of somites provides an excellent illustration of the LR coordination of
patterning processes in the vertebrate embryo. Somites form synchronously on the le
and right side from the presomitic mesoderm (PSM) and t patterning processes in the vertebrate embryo. Somites form synchronously on
and right side from the presomitic mesoderm (PSM) and they differentiate in a
synchronized manner to produce the bilaterally symmetrical vertebr and right side from the presomitic mesoderm (PSM) and they differentiate in a
synchronized manner to produce the bilaterally symmetrical vertebral column. The LR
symmetry of somite formation is controlled by a bilaterally synchronized manner to produce the bilaterally symmetrical vertebral column.
symmetry of somite formation is controlled by a bilaterally symmetrical gradier
Fibroblast Growth Factor (FGF) signaling peaking in the posterio symmetry of somite formation is controlled by a bilaterally symmetrical gradient of
Fibroblast Growth Factor (FGF) signaling peaking in the posterior PSM 1,2 . This FGF
gradient is antagonized by RA signaling produced Fibroblast Growth Factor (FGF) signaling peaking in the posterior PSM ^{1,2}. This FGF gradient is antagonized by RA signaling produced by the forming somites anteriorly Strikingly, in RA-deficient mice lacking the RA bios gradient is antagonized by RA signaling produced by the forming somites anteriorly ^{9,4}.
Strikingly, in RA-deficient mice lacking the RA biosynthetic enzyme Raldh2, the *Fgf8*
gradient in the PSM becomes asymmetric, resu Strikingly, in RA-deficient mice lacking the RA biosynthetic enzyme Raldh2, the *Fgf8* gradient in the PSM becomes asymmetric, resulting in a delay of somitogenesis in the right side ^{5,6}. A similar phenotype is observed right side 5,6 . A similar phenotype is observed in mice mutant for the RA coactivator
Rere, in which RA signaling is decreased ⁷. In the viscera, bilateral coordination of the
development of the two embryonic sides Rere, in which RA signaling is decreased '. In the viscera, bilateral coordination of the
development of the two embryonic sides is altered in response to a LR signaling
pathway that controls their asymmetric development pathway that controls their asymmetric development ⁸. In mouse and zebrafish, an Fgf8-
dependent signal derived from the Node activates Nodal in the left lateral plate to
specify the left embryonic identity ^{9,10}. Muta specify the left embryonic identity ^{9,10}. Mutation of *Fgf8* or *Nodal* can result in *situs* inversus where visceral organs become organized in a mirror-image fashion ⁸⁻¹⁰. The right-sided somitogenesis defect observ inversus where visceral organs become organized in a mirror-image fashion ⁹⁻¹⁰. The right-sided somitogenesis defect observed in RA-deficient mice can be reversed by crossing them with the *inversus viscerum* (*iv*) muta crossing them with the *inversus viscerum* (*iv*) mutant mice in which embryos exhibi
situs inversus 7,11 . Together, these experiments suggest that Rere-dependent RA
signaling interacts with FGF to buffer the action o crossing them with the *inversus viscerum* (*iv*) mutant lines in which embryos exhibit
situs inversus ^{7,11}. Together, these experiments suggest that Rere-dependent RA
signaling interacts with FGF to buffer the action of situs inversus
signaling inter
bilateral symn
between RA a
Whether the investigated. $\%$
The Transformalist Carry Constants Reflects with FGF to buffer the action of the LR pathway and maintain
netry of somites. The molecular mechanism underlying the interact
nd FGF signaling which maintains somites symm signaling interacts with Figures with Figures interaction between RA and FGF signaling which maintains somites symmetry is currently unkno
between RA and FGF signaling which maintains somites symmetry is currently unkno
Wh between RA and FGF signaling which maintains somites symmetry is currently unkno
Whether the role of RA in bilateral symmetry extends beyond somites has not been
investigated. between RA and FGF signaling which maintains somiting the maintain which may also
Whether the role of RA in bilateral symmetry extends beyond somites has not been
investigated. Whether the role of RA in bilateral symmetry extends beyond statements in the role of μ investigated.

gradients of FGF and RA signaling, raising the possibility that these pathways might als
be implicated in the control of bilateral symmetry in these structures $12,13$. The posteria
gradient of RA involved in hindbrain pa be implicated in the control of bilateral symmetry in these structures 12,13 . The posterior gradient of RA involved in hindbrain patterning arises from the somites and it interacts with FGF signaling from the midbrain be implicated in the control of bilateral symmetry in these structures 12,13 . The posterior gradient of RA involved in hindbrain patterning arises from the somites and it interacts with FGF signaling from the midbrain with FGF signaling from the midbrain-hindbrain (isthmic) organizer to specify the
identity of the forming rhombomeres ¹⁴. To explore whether RA is also involved in the
control of bilateral symmetry of the hindbrain, we identity of the forming rhombomeres 14 . To explore whether RA is also involved in the control of bilateral symmetry of the hindbrain, we incubated zebrafish embryos beginning at the 8-128 cell stage (prior to 4h postbeginning at the 8-128 cell stage (prior to 4h post-fertilization) with the pan-RA is antagonist, BMS-204493 (BMS)¹⁵. We first examined expression of the segmenta gene *deltaC*, which is expressed in a bilaterally symme **Example 12** cell state 128 cell state in the 8-128 cell state and the segmentation gene *deltaC*, which is expressed in a bilaterally symmetrical fashion in the PSM ¹⁶. As expected, BMS treatment resulted in expression gene *deltaC,* which is expressed in a bilaterally symmetrical fashion in the PSM ⁺°. As
expected, BMS treatment resulted in expression of *deltaC* becoming asymmetric
between the left and right PSM, suggesting that LR expected, BMS treatment resulted in expression of defide becoming asymmetric
between the left and right PSM, suggesting that LR synchronization of somite
segmentation is disrupted (Fig. 1a-b). We also observed striking bil between the left and right PHS, and S observed striking bilateral sym
defects in the hindbrain using the rhombomere (r) 3 and 5 marker *krox20 (egn*
1c-e, Supplementary Table 1, Extended data figure 1a-f). In r5, asymmetr defects in the hindbrain using the rhombomere (r) 3 and 5 marker *krox20 (egr2a)* (Fi
1c-e, Supplementary Table 1, Extended data figure 1a-f). In r5, asymmetries were
variable in pattern but present as a left or right hem defects in the hindbrain using the mombointere (r) 3 and 3 marker krox20 (egr2a) (rig.
1c-e, Supplementary Table 1, Extended data figure 1a-f). In r5, asymmetries were
variable in pattern but present as a left or right he variable in pattern but present as a left or right hemi-segmental absence of *krox2l*
expression in the extreme (Fig.1c-d, Extended data Fig. 1a-f). The *krox20* r3 stripe
not affected. BMS treatment also reproduced the p variable in pattern but present as a left or right hemi-segmental absence of krox20
expression in the extreme (Fig. 1c-d, Extended data Fig. 1a-f). The *krox20* r3 stripe w
not affected. BMS treatment also reproduced the

expression in the extreme (Fig.1c-d, Extended data Fig.1c-d). The Krox2013 stripe was
not affected. BMS treatment also reproduced the previously published *krox20*
expression phenotypes resulting from hindbrain anterioriz not affected. BMS treatment also reproduced the previously published krokzo
expression phenotypes resulting from hindbrain anteriorization, including a col
loss of r5 expression (Extended data Figure 1c-d)¹⁷⁻²⁰.
Next, t loss of r5 expression (Extended data Figure 1c-d)¹⁷⁻²⁰.
Next, to probe the effect of RA excess, we analyzed *krox20* expression after inhibition
the Cyp26-class of cytochrome P450 enzymes, which are involved in the degr loss of r5 expression (Extended data Figure 1c-d)²⁷⁻²⁰.
Next, to probe the effect of RA excess, we analyzed *kt*
the Cyp26-class of cytochrome P450 enzymes, which RA *in vivo* ²¹. As observed with BMS, treatment with Next, to probe the effect of NA excess, we analyzed *RIO*220 expression after inhibition of
the Cyp26-class of cytochrome P450 enzymes, which are involved in the degradation of
RA *in vivo*²¹. As observed with BMS, trea RA in vivo ²¹. As observed with BMS, treatment with the Cyp26 inhibitor MCC154 led to
desynchronized expression of *deltaC* between the left and right PSM (Fig. 1f). In the
hindbrain, we also observed a striking phenoty RA *in vivo*
desynchro
hindbrain,
embryos e
either on t
the bilater
there were
reduced sy
2a). in agre ²¹. As observed with BMS, treatment with the Cyp26 inhibitor MCC154 led to nized expression of *deltaC* between the left and right PSM (Fig. 1f). In the we also observed a striking phenotype where some MCC154-treated wh desynchronized expression of defide between the left and right PSM (Fig. 1r). In the
hindbrain, we also observed a striking phenotype where some MCC154-treated
embryos exhibit LR asymmetric expression of *krox20*, which in embryos exhibit LR asymmetric expression of *krox20*, which in extreme cases ap
either on the left or the right side in r3 (Fig. 1g, Supplementary Table 2a). In con
the bilateral symmetry of the *krox20* r5 stripe was not embryos exhibit En asymmetric expression of *Krox20*, which in extreme cases appears
either on the left or the right side in r3 (Fig. 1g, Supplementary Table 2a). In contrast,
the bilateral symmetry of the *krox20* r5 str the bilateral symmetry of the *krox20* r5 stripe was not significantly affected. In additior
there were classes of MCC154-treated embryos where *krox20* expression in r3 was
reduced symmetrically or completely lost (data there were classes of MCC154-treated embryos where *krox20* expression in r3 was
reduced symmetrically or completely lost (data not shown and Supplementary Table
2a), in agreement with prior observations ^{22,23}. These re there were classes of MCC154 treated embryos where kroazb expression in r3 was
reduced symmetrically or completely lost (data not shown and Supplementary Tabl
2a), in agreement with prior observations 22,23 . These re 2a), in agreement with prior observations $22,23$. These results were confirmed genetic
by analyzing *krox20* expression in the *cyp26a1* mutant, *giraffe* 24 . 2% (5 of 318) of the
embryos from gir^{WZ16} incrosses show 2a), in agreement with prior observations 22.2 . These results were confirmed genetically
by analyzing *krox*20 expression in the *cyp26a1* mutant, *giraffe* 24 . 2% (5 of 318) of the
embryos from gir^{w716} incrosses by analyzing Krox20 expression in the cyp26a1 mutant, graffer embryos from gir^{rw716} incrosses showed a LR asymmetric r3 phorof them genotyped as homozygous *gir* mutants (Fig. 1i-j, Suppl
Another 2% showed phenotypes co $24.$ 2% (5 of 318) of the
enotype for *krox20* and
ementary table 2b).
ain-of-function studies,
maining embryos had
ce of the asymmetric r3
y with cyp26b1 and
26 inhibitor MCC154
frequency (Fig. 1h. embryos from gir^{m-226} incrosses showed a LR asymmetric r3 phenotype for *krox20* and all
of them genotyped as homozygous gir mutants (Fig. 1i-j, Supplementary table 2b).
Another 2% showed phenotypes consistent with prev of them genotyped as homozygous gir mutants (Fig. 11), supplementary table 2b).
Another 2% showed phenotypes consistent with previous RA gain-of-function studinamely a symmetric reduction of *krox20* in r3²² (Fig. 1k). namely a symmetric reduction of *krox20* in r3²² (Fig. 1k). All remaining embryos had
symmetric *krox20* expression in r3 and r5²³. The low penetrance of the asymmetric r3
phenotype in *cyp26a1* mutants suggests funct symmetric *krox20* expression in r3 and r5²³. The low penetrance of the asymmetric r3
phenotype in cyp26a1 mutants suggests functional redundancy with cyp26b1 and
cy26c1 and is consistent with the observation that the p symmetric *krox20* expression in r3 and r5 ²⁵. The low penetrance of the asymmetric r3
phenotype in *cyp26a1* mutants suggests functional redundancy with *cyp26b1* and
cy26c1 and is consistent with the observation that phenotype in cyp26a1 mutants suggests runctional redundancy with cyp26b1 and
cy26c1 and is consistent with the observation that the pan-Cyp26 inhibitor MCC15
generates asymmetric *krox20* r3 phenotypes at a much higher fre cy20c1 and is consistent with the observation that the pan-Cyp20 inhibitor MCC154
generates asymmetric *krox20* r3 phenotypes at a much higher frequency (Fig. 1h,
Supplementary Table 2a). Therefore, our data show that incr generates asymmetric krox20 r3 phenotypes at a much higher frequency (Fig. 1h,
Supplementary Table 2a). Therefore, our data show that increasing or decreasing
signaling disrupts both somite and hindbrain bilateral symmetry Supplementary Table 2a). Therefore, our data show that increasing or decreasing the signaling disrupts both somite and hindbrain bilateral symmetry in zebrafish embryos
signaling disrupts both somite and hindbrain bilatera signaling disrupts both somite and hindbrain bilateral symmetry in zebrafish embryos.

specialization of the lateral habenula and parapineal gland-- is controlled by the signaling pathway 25 , we next tested whether RA is involved in buffering asym Nodal signaling. In zebrafish, the Nodal protein Southpa signaling pathway ²⁵, we next tested whether RA is involved in buffering asymmetric
Nodal signaling. In zebrafish, the Nodal protein Southpaw is expressed in the left latera
plate and it acts in the left diencephalon vi plate and it acts in the left diencephalon via another Nodal protein, Cyclops to control
LR asymmetry of the epithalamus 25,26 . In BMS-treated embryos, there was no
pronounced sidedness bias to the hindbrain *krox20* LR asymmetry of the epithalamus 25,26 . In BMS-treated embryos, there was no
pronounced sidedness bias to the hindbrain *krox20* asymmetries [56% of cases with
stronger disruption of *krox20* on the right side (176/316 LR asymmetry of the epithalamus 2,26 . In BMS-treated embryos, there was no
pronounced sidedness bias to the hindbrain $krox20$ asymmetries [56% of cases
stronger disruption of $krox20$ on the right side (176/316 embryos), pronounced sidedness bias to the immusium krokzo dsymmetries [56% of cases with
stronger disruption of krox20 on the right side (176/316 embryos), 44% with strong
disruption on the left side (140/316 embryos)] (Supplementa stronger disruption of krox20 on the right side (176/ 310 embryos), 44% with stronger
disruption on the left side (140/316 embryos)] (Supplementary Table 1 and data not
shown). Expression of *lefty1*, which is induced by C shown). Expression of *lefty1*, which is induced by Cyclops in the left diencephalon,
showed the same left expression bias in embryos treated with BMS and in control
embryos (Fig. 1 l-n, Supplementary Table 3). Finally, we shown). Expression of *lefty1*, which is induced by Cyclops in the left diencephalon,
showed the same left expression bias in embryos treated with BMS and in control
embryos (Fig. 1 l-n, Supplementary Table 3). Finally, we embryos (Fig. 1 I-n, Supplementary Table 3). Finally, we observed asymmetric r5
expression of *krox20* in the left or in the right side of BMS-treated embryos expres
Southpaw in the left lateral plate (Fig.1o-p). Thus, th

expression of *krox20* in the left or in the right side of BMS-treated embryos expression of *krox20* in the left or in the right side of BMS-treated embryos expression of *krox20* in the left lateral plate (Fig.1o-p). Th Southpaw in the left lateral plate (Fig.1o-p). Thus, the hindbrain asymmetries in RA-
deficient embryos appear independent of the Nodal pathway.
The RA pathway involved in the control of somite symmetry requires the Rere
 Southpaw in the left lateral plate (Fig.1o-p). Thus, the inhibitant asymmetries in RA-
deficient embryos appear independent of the Nodal pathway.
The RA pathway involved in the control of somite symmetry requires the Rere The RA pathway involved in the control of somite symmetry re
chromatin-remodeling protein which together with Hdac1/2 and
activator complex called WHHERE⁴. In zebrafish embryos hom
Rere homolog *rerea, babyface (bab^{tb2*} chromatin-remodeling protein which together with Hdac1/2 and Wdr5, forms
activator complex called WHHERE⁴. In zebrafish embryos homozygous mutant
Rere homolog *rerea, babyface* (*bab*^{tb210}/ *bab*^{tb210}), normal somit *bab*^{tb210} embryos, we did not detect asymmetries of *krox*20 expression (data not shown). activator complex called WHHERE ⁻. In zebratish embryos homozygous mutant for the
Rere homolog *rerea, babyface (bab^{tb210}) bab^{tb210})*, normal somite symmetry was
observed, possibly reflecting compensation by a mater observed, possibly reflecting compensation by a maternal pool of *rerea* mRNA
(Supplementary Table 3)²⁷. Nevertheless, close to half of the homozygous mutant
embryos (58 of 114 homozygotes) showed asymmetric pectoral fi (Supplementary Table 3) ²⁷. Nevertheless, close to half of the homozygous mut
embryos (58 of 114 homozygotes) showed asymmetric pectoral fins at 5 days p
fertilization (dpf) (Fig.2a-b). Remarkably, a similar limb sidedn (Supplementary Table 3) ²⁷. Nevertheless, close to half of the homozygous mutant
embryos (58 of 114 homozygotes) showed asymmetric pectoral fins at 5 days post-
fertilization (dpf) (Fig.2a-b). Remarkably, a similar limb remained present and intact on both sides (Fig. 2c-d). However, in bab^{tb210}/I embry Fertilization (dpf) (Fig.2a-b). Remarkably, a mutants 28.29 . In the hindbrain of bab^{tb210}/bab^{tb210} and bb^{tb210} and bb^{tb210} we examined the distribution of reticulospinal neurons, which are hindbrain neurons relaying bab²²¹² embryos, we did not detect asymmetries of *krox20* expression (data not shown).
We examined the distribution of reticulospinal neurons, which are hindbrain neurons
relaying motor commands from the brain to the s relaying motor commands from the brain to the spinal cord and are arranged in a
segmented, bilaterally symmetrical fashion 30 . In *bab^{tb210} bab^{tb210}* embryos, all
reticulospinal neurons were missing except for t relaying motology symmetrical fashion 30 . In bab^{tb210}/bab^{tb210} embryos, all reticulospinal neurons were missing except for the Mauthner neuron in r4, which remained present and intact on both sides (Fig.2c-d). However, i segmented, bilaterally symmetrical fashion ³⁰. In *bab⁸²²²⁹/ bab*⁸²²² embryos, all
reticulospinal neurons were missing except for the Mauthner neuron in r4, whi
remained present and intact on both sides (Fig.2c-d). remained present and intact on both sides (Fig.2c-d). However, in $bab^{tb210}/+$ embr
we observed unilateral absence of one reticulospinal neuron in r6 in 43+/-1% of ca
(n=37). The phenotype was observed either on the left or remained present and intact on both sides (Fig.2c-d). However, in *bab*⁸²¹⁰/+ embryos,
we observed unilateral absence of one reticulospinal neuron in r6 in 43+/-1% of cases
(n=37). The phenotype was observed either on th (n=37). The phenotype was observed either on the left or on the right side
(Supplementary Table 4). The same phenotype was only observed in 15.5+/-1.5% of
control wild type sibling (WT) embryos (n=13) (Fig.2c, e; Suppleme (n=37). The phenotype was observed either on the left or on the right side
(Supplementary Table 4). The same phenotype was only observed in 15.5+/-1.5% of
control wild type sibling (WT) embryos (n=13) (Fig.2c, e; Suppleme (supplementary Table 4).

Control wild type sibling (WT) embryos (n=13) (Fig.2c, e; Supplementary Table 4).

Together, these data show that disruption of the RA coactivator Rerea function can

result in LR asymmetries in However, Nodal-dependent epithalamic LR asymmetry, as evaluated with *lefty1,*
appears normal in *rerea* mutants (Supplementary Table 3). This suggests that Rerea acts
largely independently of Nodal to control bilateral sy Together, these data show that disruption of the RA coactivator Rerea function can
result in LR asymmetries in a variety of embryonic tissues including limbs and hindbi
However, Nodal-dependent epithalamic LR asymmetry, a Fowever, Nodal-dependent epithalamic LR asymmetry, as evaluated with *lefty1*, appears normal in *rerea* mutants (Supplementary Table 3). This suggests that Rerea acts largely independently of Nodal to control bilateral s However, Nodal-dependent epithalamic LN asymmetry, as evaluated with *lefty1,*
appears normal in *rerea* mutants (Supplementary Table 3). This suggests that Rer
largely independently of Nodal to control bilateral symmetry

appears normal in rerea mutants (Supplementary Table 3). This suggests that Rerea acts
largely independently of Nodal to control bilateral symmetry of the developing zebrafish
hindbrain.
As a functional output, we next tes hindbrain.
As a functional output, we next tested whether *rerea* is required for normal, lateralized
behavior in adult zebrafish. Since larvae homozygous for *rerea* mutations die around 8
days post-fertilization, we comp hindbrain. As a functional output, we next tested whether rereal is required for normal, lateralized
behavior in adult zebrafish. Since larvae homozygous for *rerea* mutations die around 8
days post-fertilization, we compared popula behavior in adult zebrafish. Since larvae homozygous for rerea mutations die around 8
days post-fertilization, we compared populations of WT adult fish with bab^{tb210} days post-fertilization, we compared populations of WT adult fish with *bab^{tane}*
days post-fertilization, we compared populations of WT adult fish with *bab^{tane}*
.

clockwise vs counter-clockwise swimming preference in circular swim tanks (Extend
data Fig. 2 and Supplementary Movie 1). About forty *bab* heterozygotes and forty V
fish were assayed for each sex. Consistent with publish data Fig. 2 and Supplementary Movie 1). About forty *bab* heterozygotes and forty WT
fish were assayed for each sex. Consistent with published reports 31 , WT females
showed a preference for clockwise swimming at the p data Fig. 2 and Supplementary Wovie 1). About forty bab ineterozygotes and forty WT
fish were assayed for each sex. Consistent with published reports ³¹, WT females
showed a preference for clockwise swimming at the popu fish were assayed for each sex. Consistent with published reports α , WT females
showed a preference for clockwise swimming at the population level (mean Later
Index, L.I. = 0.58; Fig. 2f). This population bias for cloc Index, L.I. = 0.58; Fig. 2f). This population bias for clockwise swimming was lost in *bab*
heterozygote females (mean L.I. = 0.49; $p = 0.04$; Fig. 2f). In contrast to published
observations ³¹, we saw no directional cl Index, L.I. = 0.58; Fig. 2f). This population bias for clockwise swimming was lost in bub-
heterozygote females (mean L.I. = 0.49; p = 0.04; Fig. 2f). In contrast to published
observations ³¹, we saw no directional cloc observations ³¹, we saw no directional clockwise bias for WT and heterozygote males
(Fig. 2g). We did not detect any change in the degree of lateralization (which measure
the strength of the preference to swim in clockw (Fig. 2g). The preference to swim in clockwise or counterclockwise direction)
the strength of the preference to swim in clockwise or counterclockwise direction)
between *bab* heterozygote and WT females (Fig. 2h; $p = 0.7$ the strength of the processes and WT females (Fig. 2h; $p = 0.7$) while a trend toward retarization was observed in male heterozygotes compared to WT (Fig. 2i; $p = 0.03$ Sex-specific differences in lateralized behaviors ar between bab heterozygote and WT females (Fig. 2n, p = 0.7) while a trend toward more
lateralization was observed in male heterozygotes compared to WT (Fig. 2i; p = 0.08).
Sex-specific differences in lateralized behaviors a Sex-specific differences in lateralized behaviors are common and may reflect
interactions with sex-specific hormones and other factors ³². For both sexes, there was
no statistically significant difference in swimming spe interactions with sex-specific hormones and other factors ³². For both sexes, t
no statistically significant difference in swimming speed and swimming distan
between *bab* heterozygotes and WT (Extended Data Fig. 3). Ana interactions with sex-specific hormones and other factors ³². For both sexes, there was
no statistically significant difference in swimming speed and swimming distance
between *bab* heterozygotes and WT (Extended Data Fi no statistically significant MT (Extended Data Fig. 3). Analysis of brain sectioned that gross LR asymmetry of the adult dorsal habenular nuclei was norms sampling of the *bab* heterozygote females that had been assayed fo between bab heterozygotes and WT (Extended Data Fig. 3). Analysis of brain sections
showed that gross LR asymmetry of the adult dorsal habenular nuclei was normal in a
sampling of the *bab* heterozygote females that had be sampling of the *bab* heterozygote females that had been assayed for swimming
laterality (9 of 9 fish, data not shown) and did not correlate with swimming direction.
Moreover, strongly clockwise and strongly counter-clockw sampling of the *bab* heterozygote remales that had been assayed for swimming
laterality (9 of 9 fish, data not shown) and did not correlate with swimming director-
Moreover, strongly clockwise and strongly counter-clockwi Moreover, strongly clockwise and strongly counter-clockwise WT fish exhibited the
same habenular LR asymmetry (Fig. 2j-k). Collectively, these results argue that *rered*
modulates behavioral laterality through habenula- an same habenular LR asymmetry (Fig. 2j-k). Collectively, these results argue that *rerec*
modulates behavioral laterality through habenula- and Nodal-independent pathway
However, the limited understanding of zebrafish functi same habenular LN asymmetry (Fig. 2j-k). Concetively, these results argue that rereation dualities behavioral laterality through habenula- and Nodal-independent pathways However, the limited understanding of zebrafish func

modulates behavior and the habenula habenula- penalty-penalty-through habenula-
However, the limited understanding of zebrafish functional neuroanatomy precludes
drawing any link between early hindbrain phenotypes and pref drawing any link between early hindbrain phenotypes and preferences in swimming
orientation.
The data above prompted us to test whether the asymmetry-buffering role of RA
discovered in the zebrafish brain extends to mammal drawing any minimized early himselving preferency per any preferences in summing
orientation.
The data above prompted us to test whether the asymmetry-buffering role of RA
discovered in the zebrafish brain extends to mamma The data about
The data about
discovered in
a bilateral gr
neural ridge
different fun
antero-medi
laterally. Thi ך
2 ה c arc discovered in the zebrafish brain extends to mammals. In the developing mouse c
a bilateral gradient of FGF signaling arising from the rostral patterning center (ant
neural ridge early and commissural plate later) plays a a bilateral gradient of FGF signaling arising from the rostral patterning center (anterior
neural ridge early and commissural plate later) plays a key role in specifying the
different functional areas 33 . High Fgf8 le neural ridge early and commissural plate later) plays a key role in specifying the
different functional areas 33 . High Fgf8 levels are required for motor cortex specification
antero-medially whereas lower levels resul different functional areas ³³. High Fgf8 levels are required for motor cortex specification
antero-medially whereas lower levels result in specification of sensory areas postero-
laterally. This results in the establish signal in the forebrain was strongly down-regulated $\frac{7}{1}$. In addition, chromatin immunosymmetrical at this stage. Like in somites, this *Fgf8* gradient is also antagonized by RA, which acts to pattern the developing forebrain ³⁴ in part through COUP-TFI (Nr2f1) which specifies the caudal part of the corte symmetrical at this stage. Like in somites, this Fyg gradient is also antagonized by KA,
which acts to pattern the developing forebrain ³⁴ in part through COUP-TFI (Nr2f1)
which specifies the caudal part of the cortex i which acts to pattern the developing forebrain ³⁴ in part through COUP-TFI (Nr2f1)
which specifies the caudal part of the cortex including the sensory and visual areas
Consistently, an RARE-LacZ reporter ³⁶, which det which specifies the caudal part of the cortex including the sensory and visual areas ∞ .
Consistently, an RARE-LacZ reporter 36 , which detects RA activity *in vivo,* is strongly
expressed in the mouse developing fo Consistently, an RARE-LacZ reporter 56 , which detects RA activity *in vivo,* is strongly expressed in the mouse developing forebrain and cortex (Fig. 3a) 34,37 . When mouse *Rere* mutants *openmind* (*Rere^{om/om}*) expressed in the mouse developing forebrain and cortex (Fig. 3a) ^{34,37}. When mouse null
Rere mutants *openmind* (*Rere^{om/om}*) ³⁸ were crossed to the *RARE-LacZ* reporter, the LacZ
signal in the forebrain was stron *Rere* mutants *openmind* (*Rere^{om/om}*) ³⁶ were crossed to the *RARE-LacZ* reporter, the LacZ signal in the forebrain was strongly down-regulated ⁷. In addition, chromatin immuno-
precipitation (ChIP) revealed that signal in the forebrain was strongly down-regulated '
precipitation (ChIP) revealed that the Retinoic Acid Re
of the WHHERE complex are bound to the RARE-LacZ
cortex (Fig. 3b-c). Finally, defects in forebrain and cor
repor prequences that the Recomplex are bound to the RARE-LacZ promoter in the E13.5 mouse
cortex (Fig. 3b-c). Finally, defects in forebrain and cortex development have been
reported for RA-deficient mice and *Rere* mutants ^{7,} cortex (Fig. 3b-c). Finally, defects in forebrain and cortex development have been
reported for RA-deficient mice and *Rere* mutants ^{7,38-43}. Together, these observation
reported for RA-deficient mice and *Rere* mutants reported for RA-deficient mice and *Rere* mutants $7,38-43$. Together, these observation reported for RA-deficient mice and *Rere* mutants $7,38-43$. reported for RA-deficient mice and *Rere* mutants ^{7,38} ⁴³. Together, these observations
intervalse on the series of the

suggest that Rere-dependent RA signaling plays a role in patterning the developing
mouse cortex. Because *Rere^{om/om}* mutants die at E10.5 before cortex formation ³⁸, we
analyzed cortical patterning in *Rere^{+/om}* het mouse cortex. Because *Rere^{smoom}* mutants die at E10.5 before cortex formation ³⁵, we analyzed cortical patterning in *Rere^{+/om}* heterozygotes which are viable and fertile and which do not show any obvious behaviora analyzed cortical patterning in *Rere^{+/om}* heterozygotes which are viable and fertile and
which do not show any obvious behavioral difference with their WT littermates⁴⁴.
Analysis of *Fgf8* expression using qPCR in di which do not show any obvious behavioral difference with their WT littermates $\tilde{ }$.
Analysis of *Fgf8* expression using qPCR in dissected E10.5 brains showed an increas
(~8%) in *Rere* $\tilde{ }$ /^{om} heterozygous mutants Analysis of Fgf8 expression using q1 CR in dissected E10.5 brains showed an increase (~8%) in *Rere*^{+/om} heterozygous mutants compared to WT sibling embryos, consistent with *Rere* antagonizing *Fgf8* expression as repo (~8%) in *Rere*^{-/om} heterozygous mutants compared to WT sibling embryos, consistent
with *Rere* antagonizing *Fgf8* expression as reported in homozygote mutants ³⁸(Fig. 3d-
At post-embryonic day 7 (P7), the motor cort with *Rere* antagonizing *Fgf8* expression as reported in homozygote mutants ³⁰(Fig. 3d-f).
At post-embryonic day 7 (P7), the motor cortex, whose boundaries are defined by the
expression of *Lmo4*⁴⁵, is expanded by ~1 expression of *Lmo4*⁴⁵, is expanded by ~10% in *Rere^{+/om}* brains when compared to WT (Fig. 3g-i). A similar increase is observed in mutants deficient for RA signaling in the cortex³⁹. In WT brains, the left and righ expression of *Lmo4*³⁵, is expanded by ~10% in *Rere*^{-/om} brains when compared to WT (Fig. 3g-i). A similar increase is observed in mutants deficient for RA signaling in the cortex ³⁹. In WT brains, the left and rig (Fig. 3⁹. In WT brains, the left and right motor cortices are of similar size, while in *Rere^{+/om}* brains, the right motor cortex is ~5% larger than the left (Fig. 3g-h,j-l). We unagnetic resonance imaging (MRI) to id *Rere^{176m}* brains, the right motor cortex is ~5% larger than the left (Fig. 3g-h,j-l). We used magnetic resonance imaging (MRI) to identify cortical regions showing bilateral asymmetries of the gray matter in live WT an asymmetries of the gray matter in live WT and *Rere^{+/om}* adult brains. The volume of gray matter in the motor cortex in *Rere^{+/om}* was expanded asymmetrically on the right side compared to WT (Fig. 3m), consistent wit matter in the motor cortex in *Rere⁻⁷⁶¹¹* was expanded asymmetrically on the right side
compared to WT (Fig. 3m), consistent with the enlargement of the *Lmo4* motor domai
observed in newborn (P7) *Rere^{+/om}* brains. T

compared to WT (Fig. 3m), consistent with the emargement of the *Emo-*4 motor domain
observed in newborn (P7) *Rere^{+/om}* brains. Together, these results demonstrate that *Rere*
antagonizes *Fgf8* expression during corti observed in newborn (P7) *Rere^{-yom}* brains. Together, these results demonstrate that *Rere*
antagonizes *Fgf8* expression during cortical patterning. This also shows that *Rere* is
involved in the control of motor corte involved in the control of motor cortex bilateral symmetry during development.
This prompted us to investigate whether *Rere* also controls the asymmetric localiza
of higher functions in the mammalian cortex. Handedness is This prompted us to investigate whether *Rere* also controls the asymmetric local
of higher functions in the mammalian cortex. Handedness is a well-known latera
behavior controlled by the motor cortex ⁴⁶. Humans are the T c k t l i t s This prompted us to investigate whether here also controls the asymmetric localization
of higher functions in the mammalian cortex ⁴⁶. Humans are the only species exhibiting close
to 90% preferential bias toward usage o behavior controlled by the motor cortex ⁴⁶. Humans are the only species exhibiting close to 90% preferential bias toward usage of the right hand, a behavior controlled by the left hemisphere ⁴⁶. In contrast, in WT mou behavior controlled by the motor cortex ⁴⁰. Humans are the only species exhibiting close
to 90% preferential bias toward usage of the right hand, a behavior controlled by the
left hemisphere ⁴⁶. In contrast, in WT mou left hemisphere ⁴⁶. In contrast, in WT mouse lines such as C57BL/6, around half of the individuals of a population show a consistent preference for the left paw (sinistral) when tested specific behavioral assays ⁴⁷. W the other half exhibits a consistent preference for the right paw (dextral) when tested in
specific behavioral assays ⁴⁷. We analyzed forelimb usage in *Rere^{+/om}* mice using an
established assay of paw usage preferenc specific behavioral assays ⁴⁷. We analyzed forelimb usage in *Rere^{+/om}* mice using an established assay of paw usage preference in adult mice (Mouse Reaching and Grasping – MoRaG, Supplementary movie 2)⁴⁸. While ~35 – MoRaG, Supplementary movie 2)⁴⁸. While ~35% (8 out of 24) of both WT females and
males use their right paw to grab food pellets, in *Rere^{+/om}* animals this proportion
reached ~80% (19 out of 24) for both genders (Fi males use their right paw to grab food pellets, in *Rere^{+/om}* animals this proportion
reached ~80% (19 out of 24) for both genders (Fig. 4a-d). No statistically significant
difference in lateralization (defined as the c males use their right paw to grab food pellets, in *Rere^{-76m}* animals this proportion
reached ~80% (19 out of 24) for both genders (Fig. 4a-d). No statistically significar
difference in lateralization (defined as the co difference in lateralization (defined as the consistency of paw usage within individu
was observed between WT and *Rere^{+/om}* (Fig. 4e-f). Overall, this analysis suggests th
Rere controls brain functional asymmetry and h

was observed between WT and *Rere^{+/om}* (Fig. 4e-f). Overall, this analysis suggests that
Rere controls brain functional asymmetry and hence the probability for an animal to us
the right forelimb in a food reaching task the right forelimb in a food reaching task at the population level.
This led us to explore the brain structure of adult $Rere^{t/om}$ animals. We used MRI to
compare brain symmetry in a cohort of live WT and $Rere^{t/om}$ mice ph This led us to explore the brain structure of adult $Rere^{t/om}$ animals. We used MRI to compare brain symmetry in a cohort of live WT and $Rere^{t/om}$ mice phenotyped for forelimb usage. Left and right comparison of the brains compare brain symmetry in a cohort of live WT and *Rere^{-yom}* mice phenotyped for
forelimb usage. Left and right comparison of the brains of sinistral and dextral WT
by voxel-based morphometry (VBM) identified asymmetric by voxel-based morphometry (VBM) identified asymmetric differences in a region
encompassing the right motor and somatosensory cortex (right sensorimotor cortex)
only in animals using preferentially the right forelimb (Fig. by compassing the right motor and somatosensory cortex (right sensorimotor corter only in animals using preferentially the right forelimb (Fig. 5a middle panels, c). The asymmetric sensorimotor region also included the cor only in animals using preferentially the right forelimb (Fig. 5a middle panels, c). This asymmetric sensorimotor region also included the cortex forelimb representation 49 . I clear structural asymmetries were identifi only asymmetric sensorimotor region also included the cortex forelimb representation ⁴⁹.

Shear structural asymmetries were identified in the sensorimotor cortex of sinistral V

Shear structural asymmetries were identifi asymmetric sensorimotor region also included the cortex forelimb representation ⁻⁻. No
clear structural asymmetries were identified in the sensorimotor cortex of sinistral WT
clear structural asymmetries were identified clear structural asymmetries were identified in the sensorimotor cortex of sinistral WT and the sensorimotor c
The sensorimotor cortex of sinistral WT and WT
T

animals (Fig. 5a, top panels, c). A larger structurally asymmetrical region including the right sensorimotor cortex was identified in *Rere^{+/om}* animals at the same position as the region identified in the brain of WT d right sensorimotor cortex was identified in *Rere*^{-/om} animals at the same position as the region identified in the brain of WT dextral animals (Fig. 5a, bottom panels, c).
The apparent increase in the right motor corte The apparent increase in the right motor cortex volume in *Rere^{+/om}* and dextral animals is unexpected as the right paw-preference is controlled by the left hemisphere. In cortices of mice mutant for *COUP-TFI*, the mot is until the right-payther COUP-TFI, the motor cortex expands into the sensory and resulting in improper patterning of the cortico-spinal motor neurons (CSMN) in the ectopically motorized area ⁵⁰. This leads to an impai cortices of line mutant for COUP-TFI, the motor cortex expands into the sensory area
resulting in improper patterning of the cortico-spinal motor neurons (CSMN) in the
ectopically motorized area ⁵⁰. This leads to an imp ectopically motorized area ⁵⁰. This leads to an impairment of fine motor skills in mutant
mice. The slight caudal expansion of the right motor cortex in *Rere^{+/om}* mice could
similarly lead to inappropriate specificat mice. The slight caudal expansion of the right motor cortex in *Rere^{+/om}* mice could
similarly lead to inappropriate specification of a subset of CSMN in the left forelim
projection area thus resulting in decreased dext projection area thus resulting in decreased dexterity associated to the left paw. To
investigate CSMN specification in *Rere^{+/om}* mutant brains, we analyzed the expressio
Ctip2 (which strongly labels the CSMN of layer V program investigate CSMN specification in *Rere* $^{+}/0m$ mutant brains, we analyzed the expression Ctip2 (which strongly labels the CSMN of layer V) and Tbr1 (which is expressed by la VI neurons), in sections of the motor investigate CSMN specification in *Rere^{-76m}* mutant brains, we analyzed the expression of Ctip2 (which strongly labels the CSMN of layer V) and Tbr1 (which is expressed by layer VI neurons), in sections of the motor cor VI neurons), in sections of the motor cortex region showing asymmetries in mouse P7
brains. We found that compared to WT, *Rere^{+/om}* mutants show similar total numbers of
neurons, of Tbr1 neurons, and of Ctip2-Tbr1 doub brains. We found that compared to WT, $Rere^{t/om}$ mutants show similar total numbers on the left and right side, but a slight yet significant decrease in Ctip2-positive neurons on the left and right side, but a slight yet s brains. We found that compared to WT, *Rere^{+/om}* mutants show similar total numbers of
neurons, of Tbr1 neurons, and of Ctip2-Tbr1 double-positive neurons on the left and
right side, but a slight yet significant decrease right side, but a slight yet significant decrease in Ctip2-positive neurons in the right si
(Extended data Figure 4). Thus, our data show subtle abnormal LR patterning of the
CSMN neurons in the right side of *Rere^{+/om}*

(Extended data Figure 4). Thus, our data show subtle abnormal LR patterning of the
CSMN neurons in the right side of *Rere^{+/om}* mutant brains which could account for the
right dominance of the heterozygous mutant popula CSMN neurons in the right side of *Rere^{+/om}* mutant brains which could account for the right dominance of the heterozygous mutant population.
We next performed brain perfusion Single Photon Emission Computed Tomography CSMN neurons in the right side of *Rere^{-/om}* mutant brains which could account for the right dominance of the heterozygous mutant population.
We next performed brain perfusion Single Photon Emission Computed Tomography (We next performed brain perfusion Single Photon Emissic
(SPECT) to analyze regional cerebral blood flow as an over
activity. In *Rere^{+/om}* but not in WT mice, we observed asyn
deep region including part of the motor and (SPECT) to analyze regional cerebral blood flow as an overall indicator of neuronal activity. In *Rere^{+/om}* but not in WT mice, we observed asymmetric tracer uptake in a deep region including part of the motor and somat extivity. In *Rere^{+/om}* but not in WT mice, we observed asymmetric tracer uptake in a deep region including part of the motor and somatosensory cortex in the left hemisphere (Fig. 5b,g). Interestingly, the tracer uptake deep region including particle interestingly, the tracer uptake signal was approximat
same antero-posterior level of the sensorimotor cortex (albeit on the contrala
as the structural asymmetry detected by MRI (Fig. 5a,c). same antero-posterior level of the sensorimotor cortex (albeit on the contralateral side
as the structural asymmetry detected by MRI (Fig. 5a,c). This data suggests increased
brain activity in the left hemisphere of *Rere*

as the structural asymmetry detected by MRI (Fig. 5a,c). This data suggests increased
brain activity in the left hemisphere of *Rere^{+/om}* brains, consistent with their preference
in using the right forelimb.
Taken toget as the structural asymmetry detected by $\frac{1}{2}$ ($\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ and $\frac{1}{2}$ and $\frac{1}{2}$ are preferent in using the right forelimb.

Taken together, our work shows th brain activity in the left hemisphere of *Rere^{-/om}* brains, consistent with their preference
in using the right forelimb.
Taken together, our work shows that the level of Rere-dependent RA signaling controls
the degree o Taken together, our work s
the degree of brain asymm
and mouse embryos. Our d
the FGF signaling gradients
as a left determinant¹⁰ whic
humans, SETDB2 (which an
has been implicated in the ヿ t a t a H H The degree of brain asymmetry at the morphological and functional levels in zebrafish
the degree of brain asymmetry at the morphological and functional levels in zebrafish
and mouse embryos. Our data suggest that this pat and mouse embryos. Our data suggest that this pathway controls the LR symmetry of
the FGF signaling gradients involved in brain patterning. In zebrafish, FGF signaling acts
as a left determinant¹⁰ which also controls as the FGF signaling gradients involved in brain patterning. In zebrafish, FGF signaling act
as a left determinant¹⁰ which also controls aspects of epithalamus asymmetry $51,52$. In
humans, SETDB2 (which antagonizes FGF si as a left determinant¹⁰ which also controls aspects of epithalamus asymmetry $51,52$. In
humans, SETDB2 (which antagonizes FGF signaling to control visceral asymmetry in fish)
has been implicated in the control of hande as a left determinant¹⁰ which also controls aspects of epithalamus asymmetry ^{51,52}. In
humans, SETDB2 (which antagonizes FGF signaling to control visceral asymmetry in fis
has been implicated in the control of handedne has been implicated in the control of handedness ^{9,53-55}. Our studies show that altering
RA levels can generate LR asymmetries in the zebrafish hindbrain independently of
Nodal. In mammals, evidence for implication of No Nodal. In mammals, evidence for implication of Nodal signaling in brain asymmetry
very limited 53 . The frequency of left-handed individuals is not increased in patients
situs inversus totalis arguing against a role fo very limited ⁵³. The frequency of left-handed individuals is not increased in patients w
situs inversus totalis arguing against a role for this pathway in the control of handedne
⁵⁶. Together, these data suggest that t very limited ³³. The frequency of left-handed individuals is not increased in patients with
situs inversus totalis arguing against a role for this pathway in the control of handedness
⁵⁶. Together, these data suggest t situs inversus totalis arguing against a role for this pathway in the control of handedness
⁵⁶. Together, these data suggest that the Rere-dependent RA pathway controls brain
asymmetry independently of Nodal. 56. Together, these data suggest that the Rere-dependent RA pathway controls brain asymmetry independently of Nodal.

dramatically shift handedness to the right at the population level in mouse. While
handedness has long been known to exhibit a genetic component, its transmission
follows a complex non-Mendelian or polygenic mechanism whic handedness has long been known to exhibit a genetic component, its transmission
follows a complex non-Mendelian or polygenic mechanism which is not understoo
classical model of genetic inheritance of handedness called the follows a complex non-Mendelian or polygenic mechanism which is not understood
classical model of genetic inheritance of handedness called the right shift theory
proposed that handedness is defined stochastically, resultin follows a complex non-Mendelian or polygenic mechanism which is not understood ". A
classical model of genetic inheritance of handedness called the right shift theory
proposed that handedness is defined stochastically, res proposed that handedness is defined stochastically, resulting in the roughly equiver distribution of dextral and sinistral individuals as observed in most animal species humans however, a right shift gene would skew the di proposed in most animal species ⁵⁸. In
distribution of dextral and sinistral individuals as observed in most animal species ⁵⁸. In
humans however, a right shift gene would skew the distribution to the right, leading to distribution of dextral and sinistral individuals as observed in most animal species ⁵⁹. In
humans however, a right shift gene would skew the distribution to the right, leading to
the strong right bias observed. Our data the strong right bias observed. Our data show that *Rere* behaves as such a right-shift gene. Genetically, RA levels can be modulated in many different ways, which could account for a multifactorial genetic control of han the strong right bias observed. Our data show that Rere behaves as such a right-shift
gene. Genetically, RA levels can be modulated in many different ways, which could
account for a multifactorial genetic control of handed generation, μ is account for a multifactorial genetic control of handedness. The ability of RA levels t
affect bilateral symmetry of the hindbrain region observed in zebrafish might also b
relevant to handedness as rec affect bilateral symmetry of the hindbrain region observed in zebrafish might also be
relevant to handedness as recent studies argue that hindbrain and spinal cord
asymmetries may be the earliest steps in the mechanism es relevant to handedness as recent studies argue that hindbrain and spinal cord
asymmetries may be the earliest steps in the mechanism establishing human
handedness 59,60 . *RERE* has been identified in GWAS studies as a asymmetries may be the earliest steps in the mechanism establishing human
handedness ^{59,60}. *RERE* has been identified in GWAS studies as a susceptibility l
schizophrenia ⁶¹. This neurological disease is associated wi handedness 59,60 . *RERE* has been identified in GWAS studies as a susceptibility
schizophrenia ⁶¹. This neurological disease is associated with brain symmetry of
and increased numbers of left-handed and mixed-handed schizophrenia ^{on}. This neurological disease is associated with brain symmetry defects
and increased numbers of left-handed and mixed-handedness individuals suggesting
that RERE and the RA signaling pathway could be invol that RERE and the RA signaling pathway could be involved in their etiology ⁶². Thus, o
study provides a basis to understand the lateralization of behaviors such as handedne
in the mammalian cortex. This mechanism might a that RERE and the RA signaling pathway could be involved in their etiology °-°. Thus, our study provides a basis to understand the lateralization of behaviors such as handedness
in the mammalian cortex. This mechanism migh in the mammalian cortex. This mechanism might also apply to the asymmetric
distribution of higher cognitive functions such as speech in the human brain.
AUTHOR CONTRIBUTIONS
Authors' contributions: M.R. and G.C.V.-N respec

AUTHOR CONTRIBUTIONS

handedness "Commission". *RERE* has been identified in GWAS studies as a susceptibility locus for
schizophenia ⁶¹. This neurological disease is associated with brain symmetry defects
tand increased numbers of left-handed distribution of higher cognitive functions such as speech in the human brain.
 AUTHOR CONTRIBUTIONS
 Authors' contributions: M.R. and G.C.V.-N respectively designed, performed an

analyzed the zebrafish and mouse exper AUTHOR CONTRIBUTIONS
AUTHOR CONTRIBUTIONS
Authors' contributions: M.R. and G.C.V.-N respectively designed, performed
analyzed the zebrafish and mouse experiments with O.P.. M.R. performed the
embryo treatments and mutant p Authors' contributions: M.R. and G.C.V.-N respectively designed, performed and
analyzed the zebrafish and mouse experiments with O.P.. M.R. performed the zebrafish
embryo treatments and mutant production and analysis with ノミ くししょう Authors' contributions: M.R. and G.C.V. Witcometry designed, performed and
analyzed the zebrafish and mouse experiments with O.P.. M.R. performed the zek
embryo treatments and mutant production and analysis with help from analyzed the ZP2CF inhibitor MCC154. M.L., L. B-C and M. R. performed the
C.S. prepared the CYP26 inhibitor MCC154. M.L., L. B-C and M. R. performed the
behavioral tests. D.C., S.B. and N.D. performed the analysis of retic embry- epared the CYP26 inhibitor MCC154. M.L., L. B-C and M. R. performed the
behavioral tests. D.C., S.B. and N.D. performed the analysis of reticulospinal neurons. J.-
L.P .and G.C.V.-N. performed the qPCR and qChIP exp behavioral tests. D.C., S.B. and N.D. performed the analysis of reticulospinal neur
L.P.and G.C.V.-N. performed the qPCR and qChIP experiments. G.C.V.-N. did the
embryo and brain analysis. A.M. performed the neuron quantif L.P .and G.C.V.-N. performed the qPCR and qChIP experiments. G.C.V.-N. did the mouse
embryo and brain analysis. A.M. performed the neuron quantifications. F.R. and H.M.
performed the MoRaG test. A.P. and S.L. designed, per embryo and brain analysis. A.M. performed the neuron quantifications. F.R. and H.M.
performed the MoRaG test. A.P. and S.L. designed, performed and analyzed the MRI
study. P.L. and D.B. performed the SPECT experiments. V.N performed the MoRaG test. A.P. and S.L. designed, performed and analyzed the MRI
study. P.L. and D.B. performed the SPECT experiments. V.N. performed the SPECT ima
analysis. G.C.V.-N. designed and analyzed the MoRaG, MRI a performed the SPECT experiments. V.N. performed the SPECT im-
analysis. G.C.V.-N. designed and analyzed the MoRaG, MRI and SPECT experiments are
sults. M. R., G.C.V.-N. and O.P. wrote the manuscript and OP supervised the p

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Figure 1: Inhibition and activation of RA signaling disrupts bilateral symmetry of the posterior hindbrain independently of Nodal signaling in zebrafish embryos

Fixed at the 8-10 somite stage and hybridized with a *deltaC* (a-b) or a *krox20* probe (c-d)
Arrowheads mark the tip of the traveling waves in the presomitic mesoderm. Scale bars
100 µm
(e) Percent of *krox20* bilateral fixed at the 8-10 somite stage and hybridized with a defide (a-b) or a krox20 probe (c-d).
Arrowheads mark the tip of the traveling waves in the presomitic mesoderm. Scale bars:
100 µm
(e) Percent of *krox20* bilateral sym 100 μ m

(e) Percent of *krox20* bilateral symmetry phenotypes observed at 8 -10 somites

following 10 μ M BMS treatment at the 8-128 cell stage (right bars) compared to control

treated with 1% or 0.1% DMSO (left bla 100 μm
(e) Perc
followin
treated
Sym: syr
any left-
enlarged ft tsicker (e) Tereent of krox20 bilateral symmetry phenotypes observed at 8 -10 somites
following 10 μ M BMS treatment at the 8-128 cell stage (right bars) compared to it
reated with 1% or 0.1% DMSO (left black bars). Three diffe treated with 1% or 0.1% DMSO (left black bars). Three different experiments are shown.
Sym: symmetrical expression, Sym red: r5 expression reduced but symmetrical, Asymr5:
any left-right asymmetric pattern of *krox20* in Sym: symmetrical expression, Sym red: r5 expression reduced but symmetrical, Asymr5:
any left-right asymmetric pattern of *krox20* in r5, No r5: r5 absent & r3-like stripe is
enlarged due to anteriorization of the hindbra

Symmetrical expression, Symmetric Dieposition reduced and Symmetrical expression, Asymptotic pattern of *krox20* in r5, No r5: r5 absent & r3-like stripe is
enlarged due to anteriorization of the hindbrain (p = 0.0001 for any left-right asymmetric pattern of krox20 in r.5, No r5: r5 absent & r5-like stripe is
enlarged due to anteriorization of the hindbrain ($p = 0.0001$ for for Sym, vs Asym)
(f, g) Zebrafish embryos treated with MCC154 (MC (f, g) Zebrafish embryos treated with MCC154 (MCC) (25 μ M) fixed at the 8-10 son
stage and hybridized with a *deltaC* (f) or a *krox20* probe (g). Arrowheads mark the
the traveling waves in the presomitic mesoderm. Sca

(s
t
(f (c (f) S) Zebrahim Embryos treated in the Exercy (disputed with a deltaC (f) or a krox20 probe (g). Arrowheads mark the tip of
the traveling waves in the presomitic mesoderm. Scale bars: 100 µm
(h) Percent of krox20 bilateral stage and hybridized with a defide (f) or a krox20 probe (g). Arrowheads mark the tip or
the traveling waves in the presomitic mesoderm. Scale bars: 100 µm
(h) Percent of *krox20* bilateral symmetry phenotypes observed at the traveling waves in the presomitic mesoderm. Scale bars: 100 μm
(h) Percent of *krox20* bilateral symmetry phenotypes observed at 8 -
following treatment with various concentrations of MCC154 (MCC). I
(right bars) comp (f
f (c t k こ (h) Percent of *krox20* bilateral symmetry phenotypes observed at 8 -10 somites
following treatment with various concentrations of MCC154 (MCC). Drug treatm
(right bars) compared to control treated with 1% or 0.4% EtOH(le (right bars) compared to control treated with 1% or 0.4% EtOH(left black bars). Two
different experiments are shown: drug/vehicle addition at 50% epiboly (left side) or
the 128-cell stage (right). Sym: symmetrical express (right bars) compared to the different experiments are shown: drug/vehicle addition at 50% epiboly (left side) or
the 128-cell stage (right). Sym: symmetrical expression, Sym red: r3 expression reduced
but symmetrical, As

the 128-cell stage (right). Sym: symmetrical expression, Sym red: r3 expression reduced
but symmetrical, Asymr3: any left-right asymmetric pattern of *krox20* in r3, No r3: r3
absent (p < 0.004 for for Sym. vs Asym.)
(i-k but symmetrical, Asymr3: any left-right asymmetric pattern of *krox20* in r3, No r3: r3
absent (p < 0.004 for for Sym. vs Asym.)
(i-k) *krox 20* expression in 22-26 somites embryos from an incross of *giraffe^{rw716}*
cyp2 but symmetrical, Asymr3: any left-right asymmetric pattern of kroszo in r3, No r3: r3
absent (p < 0.004 for for Sym. vs Asym.)
(i-k) $krox 20$ expression in 22-26 somites embryos from an incross of giraffe^{rw716}
cyp26a1 he (i-k) krox 20 expression in 22-26 somite
cyp26a1 heterozygous mutants. (i) wild
hemisegmental LR asymmetry of krox 20
bars: 50 µm
(l-n) 24-25 somites zebrafish embryos tr
and *lefty* probes. Red arrowhead shows c
|-
|-
|-(i-k) *krox 20* expression in 22-26 somites embryos from an incross of *giraffe* recyp26a1 heterozygous mutants. (i) wild-type sib. (j) *gir-/*- homozygote with hemisegmental LR asymmetry of *krox 20* expression in r3. (k

cyp26a1 heterozygous mutants. (i) what type sib. (j) gir-/ homozygote with
hemisegmental LR asymmetry of *krox 20* expression in r3. (k) *gir-/-* homozygo
bars: 50 µm
(l-n) 24-25 somites zebrafish embryos treated with 10 µ hemisegmental EN asymmetry of krox 20 expression in 13. (k) gir-/- homozygote. Scale
bars: 50 µm
(l-n) 24-25 somites zebrafish embryos treated with 10 µM BMS, hybridized with *krox2C*
and *lefty* probes. Red arrowhead show bars: 50 μm
(l-n) 24-25 s
and *lefty* pro
black arrowh
Supplement:
(o-p) 18-19 s
krox20 and s (a
| a
| s
| f

(iii) 24-25 somites zebrafish embryos treated with 10 µM BMS, hybridized with krokzo
and *lefty* probes. Red arrowhead shows *lefty1* expression in the diencephalon whereas
black arrowhead in I and n shows asymmetric *krox* and *lefty* probes. Red arrowhead shows *lefty1* expression in the diencephalon whereas
black arrowhead in I and n shows asymmetric *krox20* expression in r5 (see
Supplementary Table 3 for quantification). Scale bars: 100 Supplementary Table 3 for quantification). Scale bars: 100 μ m
(o-p) 18-19 somites zebrafish embryos treated with 10 μ M BMS, and hybric
krox20 and *southpaw* probes. Red arrowhead marks *southpaw* expression
plate Supplementary Table 3 for quantification). Scale bars: 100 μm
(o-p) 18-19 somites zebrafish embryos treated with 10 μM BM
krox20 and *southpaw* probes. Red arrowhead marks *southpaw*
plate mesoderm (LPM) whereas black a (or 20) and southpaw probes. Red arrowhead marks southpaw expression in the late
plate mesoderm (LPM) whereas black arrowhead shows asymmetric *krox20* expression
in r5. Scale bars: 100 µm
(a-g, i-p) Dorsal views. L: left, krox20 and southpaw probes. Red arrowhead marks southpaw expression in the lateral
plate mesoderm (LPM) whereas black arrowhead shows asymmetric *krox20* expression
in r5. Scale bars: 100 µm
(a-g, i-p) Dorsal views. L: lef plate mesoderm (LPM) whereas black arrowhead shows asymmetric krox20 expression
in r5. Scale bars: 100 µm
(a-g, i-p) Dorsal views. L: left, R: right. Anterior to the top.

in r5. Scale bars: 100 μm
(a-g, i-p) Dorsal views. L:
) \overline{a} (a-g, i-p) Dorsal views. L: left, R: right. Anterior to the top.

$\ddot{}$ Figure 2: Laterality defects in the rerea RA co-activator zebrafish mutant babyface $($ bab^{tb210})
(a, b) LR asymmetric fin (arrowhead) phenotype in *bab* homozygous mutant (b)

compared to wild-type (WT) sib (a) in 8 dpf zebrafish larvae. Scale bar:50 μ m $\frac{m}{\sqrt{2}}$ we have $\frac{m}{\sqrt{2}}$ when $\frac{m}{\sqrt{2}}$ was $\frac{m}{\sqrt{2}}$ Scale bar:50 μ m compared to wild-type (WT) sib (a) in 8 dpf zebrafish larvae. Scale bar:50 μm
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It is made available under a [CC-BY-NC-ND 4.0 International license.](http://creativecommons.org/licenses/by-nc-nd/4.0/) (which was not peer-reviewed) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. bioRxiv preprint first posted online Mar. 15, 2019; doi: [http://dx.doi.org/10.1101/578625.](http://dx.doi.org/10.1101/578625) The copyright holder for this preprint

(c-e) Dorsal confocal views of 48 hpf WT sib (+/+), bab+/- of bab-/-embryos showing
rhodamine dextran-backfilled hindbrain reticulospinal neurons (red; left panels: merg
view with DAPI counterstaining, right panels: red ch view with DAPI counterstaining, right panels: red channel only). Note the asymmetry in
the number of r6 reticulospinal neurons in $bab+/-$ (red arrows point to reticulospinal
neurons in r6, white arrow shows the position of

the number of r6 reticulospinal neurons in *bab+/-* (red arrows point to reticulospinal
neurons in r6, white arrow shows the position of missing neurons). M: Mauthner cell
(r4). Scale bars: left panels: 50 µm, right panel the number of rotetatospinal neurons in bab+/- (red arrows point to reticulospinal
neurons in r6, white arrow shows the position of missing neurons). M: Mauthner cell
(r4). Scale bars: left panels: 50 µm, right panels: 30 (r4). Scale bars: left panels: 50 μ m, right panels: 30 μ m.
(f-g) Scatter Plots comparing population-level biases for clockwise swimming in WT
adults vs *bab* heterozygotes. (f, g) Comparison of laterality indices (L (r4). Scale bars: left panels: 50 μm, right panels: 30 μm.
(f-g) Scatter Plots comparing population-level biases for
adults vs *bab* heterozygotes. (f, g) Comparison of latera
and *bab* heterozygotes females (f) and males (ここへ c r (adults vs *bab* heterozygotes. (f, g) Comparison of laterality indices (L.I.) between W
and *bab* heterozygotes females (f) and males (g). Numbers at top: group mean L.I.
values. Horizontal black bars mark the mean L.I. a adults vs *bub* heterozygotes. (f, g) Comparison of laterality malles (E.I.) between WT
and *bab* heterozygotes females (f) and males (g). Numbers at top: group mean L.I.
values. Horizontal black bars mark the mean L.I. an and *bab* heterozygotes remales (r) and males (g). Numbers at top: group mean L.I.
values. Horizontal black bars mark the mean L.I. and Standard Error of the Mean for
group of WTs or Heterozygotes. The clockwise swimming b group of WTs or Heterozygotes. The clockwise swimming bias of females (f) is lost when
one allele of *rerea* is disrupted (p = 0.04). For both WT and *bab* heterozygous males (g),
no group-level directional swimming bias i

group of Bleis of *rerea* is disrupted ($p = 0.04$). For both WT and *bab* heterozygous males (g), no group-level directional swimming bias is evident.
(h, i) Scatter Plots comparing the mean Degree of Lateralization (D.L. one allele of rerea is disrupted (p = 0.04). Tor both WT and *bab* heterozygous males (g),
no group-level directional swimming bias is evident.
(h, i) Scatter Plots comparing the mean Degree of Lateralization (D.L.) betwee no group-level and comparing the mean Degree of Lat
 hab heterozygotes. For both sexes, the p-value is > (

between WTs vs *bab* heterozygotes.

Each gray square or circle represents the mean Later

Lateralization of one

(h) is a state of the mean alterality in the mean point of the mean point of the mean between WTs vs bab heterozygotes.

Each gray square or circle represents the mean Laterality Index or Degree of

Lateralization of one bab heterozygotes. For both sexes, the p-value is > 0.05 for group lever unlerences
between WTs vs *bab* heterozygotes.
Each gray square or circle represents the mean Laterality Index or Degree of
Lateralization of one fis Bach gray square or circle represents

Lateralization of one fish.

(j, k) Swimming Laterality is independ

Coronal sections of 2 WT adult femal

counter-clockwise swimming bias (90

the gross LR asymmetries of the dors

l Lateralization of one fish.

(j, k) Swimming Laterality is independent of gross habenular Left-Right asymm

Coronal sections of 2 WT adult female brains. Although one fish (j) had a stro

counter-clockwise swimming bias (9 (j, k) Swimming Laterality
(j, k) Swimming Laterality
Coronal sections of 2 WT a
counter-clockwise swimm
the gross LR asymmetries
larger than Right). Scale ba
(a-e) Dorsal views. L: left, ()
()
(t | Coronal sections of 2 WT adult female brains. Although one fish (j) had a strong
counter-clockwise swimming bias (90%) and the other (k) a strong clockwise bias (7
the gross LR asymmetries of the dorsal habenular nuclei a counter-clockwise swimming bias (90%) and the other (k) a strong clockwise bias
the gross LR asymmetries of the dorsal habenular nuclei are the same (Left nucle
larger than Right). Scale bar: 50 µm
(a-e) Dorsal views. L: the gross LR asymmetries of the dorsal habenular nuclei are the same (Left nucleus
larger than Right). Scale bar: 50 µm
(a-e) Dorsal views. L: left, R: right
(a-e) Dorsal views. L: left, R: right $\lvert \text{larger than Right}.$ Scale bar: 50 μ m
(a-e) Dorsal views. L: left, R: right
 $\lvert \text{const.} \rvert$ larger than Right). Scale bar: 50 μm
(a-e) Dorsal views. L: left, R: right
)
- $(a-c)$ Dorsal views. L: left, R: right

Figure 3: Retinoic acid signaling in the mouse cortex and asymmetric motor area expansion in newborn *Rere''* w brains

(b, c) ChIP analysis of the top.

(b, c) ChIP analysis of the RARE sequence on the RARE-LacZ reporter with specific

antibodies against Rar α (b) or Rere, Wdr5, Hdac1 and Hdac2 (c) in E13.5 RARE-LacZ

mouse cortices (n (b, c) ChIP analysis of the *RARE* sentibodies against Rar α (b) or Remouse cortices (n = 3). IgG: nega
(d) Schematic dorsal view of diss
neocortical primordia (Ncp) (oral

)
r
c
c (b, c) CHIP analysis of the RARE sequence on the RARE Eac2 reporter with specific
antibodies against Rar α (b) or Rere, Wdr5, Hdac1 and Hdac2 (c) in E13.5 RARE-Lac
mouse cortices (n = 3). IgG: negative control. Data rep mouse cortices (n = 3). IgG: negative control. Data represent mean ± s.e.m. $*P < 0$.

(d) Schematic dorsal view of dissected E10.5 forebrain showing the area of the

neocortical primordia (Ncp) (orange dashed line, Anteri (d) Schematic dorsal view of dissected E10.5 forebrain showing the area of the
neocortical primordia (Ncp) (orange dashed line, Anterior to the top) used for qPCR
analysis of *Fgf8* (e) and *Rere* (f) expression from wild (r こし
1
(/ neocortical primordia (Ncp) (orange dashed line, Anterior to the top) used for c
analysis of *Fgf8* (e) and *Rere* (f) expression from wild-type (n = 30) and *Rere*^{+/om}
Unpaired two-sample *t*-test, two-tailed, *Fgf8* m analysis of *Fgf8* (e) and *Rere* (f) expression from wild-type (n = 30) and *Rere^{+/om}* (n = 29).
Unpaired two-sample *t*-test, two-tailed, *Fgf8* mRNA: *P* = 0.0152 and *Rere* mRNA: *P* <
0.0001.
(g, h) Dorsal views of

0.0001.

(g, h) Dorsal views of wild-type (g) and *Rere^{+/om}* (h) P7 brains hybridized *In situ* with a
 Lmo4 probe. Left (L) and Right (R). Anterior to the top.

(i) Motor area ratio (Area of rostral *Lmo4*-positive d (g, h) Defined a probability of (i) Moto
 ≤ 0.0001
 (i) Moto ()
)
() (g, h) Dorsal views of wild-type (g) and *Rere* ¹/^{om} (h) P7 brains hybridized *ln situ* with a *Lmo4* probe. Left (L) and Right (R). Anterior to the top.

(i) Motor area ratio (Area of rostral *Lmo4*-positive domain / (i) Motor area ratio (Area of rostral Lmo4-positive dom
wild-type (n = 9) and Rere^{+/om} (n = 14) brains. Unpaired
< 0.0001.
(j) Motor asymmetry index [(Right motor area – Left motorex)] in wild-type (n = 9) and Rere^{+/om}

(i) Wotor area ratio (Area of rostral Lmo4-positive domain) Area of entire cortex) in
wild-type (n = 9) and *Rere^{+/om}* (n = 14) brains. Unpaired two-sample *t*-test, two-tailed
< 0.0001.
(j) Motor asymmetry index [(Rig cortex)] in wild-type (n = 9) and Rere^{+/om} (n = 14) brains. Unpaired two-sample t-test,

wild-type (n = 9) and *Rere^{-/om}* (n = 14) brains. Unpaired two-sample *t*-test, two-tailed, *P*

< 0.0001.

(j) Motor asymmetry index [(Right motor area – Left motor area) / Area of whole

cortex)] in wild-type (n = 9) < 0.0001.

(j) Motor asymmetry index [(Right motor area – Left motor area) / Area of whole

cortex)] in wild-type (n = 9) and *Rere^{+/om}* (n = 14) brains. Unpaired two-sample *t*-test,

two-tailed, $P = 0.0071$.

(k-l) Le cortex)] in wild-type (n = 9) and *Rere^{-/om}* (n = 14) brains. Unpaired two-sample *t*-test,
two-tailed, $P = 0.0071$.
(k-l) Left (Left motor area / Area of entire cortex) and Right (Right motor area / Area of
entire cort (k-l) Left (Left motor and

entire cortex) motor and

and *Rere^{+/om}* (n = 14) (l)
 Rere^{+/om} : $P = 0.0118$.

In all graphs, data repre

(m) Magnetic resonand

brains. (Top) Coronal se entire cortex) motor area ratio comparison within the same brain in wild-type (n = 9) (k
and *Rere^{+/om}* (n = 14) (l). Paired two-sample *t*-test, two-tailed, wild-type: $P = 0.1232$ and
Rere^{+/om}: $P = 0.0118$.
In all

and $Rere^{t/om}$ (n = 14) (l). Paired two-sample *t*-test, two-tailed, wild-type: $P = 0.1232$ and
 $Rere^{t/om}$: $P = 0.0118$.

In all graphs, data represent mean ± s.d. unless otherwise specified.

(m) Magnetic resonance imagin and *Rere^{-/om}* (n = 14) (l). Paired two-sample *t*-test, two-tailed, wild-type: $P = 0.1232$ and *Rere^{+/om}* : $P = 0.0118$.
In all graphs, data represent mean ± s.d. unless otherwise specified.
(m) Magnetic resonance im $Rere^{1cm}: P = 0.0118.$
In all graphs, data rep
(m) Magnetic resona
brains. (Top) Coronal
surface - max intensit
 $Rere^{+/om}$). (Bottom) co
background surface -
(Rere $^{+/om}$ > WT region (m) Magnetic resonance imaging from wild-type (n = 20) and $Rere^{t/om}$ (n = 18) adult
brains. (Top) Coronal sections (left panels) and 3D brain projections (below background
surface - max intensity) (right panel) representi (m) Magnetic resonance imaging from wild-type (n = 20) and *Rere^{-/om}* (n = 18) adult
brains. (Top) Coronal sections (left panels) and 3D brain projections (below backgrou
surface - max intensity) (right panel) represent surface - max intensity) (right panel) representing regions larger in wild-type (WT >
Rere^{+/om}). (Bottom) coronal sections (left panels) and 3D brain projections (below
background surface - max intensity) (right panels) *Rere^{+/om}*). (Bottom) coronal sections (left panels) and 3D brain projections (below
background surface - max intensity) (right panels) representing regions larger in *Ret*
(*Rere^{+/om}* > WT regions). The statistical p *Rere^{-/om}*). (Bottom) coronal sections (lett panels) and 3D brain projections (below
background surface - max intensity) (right panels) representing regions larger in *R*
(*Rere^{+/om}* > WT regions). The statistical par background surface - max intensity) (right panels) representing regions larger in *Rere*^{-/om}
(*Rere^{+/om}* > WT regions). The statistical parametric t-maps from Unpaired two-sample *t*-
test are thresholded at $P < 0.001$ (*Rere^{-70m}* > WT regions). The statistical parametric t-maps from Unpaired two-sample *t*-
test are thresholded at $P < 0.001$ with an extent threshold of 500 voxels. The data are
overlaid to an MRI template derived from test are thresholded at P < 0.001 with an extent threshold of 300 voxels. The data are
overlaid to an MRI template derived from Dartel normalization (using SPM8, Wellcom
Trust Center for Neuroimaging, UK, http://www.fil.io Trust Center for Neuroimaging, UK, http://www.fil.ion.ucl.ac.uk/spm) of all brain
volumes (acquisition matrix size: 384x384, pixel size: 60x60 μ m²; 72 slices, thickness:
0.22 mm). Yellow voxels indicate significant d Trust Center for Neuroimaging, UK, http://www.fil.ion.ucl.ac.uk/spm) of all brain
volumes (acquisition matrix size: 384x384, pixel size: 60x60 μ m²; 72 slices, thickness:
0.22 mm). Yellow voxels indicate significant d volumes (acquisition matrix size: 384x384, pixel size: 60x60 μm²
0.22 mm). Yellow voxels indicate significant differences with the
Increased volume of gray matter in the right hemisphere of *Rere*
by 4.6% compared to WT $\frac{1}{100}$ contralateral side.
 $e^{t/om}$ mice
s. Left (L) and Right (F Increased volume of gray matter in the right hemisphere of *Rere^{+/om}* mice
by 4.6% compared to WT mice. The color bars represent t-scores. Left (L) and Right
 $\frac{1}{2}$ Increased volume of gray matter in the right hemisphere of *Rere^{ty om}* mice
by 4.6% compared to WT mice. The color bars represent t-scores. Left (L) a
interval of the color bars represent t-scores. Left (L) a
discussed t by 4.6% compared to WT mice. The color bars represent t-scores. Left (L) and Right (R).

Figure 4. Rere controls asymmetric forelimb usage at the population level
(a-b) Heatmap detailing the mouse reaching and grasping (MORAG) behavior test in (a b) Heatmap detailing the modes reaching the grasping (MT: n = 12 and females (WT: n = 12 and Rere^{+/om}: n = 12) (a) and males (WT: n = 12 and Rere^{+/om}: n = 12) (b). Columns correspond to 130 food pellets per animal $Rere^{t/om}$: n = 12) (b). Columns correspond to 130 food pellets per animal trial. Yellow: Rere^{-/om}: n = 12) (b). Columns correspond to 130 food pellets per animal trial. Yellow:
Left-forelimb usage; Blue: Right-forelimb usage.
The same of pellets per animal trial. Yellow: $L_{\rm g}$, and $R_{\rm g}$, and $R_{\rm g}$ is a computation using usage.

(c-d) Handedness index [(Right-paw use - Left-paw use) / Total paw entries)] in WT and *Rere^{+/om}* females (c) and in WT and *Rere^{+/om}* males (d). Unpaired two-sample *t*-test, two-tailed, females: $P = 0.0039$ and male *Rere^{-/om}* temales (c) and in WT and *Rere*^{-/om} males (d). Unpaired two-sample *t*-test, two-
tailed, females: $P = 0.0039$ and males: $P = 0.0211$.
(e-f) Percentage of lateralization (highest value from either Right- o (e-f) Percentage of lateralization (highest value fro
for each animal / Total paw entries) in wild-type and
 $Rere^{t/om}$ males (f). Unpaired two-sample *t*-test, two males: $P = 0.2660$.
In all graphs, data represent mean \pm for each animal / Total paw entries) in wild-type and *Rere^{+/om}* females (e) and in WT an *Rere^{+/om}* males (f). Unpaired two-sample *t*-test, two-tailed, females: *P* = 0.1584 and males: *P* = 0.2660.
In all graphs, d *Rere^{+/om}* males (f). Unpaired two-sample *t*-test, two-tailed, females: $P = 0.1584$ and Rere^{tyom} males (f). Unpaired two-sample *t*-test, two-tailed, females: *P* = 0.1584 and
males: *P* = 0.2660.
In all graphs, data represent mean ± s.d.

In all graphs, data represent mean \pm s.d. In all graphs, data represent mean ± s.d.

l, Figure 5. Asymmetric patterning of Corticospinal Motoneurons of the sensorimotor cortex in *Rere''^{om} heterozygous mic*e

(a, c) Brain magnetic resonance imaging from wild-type (WT) sinistral (n = 12), WT dextral (n = 8) and *Rere^{+/om}* dextral (n = 18) animals. Coronal sections (a) and 3D brain projections (below background surface - max i projections (below background surface - max intensity) (c) representing structural Left-
Right asymmetry differences. Increased gray matter in the right hemisphere of dextral
WT and dextral *Rere^{+/om}* mice by 6% and 7.5 WT and dextral $Rere^{+/om}$ mice by 6% and 7.5% respectively compared to the left
hemisphere. The statistical parametric t-maps from uncorrected Paired two-sample t-
test are thresholded at $P < 0.00001$ with an extent thresho

*Rere^{+/om} (*n = 9) animals. Coronal (b) and transverse (g) sections representing Left-Right hemisphere. The statistical parametric t-maps from uncorrected Talled two-sample that the thesholded at $P < 0.00001$ with an extent threshold of 500 voxels and overlai
to the MRI template.
(b, g) Single photon emission co to the MRI template.

(b, g) Single photon emission computed tomography (SPECT) in wild-type (n = 9) and

Rere^{+/om} (n = 9) animals. Coronal (b) and transverse (g) sections representing Left-Right

asymmetry differences $Rere^{t/om}$ (n = 9) animals. Coronal (b) and transverse (g) sections representing Left-Rigl
asymmetry differences in regional cerebral blood flow. The statistical parametric t-m
from One-sample *t*-test for Left-Right asymm *Rere^{-/om}* (n = 9) animals. Coronal (b) and transverse (g) sections representing Left-Right
asymmetry differences in regional cerebral blood flow. The statistical parametric t-map
from One-sample *t*-test for Left-Right

from One-sample *t*-test for Left-Right asymmetry are thresholded at $P < 0.01$ and
overlaid to the MRI template.
The color bars represent t-scores. Left (L) and Right (R).
(e) Coronal section of the brain of a P7 Rere^{+/o} from One-sample t-test for Left-Right asymmetry are thresholded at $P \le 0.01$ and
overlaid to the MRI template.
The color bars represent t-scores. Left (L) and Right (R).
(e) Coronal section of the brain of a P7 Rere^{+/om} The color bars represent t-sco

(e) Coronal section of the brain

cortex labeled with antibodies

respectively label the layer V a

counts were performed.

(f) Comparison of the left-righ (e) Coronal section of the brain of a P7 Rere^{+/om} mouse a
cortex labeled with antibodies against Ctip2 (purple) and
respectively label the layer V and VI neurons. Orange lin
counts were performed.
(f) Comparison of the l (c r c
(c (

(e) Coronal section of the brain of a P7 Rere^{-yom} mouse at the level of the sensorimotor
cortex labeled with antibodies against Ctip2 (purple) and Tbr1 (green) which
respectively label the layer V and VI neurons. Orange respectively label the layer V and VI neurons. Orange lines delimits the area v
counts were performed.
(f) Comparison of the left-right ratio in the whole cell population and in Ctip2
double positive populations of neurons respectively label the layer V and VI neutrons. The general tender of the last since ϵ counts were performed.

(f) Comparison of the left-right ratio in the whole cell population and in Ctip2, Tbr1 and

double positive (f) Comparison of the left
double positive population
between WT and $Rere^{t/0t}$
positive cells (p=0.01, Ma
of Layer V. (c
k
F c (f) Comparison of neurons counted in the orange boxes shown in (e) between WT and *Rere^{t/om}* mice (HET). The only difference detected is for the Ctip2 positive cells (p=0.01, Mann-Whitney test) which include the cortico between WT and *Rere^{+/om}* mice (HET). The only difference detected is for the Ctip2
positive cells (p=0.01, Mann-Whitney test) which include the corticospinal motoneu
of Layer V. positive cells (p=0.01, Mann-Whitney test) which is the corticospinal motor which include the corticospinal moton
include the corticospinal motor which is the corticospinal motor which is the corticospinal motor which is t of Layer V.

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Zebrafish Lines

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Drug Treat
BMS-20449 Econalish Lines
The *giraffe^{rw716}*
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hours post-ferti
Drug Treatment
BMS-204493 (B
DMSO to a stocl
done using 1-10 The *giraffe^{rw 26}* line ²⁴was obtained from the Zebratish National BioResource Project,
Japan. The *bab^{tb210}* allele²⁷ was obtained courtesy of Nikki Plaster and Tom Schilling.
Zebrafish embryos and larvae were sta

Japan. The *bab*⁸²²² allele²⁷ was obtained courtesy of Nikki Plaster and Tom Schilling.
Zebrafish embryos and larvae were staged in cell numbers, somite numbers and in
hours post-fertilization (hpf) or days post-fertil bours post-fertilization (hpf) or days post-fertilization (dpf).
 Drug Treatments

BMS-204493 (BMS), a pan-RAR antagonist, was custom-synthesized and dissolved in

DMSO to a stock concentration of 10mM. Unless otherwise **Drug Treatments**
BMS-204493 (BMS), a pan-RAR antagonist, was custom-synt
DMSO to a stock concentration of 10mM. Unless otherwise
done using 1-10 µM BMS, a dose range that specifically inhil
embryos¹⁵. The pan-Cyp26 inh **Drug Treatments**
BMS-204493 (BMS), a pan-RAR antagonist, was custom-synthesized and dissolved in DMSO to a stock concentration of 10mM. Unless otherwise noted, experiments were
done using 1-10 μ M BMS, a dose range that specifically inhibits RA signaling in zebraf
embryos¹⁵. The pan-Cyp26 inhibitor MCC154 (3-imid done using 1-10 μ M BMS, a dose range that specifically inhibits RA signaling in zebrafis
embryos¹⁵. The pan-Cyp26 inhibitor MCC154 (3-imidazol-1-yl-2-methyl-3-[4-
(naphthalen-2-ylamino)-phenyl]-propionic acid methyl embryos¹⁵. The pan-Cyp26 inhibitor MCC154 (3-imidazol-1-yl-2-methyl-3-[4-
(naphthalen-2-ylamino)-phenyl]-propionic acid methyl ester) was synthesized as
described ⁶³ and resuspended in ethanol (6mM stock). All drug tr embryos²⁵. The pan-Cyp26 inhibitor MCC154 (3-imidazol-1-yl-2-methyl-3-[4-
(naphthalen-2-ylamino)-phenyl]-propionic acid methyl ester) was synthesized
described ⁶³ and resuspended in ethanol (6mM stock). All drug treat described 63 and resuspended in ethanol (6mM stock). All drug treatments used
alone (DMSO or EtOH) as the negative control. BMS was added at the 8-128 cell
(unless otherwise noted) in fish system water and embryos wer described ⁸⁵ and resuspended in ethanol (6mM stock). All drug treatments used vehicle
alone (DMSO or EtOH) as the negative control. BMS was added at the 8-128 cell stage
(unless otherwise noted) in fish system water and (unless otherwise noted) in fish system water and embryos were incubated in BMS at 28.5°C until the time of analysis. For a few experiments, development was slowed dow
by incubation at 23.5C°C. MCC154 was added at either $^{28.5^{\circ}C}$ until the time of analysis. For a few experiments, development was slowed dow
by incubation at 23.5C°C. MCC154 was added at either 3 hpf or 6 hpf, at doses from 0.
to 64 µM. To inhibit pigment formation, PTU by incubation at 23.5C°C. MCC154 was added at either 3 hpf or 6 hpf, at doses from 0.25
to 64 μ M. To inhibit pigment formation, PTU (1-phenyl 2-thiourea) was added at 10hpf
(bud stage) to a final concentration of 0.003 to 64 μM. To inhibit pigment formation, PTU (1-phenyl 2-thiourea) was added at 10hpf
(bud stage) to a final concentration of 0.003%. Fish used in the swimming behavior
assay were never exposed to PTU during their lifetim

Zebrafish *in situ* hybridization, histology and reticulospinal neurons retrograde

to 64 μ M. To inhibit pigment formation, PTU (1-phenyl 2-thiourea) was added at 10hpf
(bud stage) to a final concentration of 0.003%. Fish used in the swimming behavior
assay were never exposed to PTU during their lifet assay were never exposed to PTU during their lifetimes.
Zebrafish *in situ* **hybridization, histology and reticulospinal neurons retrograde**
labeling
Whole mount *in situs* with digoxygenin-labelled RNA probes and develop **Zebrafish in situ hybridization, histology and reticulospi**
labeling
Whole mount in situs with digoxygenin-labelled RNA pro
BMPurple (Roche) were performed as described previou
pBluescript KS+Krox 20 (Sal I digest; T7 Pol Whole mount in situs with digoxygenin-labelled RNA probes and development with Whole mount *in situs* with digoxygenin-labelled NNA probes and development with
BMPurple (Roche) were performed as described previously ⁶⁴. *krox 20* probe,
pBluescript KS+Krox 20 (Sal I digest; T7 Pol); southpaw prob BMPurple (Roche) were performed as described previously σ . *krox 20* probe,
pBluescript KS+Krox 20 (Sal I digest; T7 Pol); southpaw probe, *pGEMT-Southpa*
1; T7 Pol); *delta C* probe, *pL3-Delta C* (Xba 1; T7 Pol); *u*

pBluescript KS+Krox 20 (Sal I digest; T7 Tol); soddifipaw probe, pCEMT-Southpaw1.4 (Spe
I; T7 Pol); delta C probe, pL3-Delta C (Xba I; T7 Pol); unxc4.1 probe (EcoRV; SP6 Pol);
lefty1 probe; pAD-Gal4Lefty1 (Mlu I; T7 Pol); I; T7 Pol); delta C probe, pL3-Delta C (Xba I; T7 Pol); dixe4.1 probe (Leonv, SP6 Pol);
lefty1 probe; pAD-Gal4Lefty1 (Mlu I; T7 Pol); hoxb1a probe, pBluescript KS+hoxb1a (
T7 Pol) (clone ibd3532).
Adult brains sections lefty1 probe; *pAD*-Gal4Lefty1 (Mlu 1, 17 To1), hoxb1u probe, *pBluescript KS+hoxb1u* (Sal 1,
T7 Pol) (clone ibd3532).
Adult brains sections were prepared as follows: adult zebrafish were euthanized; the
heads were cut of Adult brains sections wer
heads were cut off with r
dissected and stored in 1
in paraffin, sectioned at 7
contrast of the habenula
Retrograde labeling: retio
10,000 MW Tetramethylicord transection posteric heads were cut off with razor blades and fixed in 4% PFA for 24-48 hours. Brains were
dissected and stored in 1XPBS + azide until use. Brains were dehydrated and embedd
in paraffin, sectioned at 7 µM and stained with hemat

dissected and stored in 1XPBS + azide until use. Brains were dehydrated and embedded
in paraffin, sectioned at 7 µM and stained with hematoxylin-eosin which gave better
contrast of the habenulae than the standard Nissl sta in paraffin, sectioned at 7 μ M and stained with hematoxylin-eosin which gave better
contrast of the habenulae than the standard Nissl staining.
Retrograde labeling: reticulospinal neurons were backfilled by applying a contrast of the habenulae than the standard Nissl staining.

Retrograde labeling: reticulospinal neurons were backfilled by applying a 5% solution

10,000 MW Tetramethylrhodamine Dextran (Thermo Fischer) at the level of a **Example International International International Retrograde Iabeling:** reticulospinal neurons were backfilled
10,000 MW Tetramethylrhodamine Dextran (Thermo Fisch
cord transection posterior to the hindbrain in 48hpf live 10,000 MW Tetramethylrhodamine Dextran (Thermo Fischer) at the level of a spinal
cord transection posterior to the hindbrain in 48hpf live embryos. The dye was allowed
to diffuse for 6 hours, after which time the embryos w ord transection posterior to the hindbrain in 48hpf live embryos. The dye was allow
to diffuse for 6 hours, after which time the embryos were fixed, incubated in DAPI ar
processed for whole-mount confocal scanning.
Genotyp cord transferred posterior to the hindbrain in 180, protocome, per line and processed for 6 hours, after which time the embryos were fixed, incubated in DAPI and processed for whole-mount confocal scanning.
 Genotyping

processed for whole-mount confocal scanning.
 Genotyping

The following combinations of genomic PCR primers and restriction digests were used

for genotyping zebrafish adults or embryos: processed for the following combinations of genomic PCR pri
for genotyping zebrafish adults or embryos:
for genotyping zebrafish adults or embryos: (
|
| **Genotyping**
The following combinations of genomic PCR primers and restriction digests were used The following communities of generations of primers and restriction digests were used
for genotyping zebrafish adults or embryos: for genotyping zebrafish adults or embryos:

*bab*²²¹² allele (mutation causes the loss of an Rsal site within the PCR amplified
region)²⁷:
REREAExtFwd: 5'-GTATATGTAGTTCTTGATGTCAGTTGTTATGGG-3'
REREAExtRev: 5'-GTGATTCCGTACCGAAGTTAAAGTTTTGTGC-3'
gir^{rw716} allele region)²⁷:
REREAExtl
REREAExtl
gir^{rw716} alle
cyp26A1G
cyp26A1G
After *in sit*lextracted
extracted REREAExtRev: 5'-GTGATTCCGTACCGAAGTTAAAGTTTTGTGC-3'

gir^{rw716} allele (mutation creates an Xbal site within the PCR amplific

cyp26A1GF1: 5'-CAGGGTTTGAGGGCACGCAATTT-3'

cyp26A1GR1: 5'-GCTGCTTCTTTCATCGCCTAAGC-3'

After *in* gir^{rw716} allele (mutation creates an Xbal site within the PCR amp
cyp26A1GF1: 5'-CAGGGTTTGAGGGCACGCAATTT-3'
cyp26A1GR1: 5'-GCTGCTTCTTTCATCGCCTAAGC-3'
After *in situ* hybridization and color development of embryos, g
extr gir^{w716} allele (mutation creates an XbaI site within the PCR amplified region):
cyp26A1GF1: 5'-CAGGGTTTGAGGGCACGCAATTT-3'
cyp26A1GR1: 5'-GCTGCTTCTTTCATCGCCTAAGC-3'
After *in situ* hybridization and color development of eyp26A1GR1: 5'-GCTGCTTCTTTCATCGCCTAAGC-3'
After *in situ* hybridization and color development of
extracted from the embryos using the following prot
personal communication): 1X Extraction Buffer: 1.5
50 mM KCl, 0.3% Tween After *in situ* hybridization and color development o
extracted from the embryos using the following propersonal communication): 1X Extraction Buffer: 1.
50 mM KCl, 0.3% Tween 20, 0.3% NP-40. Single em
of extraction buffer extracted from the embryos using the following protocol (method from M. Halpe
personal communication): 1X Extraction Buffer: 1.5 mM MgCl₂,10 mM Tris HCl p
50 mM KCl, 0.3% Tween 20, 0.3% NP-40. Single embryos were rinsed personal communication): 1X Extraction Buffer: 1.5 mM MgCl₂,10 mM Tris HCl pH 8.3,
50 mM KCl, 0.3% Tween 20, 0.3% NP-40. Single embryos were rinsed with 1X PBS. 50 µ
of extraction buffer was added to each embryo, heated personal KCl, 0.3% Tween 20, 0.3% NP-40. Single embryos were rinsed with 1X PBS. 50 μ
of extraction buffer was added to each embryo, heated to 98°C to 100°C for 10 minutes
and then incubated for 2.5 hours at 55°C with Pr of extraction buffer was added to each embryo, heated to 98°C to 100°C for 10 minutes
and then incubated for 2.5 hours at 55°C with Proteinase K added to a final
concentration of 2 mg/ml. After heating at 98°C for 10 minu and then incubated for 2.5 hours at 55°C with Proteinase K added to a final
concentration of 2 mg/ml. After heating at 98°C for 10 minutes, the extracts were
centrifuged briefly, and the supernatants were transferred to n concentration of 2 mg/ml. After heating at 98°C for 10 minutes, the extract
centrifuged briefly, and the supernatants were transferred to new tubes and
20°C until use. PCRs were carried out in 50 µl reactions using 1X Pro centrifuged briefly, and the supernatants were transferred to new tubes and stored
20°C until use. PCRs were carried out in 50 µl reactions using 1X Promega GoTaq G
Flexi Buffer, 1 ng/nl of each primer, 2 mM MgCl₂, 200 20°C until use. PCRs were carried out in 50 µl reactions using 1X Promega GoTaq Green
Flexi Buffer, 1 ng/nl of each primer, 2 mM MgCl₂, 200 µM dNTPs, 5 U Taq and 2 to 10 µl
of extract. Genomic PCR reaction conditions we

Flexi Buffer, 1 ng/nl of each primer, 2 mM MgCl₂, 200 µM dNTPs, 5 U Taq and 2 to 10 µl
of extract. Genomic PCR reaction conditions were 92°C, 2 min followed by 44 cycles of
95°C, 60s, 65°C, 30s, 73°C, 60s then 4°C hold. of extract. Genomic PCR reaction conditions were 92°C, 2 min followed by 44 cycles of
95°C, 60s, 65°C, 30s, 73°C, 60s then 4°C hold.
Laterality Behavioral Assay
Assays were done at the CNRS AMATRACE Behavior Core Facility 95°C, 60s, 65°C, 30s, 73°C, 60s then 4°C hold.
 Laterality Behavioral Assay

Assays were done at the CNRS AMATRACE Behavior Core Facility in the Zebrafish

Neurogenetics Lab at Gif-sur-Yvette (France). Zebrafish embryos Laterality Behavioral Assay
Assays were done at the CNRS AMATRACE Be
Neurogenetics Lab at Gif-sur-Yvette (France).
several *bab^{tb210}* heterozygotes (in the AB back
tanks under defined day-night conditions and
into wild-t Laterality Behavioral Assay
Assays were done at the CNRS AMATRACE Behavior Core Facility in the Zebrafish Neurogenetics Lab at Gif-sur-Yvette (France). Zebrafish embryos from outcrosses
several bab^{bb210} heterozygotes (in the AB background) to AB fish were raised in 40
tanks under defined day-night conditions and sorted at ap Several *bab*^{tb210} heterozygotes (in the AB background) to AB fish were raised in 40-lite tanks under defined day-night conditions and sorted at approximately 9 months of a into wild-type $(+)+$ and *bab* heterozygote fis several *bab*²²¹⁰ heterozygotes (in the AB background) to AB fish were raised in 40-liter
tanks under defined day-night conditions and sorted at approximately 9 months of age
into wild-type (+/+) and *bab* heterozygote into wild-type $(+/+)$ and *bab* heterozygote fish. Because we planned to use the adults in
swimming assays, we decided not to genotype the fish by PCR on DNA from fin clips.
This was to avoid any confounding variables due into wild type (+/+) and *bub* heterozygote fish. Because we planned to use the adults in
swimming assays, we decided not to genotype the fish by PCR on DNA from fin clips.
This was to avoid any confounding variables due This was to avoid any confounding variables due to variable fin regeneration and
trauma. Consequently, the genotype of each fish was verified by two ID crosses, scor
for the presence of at least two out of three visible p Trauma. Consequently, the genotype of each fish was verified by two ID crosses, for the presence of at least two out of three visible phenotypes in the progeny (reduction of pectoral fins, outgrowth of the Retinal Pigment trauma. Consequently, the genotype of each fish was verified by two ID crosses, scoring
for the presence of at least two out of three visible phenotypes in the progeny
(reduction of pectoral fins, outgrowth of the Retinal (reduction of pectoral fins, outgrowth of the Retinal Pigmented Epithelium (RP)
jaw defects), which would indicate the presence of homozygous bab^{tb210} mutar
embryos and of heterozygous parents. Diseased and non-thriving (iaw defects), which would indicate the presence of homozygous bab^{tb210} mutant
embryos and of heterozygous parents. Diseased and non-thriving fish were discarded
The sex of each fish used in the laterality assay was dete jaw defects), which would indicate the presence of homozygous *bab*²²²² mutant
embryos and of heterozygous parents. Diseased and non-thriving fish were disc
The sex of each fish used in the laterality assay was determine The sex of each fish used in the laterality assay was determined by visual criteria and in
ambiguous cases, by retrospective dissection of fish. "Donut"-shaped swimming
chambers were used for assays of rotational swimming ambiguous cases, by retrospective dissection of fish. "Donut"-shaped swimming
chambers were used for assays of rotational swimming behavior and were made by
immersing a smaller plastic bucket (15 cm diameter) into the cent chambers were used for assays of rotational swimming behavior and were made by
immersing a smaller plastic bucket (15 cm diameter) into the center of a larger circular
plastic tank that was infrared-transparent [KIS Ecobow immersing a smaller plastic bucket (15 cm diameter) into the center of a larger circul
plastic tank that was infrared-transparent [KIS Ecobowl, 36 cm (bottom diameter) X
cm (top diameter) X 18 cm (height), catalog #008712, plastic tank that was infrared-transparent [KIS Ecobowl, 36 cm (bottom diameter) X 38
cm (top diameter) X 18 cm (height), catalog #008712, polyethylene]. This gave a
"donut"-shaped swimming chamber with an opaque white inn plastic tank that was transpared in the space of the plastic transparent (top diameter) X 18 cm (height), catalog #008712, polyethylene]. This gave a "donut"-shaped swimming chamber with an opaque white inner wall and a se "donut"-shaped swimming chamber with an opaque white inner wall and a semi-
translucent blue outer wall and floor. Both inner and outer walls were partially
reflective. Tanks were filled to a depth of 7.5 cm prior to each reflective. Tanks were filled to a depth of 7.5 cm prior to each assay. Each indivalult was transferred by dip net into a swimming chamber. Videos of fish in one swimming chambers were then recorded simultaneously using Vi adult was transferred by dip net into a swimming chamber. Videos of fish in one to fit
swimming chambers were then recorded simultaneously using Viewpoint Life Sciences
Infrared Cameras and Platforms with Zebralab tracking swimming chambers were then recorded simultaneously using Viewpoint Life Sciences
Infrared Cameras and Platforms with Zebralab tracking software, and the number of
The network of the field of the financies of the number of Infrared Cameras and Platforms with Zebralab tracking software, and the number of
Infrared Cameras and Platforms with Zebralab tracking software, and the number of Infrared Cameras and Platforms with Zebralab tracking software, and the number of

Viewpoint's automated rotation counting software. Program parameters for the rotation counting software were set to: minimum diameter 6.0 cm, return angle 40.0 degrees, scale calibrated to the water surface level. In any c rotation counting software were set to: minimum diameter 6.0 cm, return angle
degrees, scale calibrated to the water surface level. In any cases where infrared ti
was imprecise, the videos were played back, and the number degrees, scale calibrated to the water surface level. In any cases where infrared trackine was imprecise, the videos were played back, and the number of clockwise and counter-
clockwise rotations was scored visually. To av was imprecise, the videos were played back, and the number of clockwise and counter-
clockwise rotations was scored visually. To avoid introducing a bias, if the number of
visually scored replacement videos was different b reversal of swimming direction. The accuracy of the automated rotation counting visually scored replacement videos was different between the WT and heterozygote
samples, we replaced additional rotation scores counted by the automated software
with visually determined rotation scores, so that the total samples, we replaced additional rotation scores counted by the automated software
with visually determined rotation scores, so that the total number of visually scored
assays was the same for both the WT and the heterozygo samples, we replace addition scores, so that the total number of visually scored
assays was the same for both the WT and the heterozygous sets. For visual scoring,
complete rotations were defined as either continuous 320 where the same for both the WT and the heterozygous sets. For visual scoring,
assays was the same for both the WT and the heterozygous sets. For visual scoring,
complete rotations were defined as either continuous 320 - 36 assays complete rotations were defined as either continuous 320 - 360 degrees swims or
circular swims that were briefly interrupted by a pause in swimming or by a brief
reversal of swimming direction. The accuracy of the a circular swims that were briefly interrupted by a pause in swimming or by a brief
reversal of swimming direction. The accuracy of the automated rotation counting
software was cross-checked by comparing the scores obtained reversal of swimming direction. The accuracy of the automated rotation counting
software was cross-checked by comparing the scores obtained by automated and
counts of rotations for twenty individual assays (25 minutes reco software was cross-checked by comparing the scores obtained by automated and v
counts of rotations for twenty individual assays (25 minutes recording periods). E
fish was recorded for twenty-five minutes and the assay was counts of rotations for twenty individual assays (25 minutes recording periods). Each
fish was recorded for twenty-five minutes and the assay was repeated on 3 to 4
successive days. Fish often showed reproducible direction Fish was recorded for twenty-five minutes and the assay was repeated on 3 to 4
successive days. Fish often showed reproducible directional preferences from one day
to the next (Extended Data File Fig. 2c and Supplementary successive days. Fish often showed reproducible directional preferences from o
to the next (Extended Data File Fig. 2c and Supplementary Tables 5, 6). For each
this resulted in 3 to 4 daily laterality indices, which then to the next (Extended Data File Fig. 2c and Supplementary Tables 5, 6). For each fish,
this resulted in 3 to 4 daily laterality indices, which then were averaged to give a mean
laterality index (L.I.) for each fish, which this resulted in 3 to 4 daily laterality indices, which then were averaged to give a mean
laterality index (L.l.) for each fish, which were in turn averaged to get a mean L.l. for tl
WT or heterozygote group. The laterali laterality index (L.I.) for each fish, which were in turn averaged to get a mean L.I. for the
WT or heterozygote group. The laterality index, L.I., is defined as the ratio of the
number of complete CW rotations/swims divid WT or heterozygote group. The laterality index, L.I., is defined as the ratio of the
number of complete CW rotations/swims divided by the sum of CW + CCW rotations.
L.I. < 0.5 indicates a preference for swimming in the co number of complete CW rotations/swims divided by the sum of CW + CCW rotation
L.I. < 0.5 indicates a preference for swimming in the counter-clockwise direction.
Laterality indices were measured for about 40 wild-type fema L.I. $\lt 0.5$ indicates a preference for swimming in the counter-clockwise direction.
Laterality indices were measured for about 40 wild-type females and 40 bab^{b210}
heterozygous females and for a similar number of WT an Laterality indices were measured for about 40 wild-type females and 40 *bab^{tb210}*
heterozygous females and for a similar number of WT and heterozygous males.
laterality indices and rotation scores for all tested fish are Laterality indices were measured for about 40 wild-type females and 40 *bab*⁸²¹⁰
heterozygous females and for a similar number of WT and heterozygous males.
laterality indices and rotation scores for all tested fish are

haterality indices and rotation scores for all tested fish are listed in Supplementary
Tables 5 and 6.
We also calculated the Degree of Lateralization, D.L., which is independent of direction
Thus, a fish which completes 9 Tables 5 and 6.
We also calculated the Degree of Lateralization, D.L., which is independent of dire
Thus, a fish which completes 90 clockwise rotations and 10 counter-clockwise rota
during the assay period has the same D.L Thus 2 and 5.

We also calcula

Thus, a fish whith

during the assay

counter-clockw

were obtained b

population mea

Locomotion par 「(()「)」 しょうしょう Thus, a fish which completes 90 clockwise rotations and 10 counter-clockwise rotations
during the assay period has the same D.L. index (0.9) as a fish that completes 90
counter-clockwise rotations and 10 clockwise. Mean D. during the assay period has the same D.L. index (0.9) as a fish that completes 90
counter-clockwise rotations and 10 clockwise. Mean D.L. indices for the populations
were obtained by calculating a mean D.L. index for each

names are accurated to a following a mean D.L. indices for the populat were obtained by calculating a mean D.L. index for each fish and then obtaining to population mean D.L from those (Supplementary Table 7 and 8).
Locomo were obtained by calculating a mean D.L. index for each fish and then obtaining the
population mean D.L from those (Supplementary Table 7 and 8).
Locomotion parameters during the rotational assays (Extended data figure 3) population mean D.L from those (Supplementary Table 7 and 8).
Locomotion parameters during the rotational assays (Extended data figure 3) were
extracted using the Zebralab tracking software. We plotted the speed and distan population means a letter means (supplementary Table 1 and 2).
Locomotion parameters during the rotational assays (Extended d
extracted using the Zebralab tracking software. We plotted the sp
travelled for all fish tested

extracted using the Zebralab tracking software. We plotted the speed and distance
travelled for all fish tested in the rotational assays except for a few of the males *bal*
Hets due to some minor tracking failure.
Statist travelled for all fish tested in the rotational assays except for a few of the males *bal*
Hets due to some minor tracking failure.
Statistical Analysis: For each population of fish (wild-types or *bab* heterozygotes of
 travelled for all fish tested in the rotational assays except for a few of the males babe
Hets due to some minor tracking failure.
Statistical Analysis: For each population of fish (wild-types or *bab* heterozygotes of t Statistical Analysis: For each population
same sex), the distribution of mean latera
verified using the Shapiro Wilk and D'Ago
tests indicated the variances were similal
test was used to compare the mean later
heterozygote Statistical Analysis: For each population of fish (wild-types or *bub* heterozygotes of the
same sex), the distribution of mean laterality indices followed a normal distribution, as
verified using the Shapiro Wilk and D'Ag verified using the Shapiro Wilk and D'Agostino-Pearson omnibus tests. Since Fisher
tests indicated the variances were similar (homoscedastic), an unpaired, two-tailed t-
test was used to compare the mean laterality indices version in the Variances were similar (homoscedastic), an unpaired, two-tailed that was used to compare the mean laterality indices of the two populations (WT vs heterozygotes of the same sex) and calculate p values. For o test was used to compare the mean laterality indices of the two populations (D.L.,
heterozygotes of the same sex) and calculate p values. For other parameters (D.L., heterozygotes of the same sex) and calculate p values. For other parameters (D.L.,

determined using the Mann-Whitney test.

Mice breeding and generation of mutant embryos

The generation of the *Rere^{+/om}* mouse line was described previously ³⁸ and the line was

maintained on a C57BL/6 genetic backgr Mice breeding and generation of mutant ϵ
The generation of the *Rere^{+/om}* mouse line v
maintained on a C57BL/6 genetic backgrou
signaling, the *RARE-LacZ* reporter mice, ³⁶ i
Retinoic Acid Responsive Element (RARE) ¶ ┌ r c c F ヽ g **Mice breeding and generation of mutant embryos**
The generation of the *Rere^{+/om}* mouse line was described previously ³⁸ and the line was The generation of the *Rere^{-/om}* mouse line was described previously ³⁰ and the line was maintained on a C57BL/6 genetic background. To study the status of retinoic (RA) signaling, the *RARE-LacZ* reporter mice, 36 signaling, the *RARE-LacZ* reporter mice, 36 in which LacZ expression is driven by the
Retinoic Acid Responsive Element (RARE) of the retinoic acid receptor beta gene (*N*
Were crossed to WT C57BL/6 and the resulting em Retinoic Acid Responsive Element (RARE) of the retinoic acid receptor beta gene (RarB),
were crossed to WT C57BL/6 and the resulting embryos were analyzed for β -
galactosidase activity using X-gal (5-bromo-4-chloro-3-i Retinoic Acid Responsive Element (RARE) of the retinoic acid receptor beta gene (Rarb),
were crossed to WT C57BL/6 and the resulting embryos were analyzed for β -
galactosidase activity using X-gal (5-bromo-4-chloro-3-i

were crossed to WT C57BL/6 and the resulting embryos were analyzed for δ
galactosidase activity using X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyra
Embryos were stages in embryonic days (E).
Mouse embryo and brain who galactosidase activity using X-gal (5-bromo-4-chloro-5-indolyl-b-D-galactopyramoside).
Embryos were stages in embryonic days (E).
Mouse embryo and brain whole-mount *in situ* hybridization
In situ hybridizations were pe Mouse embryo and brain whole-mount in s

In situ hybridizations were performed as des

the literature: $Uncx4.1^{66}$, $Lfng^{67}$ and $Hes7^{68}$

length cDNA encoding mouse $Lmo4$ was clor

Real-Time PCR Assays using NIH3T3 cel *In situ* hybridizations were performed as described ⁶⁵. The pro
the literature: *Uncx4.1* ⁶⁶, *Lfng* ⁶⁷ and *Hes7* ⁶⁸. To generate the
length cDNA encoding mouse *Lmo4* was cloned into pCMV-Ta
Real-Time PCR Assa

Real-Time PCR Assays using NIH3T3 cells and dissected neocortical primordium/commissural plate from mouse embryo brain

In situ hybridizations were performed as described ∞ . The probes used are described in
the literature: Uncx4.1 ⁶⁶, Lfng ⁶⁷ and Hes 7⁶⁸. To generate the Lmo4 probe, the full-
length cDNA encoding mouse Lmo4 was c length cDNA encoding mouse *Lmo4* was cloned into pCMV-Tag2.
 Real-Time PCR Assays using NIH3T3 cells and dissected neocortical
 primordium/commissural plate from mouse embryo brain

Total RNA was isolated from NIH3T3 $\begin{array}{c} \infty, \textit{L} \textit{f} \textit{n} \textit{g} \ \end{array}$
 $\begin{array}{c} \textit{using} \ \textit{N} \textit{l} \ \textit{and} \ \textit{p} \textit{l} \ \textit{a} \textit{th} \ \end{array}$
 $\begin{array}{c} \textit{In} \ \textit{in} \ \textit{in} \ \textit{in} \ \end{array}$
 $\begin{array}{c} \textit{In} \ \textit{in} \ \textit{in} \ \end{array}$ % and Hes7
mo4 was clc.
H3T3 cells a
e from mou
H3T3 cells u
rains at E10.
e commissul
A extraction ⁸⁶. To generate the *Lmo4* probe, the full-

98. To generate the *Lmo4* probe, the full-
 **nd dissected neocortical

se embryo brain

sing the FastLane Cell One-Step Buffer S

5 were dissected in cold PBS. Only the

1. T** length cDNA encoding mouse *LMO*+ was cloned into pCNV-Tag2.
 Real-Time PCR Assays using NIH3T3 cells and dissected neocortioning and property of the property of the Total RNA was isolated from NIH3T3 cells using the Fast Total RNA was isolated from NIH3T3 cells using the FastLane Cell One-Step Buffer Set (Qiagen). The mouse embryo brains at E10.5 were dissected in cold PBS. Only the
neocortical primordium and the commissural plate were dissected and stored in RNA
Later (Qiagen) at -20°C until RNA extraction. The total RNA (Ringer, Primordium and the commissural plate were dissected and stored in R

Later (Qiagen) at -20°C until RNA extraction. The total RNA was purified using Qiaz

the RNeasy Mini Kit (Qiagen). All the qPCR experiments were Later (Qiagen) at -20°C until RNA extraction. The total RNA was purified using Qiazol a

the RNeasy Mini Kit (Qiagen). All the qPCR experiments were performed using

Quantifast SYBR Green RT-PCR Kit (Qiagen) in a LightCycl

For Faf8 expression analysis from dissected mouse brains the following primer pair was

Chromatin Immunoprecipitation (ChIP) Assay

Fgf8

Rev: CCTTGCGGGTAAAGGCCAT

noprecipitation (ChIP) Assay

h mouse embryonic tissue or brain cortices, E8.75-9.0 embryos or

were harvested from transgenic RARE-LacZ mice and 20 µg of

sed per immunoprecipitation. ChIP Rev: CONSTRUCT CONTROLLED THE STAR
RARE-LacZ mice and 20 μg of
RARE-LacZ mice and 20 μg of
NP was performed as described For ChIP assay with mouse embryonic tissue or
E12.5-13.5 brains were harvested from transge
chromatin were used per immunoprecipitation
Antibodies used for ChIP are as follows: E12.5-13.5 brains were harvested from transgenic RARE-LacZ mice and 20 µg of
chromatin were used per immunoprecipitation. ChIP was performed as described ⁷.
Antibodies used for ChIP are as follows: E12.5-13.5 brains were harvested from transgenic $RARE$ -Lacz mice and 20 μ g of
chromatin were used per immunoprecipitation. ChIP was performed as describe
Antibodies used for ChIP are as follows: chromatin were used per immunoprecipitation. ChIP was performed as described '
Antibodies used for ChIP are as follows:
The Washington of the Washington of Antibodies used for ChIP are as follows:

RARE-LacZ
Rev: TGCTGCACGCGGAAGA
ng and Grasping (MoRaG) behavior test
t consists of an assessment of reaching and grasping motor behavior
ecific fine-tuned motor abilities are altered in neurological diseases
disease. In t MARK AND THE THE TEST OF THE TEST OF THE REVIEW OF THE 【 ̄ r a c c c Mouse Reaching and Grasping (MoRaG) behavior test
The MoRaG test consists of an assessment of reaching and grasping motor behavior in mice. These specific fine-tuned motor abilities are altered in neurological diseases such
as Parkinson's disease. In this test, mice submitted to food restriction are forced to use
single paw instead of their mouth in ord as Parkinson's disease. In this test, mice submitted to food restriction are forced to use
single paw instead of their mouth in order to get a small food pellet. Mice are tested in
the Collin's apparatus ⁶⁹, which consi single paw instead of their mouth in order to get a small food pellet. Mice are tested in
the Collin's apparatus ⁶⁹, which consists of a Plexiglas chamber with dimensions of ~10
cm high, by 6 cm deep and by 6 cm wide. O the Collin's apparatus ⁶⁹, which consists of a Plexiglas chamber with dimensions of ~10
cm high, by 6 cm deep and by 6 cm wide. On the outside of the front wall of each
cubicle, a Plexiglas feeding platform, accessible the Collin's apparatus ⁵⁹, which consists of a Plexiglas chamber with dimensions of ~10 cm high, by 6 cm deep and by 6 cm wide. On the outside of the front wall of each cubicle, a Plexiglas feeding platform, accessible cubicle, a Plexiglas feeding platform, accessible only through a $^{\sim}10$ mm diameter
opening, is attached $^{\sim}5$ cm from the floor. A total of 24 wild-type (12 females and
males) and 24 *Rere^{+/om}* (12 females and 12 m opening, is attached ~5 cm from the floor. A total of 24 wild-type (12 females and
males) and 24 *Rere^{+/om}* (12 females and 12 males) were used for the MoRaG test t
identify the preferred forelimb used to retrieve the f males) and 24 *Rere^{+/om}* (12 females and 12 males) were used for the MoRaG test to identify the preferred forelimb used to retrieve the food pellet. Mice are food-deprive overnight in order to increase their motivation. males) and 24 *Rere^{+/om}* (12 females and 12 males) were used for the MoRaG test to
identify the preferred forelimb used to retrieve the food pellet. Mice are food-depri
overnight in order to increase their motivation. On overnight in order to increase their motivation. On the day of the test, the mouse is
placed in the testing box and allowed to acclimate for 5 minutes before the task begins.
A small food pellet is placed on the feeding pl placed in the testing box and allowed to acclimate for 5 minutes before the task beg
A small food pellet is placed on the feeding platform. It can be accessed through the
opening but the size of the opening is not large en placed in the feeding platform. It can be accessed through the
opening but the size of the opening is not large enough to allow the mouse to collect it
by using its mouth. The MoRaG performance scale is designed to record A spening but the size of the opening is not large enough to allow the mouse to collect
by using its mouth. The MoRaG performance scale is designed to record the upper-
forelimb movement for reaching (the forepaw proceeds opening the mouth. The MoRaG performance scale is designed to record the upper-
forelimb movement for reaching (the forepaw proceeds in the horizontal plane out of
the trunk to approach an object at a distance) and graspin $2010/63/UE86/609/CEE$ and was approved by the local animal care, use and ethics the trunk to approach an object at a distance) and grasping (the object is grasped). The
experimental procedure comprises 3 testing sessions. On the first session it was done
for 30 trials and on the next 2 sessions 50 tri the trunk to approach an original procedure comprises 3 testing sessions. On the first session it was done
for 30 trials and on the next 2 sessions 50 trials each were done for a total of 130 trials,
and accuracy (percenta experience and on the next 2 sessions 50 trials each were done for a total of 130 trials
and accuracy (percentage of success) of reaching and grasping was recorded. The mot
lateralization was also evaluated to analyse the and accuracy (percentage of success) of reaching and grasping was recorded. The motol
lateralization was also evaluated to analyse the paw of preference to reach the food
pellet. The behavioral pipeline was performed in ag ateralization was also evaluated to analyse the paw of preference to reach the food
pellet. The behavioral pipeline was performed in agreement with the EC directive
2010/63/UE86/609/CEE and was approved by the local animal pellet. The behavioral pipeline was performed in agreement with the EC directive
2010/63/UE86/609/CEE and was approved by the local animal care, use and ethics
committee of the IGBMC (Com'Eth) under accreditation number (2 pellet. The behavior of the behavior and the behavior of 2010/63/UE86/609/CEE and was approved by the local animal care, use and ethic
committee of the IGBMC (Com'Eth) under accreditation number (2012-139).
Magnetic Resona committee of the IGBMC (Com'Eth) under accreditation number (2012-139).
Magnetic Resonance Imaging (MRI)

committee of the IGBMC (Comeditation accreditation number (2012-139).
Magnetic Resonance Imaging (MRI) Magnetic Resonance Imaging (MRI)

1. MRI data acquisition
A total of 22 wild-type and 22 *Rere^{+/om}* mice were imaged after performing the MoRaG A total of 22 wild-type and 22 *Rere^{-/om}* mice were imaged after performing the MoRaG
test. All images were acquired using a Bruker Biospec 117/16 USR MRI system (BGA-9S
gradients, 750 mT/m, AVIII) running Paravision 5. gradients, 750 mT/m, AVIII) running Paravision 5.1. A 72 mm resonator was used for
signal emission and a planar surface coil for mouse heads was used for signal reception
(Bruker Biospin, Ettlingen, Germany). Structural T signal emission and a planar surface coil for mouse heads was used for signal receptio
(Bruker Biospin, Ettlingen, Germany). Structural T2-weighted images were acquired for
all mice. A two-dimensional turbo-RARE (Rapid Ac Signal emission, Ettlingen, Germany). Structural T2-weighted images were acquired for
all mice. A two-dimensional turbo-RARE (Rapid Acquisition with Relaxation
Enhancement) sequence was used with TR = 6500 ms; TE = 40 ms; (Bruker Biospin and the FARE (Rapid Acquisition with Relaxation

Enhancement) sequence was used with TR = 6500 ms; TE = 40 ms; matrix = 384 x 384;

field-of-view (FOV) = 23 x 23 mm² (60 x 60 µm² in-plane resolution); Enhancement) sequence was used with TR = 6500 ms; TE = 40 ms; matrix = field-of-view (FOV) = 23 x 23 mm² (60 x 60 µm² in-plane resolution); 72 slice
thickness = 0.22 mm; number of averages = 4; scan time = 2h 8min. Th Field-of-view (FOV) = 23 x 23 mm² (60 x 60 μ m² in-plane resolution); 72 slices; slice
thickness = 0.22 mm; number of averages = 4; scan time = 2h 8min. The animals were
anesthetized using lsoflurane at 1.5-2% mixed field-of-view (FOV) = 23 x 23 mm²
thickness = 0.22 mm; number of a
anesthetized using lsoflurane at 1
mouse cradle and their heads wer
circulating system integrated to th
physiological temperature. Their r
under their ab (60 x 60 μ m²
(verages = 4; s
.5-2% mixed i
re held fixed v
ne cradle ensu
espiration wal parameter: inded Time = 2h 8min. The animals we
in oxygen:air (1:5). They were placed
with ear bars and a tooth bar. A wate
ured that the animals were kept at
as monitored via a pressure pad place
s were monitored throughout the M anesthetized using Isoflurane at 1.5-2% mixed in oxygen:air (1:5). They were placed in
mouse cradle and their heads were held fixed with ear bars and a tooth bar. A water-
circulating system integrated to the cradle ensure mouse cradle and their heads were held fixed with ear bars and a tooth bar. A water-
circulating system integrated to the cradle ensured that the animals were kept at
physiological temperature. Their respiration was monito chromological temperature. Their respiration was monitored via a pressure pad plant under their abdomen. Physiological parameters were monitored throughout the Mexicons.
2. MRI data analysis
2.1. *Voxel-based morphometry a*

2. MRI data analysis
2.1. Voxel-based morphometry and preprocessing

We first multiplied the voxel size by a factor of 17 in order to have brain dimensions physiological temperature. They have the included throughout the MRI
under their abdomen. Physiological parameters were monitored throughout the MRI
sessions.
2. 1. *Voxel-based morphometry and preprocessing*
We first mult under their abdoments weyers given parameters were monitored to the MRI data analysis
2.1. *Voxel-based morphometry and preprocessing*
We first multiplied the voxel size by a factor of 17 in order to have brain dimensions
 2. MRI da
2.*1. Voxe.*
We first n
similar to
Mapping
<u>http://ww</u>
nerforme 14.4 // so 【上下s 2.1. *Voxer bused morphometry and preprocessing*
We first multiplied the voxel size by a factor of 17
similar to human brains to match the default mod
Mapping software (SPM8, Wellcome Trust Centre
http://www.fil.ion.ucl.ac similar to human brains to match the default mode parameter of Statistical Paramet
Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, UK,
http://www.fil.ion.ucl.ac.uk/spm). Image segmentation and normalizatio Mapping software (SPM8, Wellcome Trust Centre for Neuroimaging, UK, $\frac{http://www.fili.ion.ucl.ac.uk/spm)}{http://www.fili.ion.ucl.ac.uk/spm)}$. Image segmentation and normalization was performed in two steps using first the template given by SPMMouse and then a http://www.fil.ion.ucl.ac.uk/spm). Image segmentation and normalizatio
performed in two steps using first the template given by SPMMouse and
symmetrical template. First, SPM8 was used to segment into gray matter
white mat performed in two steps using first the template given by SPMMouse and then a
symmetrical template. First, SPM8 was used to segment into gray matter (GM)
white matter (WM) probability maps and to normalize the structural i performed in template. First, SPM8 was used to segment into gray matter (GM) a
white matter (WM) probability maps and to normalize the structural images wit
segment function and the template given by SPMMouse ⁷⁰. Dartel symmetrical template. The resulting pormalize the structural images with the segment function and the template given by SPMMouse 70 . Dartel normalization was performed using the GM and WM probability maps. Dartel norm segment function and the template given by SPMMouse ⁷⁰. Dartel normalization was
performed using the GM and WM probability maps. Dartel normalization was
performed on all structural and swapped images (to invert left and segment function and the template given by SPMMouse $^{\prime\prime}$. Dartel normalization was
performed using the GM and WM probability maps. Dartel normalization was
performed on all structural and swapped images (to invert left performed on all structural and swapped images (to invert left and right sides)
were then averaged. This provided a symmetrical template. Second, we perfor
again the segmentation and Dartel normalization on native images u performed and averaged. This provided a symmetrical template. Second, we performed
again the segmentation and Dartel normalization on native images using the
symmetrical template. The resulting normalized modulated GM imag again the segmentation and Dartel normalization on native images using the
symmetrical template. The resulting normalized modulated GM images were then
smoothed with an 8 mm kernel before performing voxel-based morphometry

again the segmentation and substract in an interaction comparison symmetrical template. The resulting normalized modulated GM images were
smoothed with an 8 mm kernel before performing voxel-based morphometry
analysis.
2.2 smoothed with an 8 mm kernel before performing voxel-based morphometry (VBN analysis.
2.2. Statistical analysis
The left and right hemispheres were considered for comparisons. Statistical analys
were conducted to assess w analysis.
2.2. Statistical analysis
The left and right hemispheres were considered for comparisons. Statistical analyses
were conducted to assess within-group differences in GM between the left and the ri
hemispheres by co 2.2. Stati.
The left a
were con
hemisphe
conducte
Both Pair
sample t-considere 2.2. Statistical analysis
The left and right hemis
were conducted to asse
hemispheres by compa
conducted to compare
Both Paired two-sample
sample t-test for betwe
considered significant a
mm. i.e. 3 voxels radius were conducted to assess within-group differences in GM between the left and the ri
hemispheres by comparing native and swapped images. Statistical analyses were also
conducted to compare wild type and *Rere^{+/om}* volume hemispheres by comparing native and swapped images. Statistical analyses were also
conducted to compare wild type and *Rere^{+/om}* volumes for between-group comparisons.
Both Paired two-sample *t*-test for within-group st conducted to compare wild type and *Rere^{+/om}* volumes for between-group comparisor
Both Paired two-sample *t*-test for within-group statistical analyses and Unpaired two-
sample *t*-test for between-group statistical an conducted to compare wild type and *Rere⁻⁷⁵¹¹* volumes for between-group comparisons.
Both Paired two-sample *t*-test for within-group statistical analyses and Unpaired two-
sample *t*-test for between-group statistical Both Faired two-sample t-test for within-group statistical analyses and Onpaired two-
sample t-test for between-group statistical analyses were run in SPM8. Clusters were
considered significant at $P < 0.05$ with small vol sample t-test for between-group statistical analyses were run in SPM8. Clusters were
considered significant at $P < 0.05$ with small volume correction (SVC, sphere radius = 3
mm, i.e. 3 voxels radius) with an uncorrected h considered significant at $P \le 0.55$ with small volume correction (SVC, sphere radius = 3
mm, i.e. 3 voxels radius) with an uncorrected height threshold of $P < 0.00001$ for within mm, i.e. 3 voxels radius) with an uncorrected height threshold of P < 0.00001 for within-

group comparisons and P < 0.001 for between-group comparisons, and an extent
threshold of 500 voxels.
2.3. 3D brain projections
For 3D brain projections (below background surface - max intensity) to compare v
type versus 2.*3. 3D brain projections*
For 3D brain projections
type versus *Rere^{+/om}* and
volume rendering was fr
Resources Clearinghouse
Single photon emission こと トート くらい 2.3. 3D brain projections
For 3D brain projections
type versus *Rere^{+/om}* and
volume rendering was fro
Resources Clearinghouse
Single photon emission of
1. SPECT/CT data acquisi
A total of 9 wild-type and For k branch and Left versus Right asymmetric differences, the software used for volume rendering was from MRIcron image viewer (Neuroimaging Informatics Tools and Resources Clearinghouse, NITRC, http://www.nitrc.org/pr

\overline{a} Single photon emission computed tomography/Computed tomography (SPECT/CT)

Resources Clearinghouse, NITRC, http://www.nitrc.org/projects/mricron, 2013 release).

Single photon emission computed tomography/Computed tomography (SPECT/CT)

1. SPECT/CT data acquisition

A total of 9 wild-type and 9 R Single photon emission computed tomography/Computed tomography (SPECT/CT)
1. SPECT/CT data acquisition
A total of 9 wild-type and 9 $Rere^{+/om}$ mice were imaged to detect regional cerebral blood
flow. In order to recognize t 1. SPECT/CT data acquisition A total of 9 wild-type and 9 *Rere^{-yom}* mice were imaged to detect regional cerebral blood
flow. In order to recognize the different organs, prior to SPECT analysis, a CT image was
recorded on the AMISSA platform, a hom recorded on the AMISSA platform, a homemade multimodality imaging system for small
animals combining X-ray, SPECT and PET devices. The µCT delivered a 3D reconstructed
volume of the animal in real time ^{71,72}. Targeting animals combining X-ray, SPECT and PET devices. The µCT delivered a 3D reconstructed
volume of the animal in real time 71,72 . Targeting of 99mTc-HMPAO was monitored via
the µSPECT imaging technique. Briefly Technetium volume of the animal in real time ^{71,72}. Targeting of 99mTc-HMPAO was monitored via
the µSPECT imaging technique. Briefly Technetium-99m, as $[{}^{99m}TcO_4]$ Na in physiological
solution, was obtained from a ${}^{99}Mo/{}^{99$ solution, was obtained from a ⁹⁹Mo/^{99m}Tc generator Elumatic® III (CisBio/IBA Molecular,
France). The [^{99m}Tc]-HMPAO was prepared using the commercial kit Cerestab (GE
Healthcare, Vélizy, France) following provider's France). The $[{}^{99m}Tc]$ -HMPAO was prepared using the commercial kit Cerestab (GE
Healthcare, Vélizy, France) following provider's recommendations for preparation and
quality control. Mice were anesthetized by intraperit France). The $[3^{5m}Tc]$ -HMPAO was prepared using the commercial kit Cerestab (GE
Healthcare, Vélizy, France) following provider's recommendations for preparation
quality control. Mice were anesthetized by intraperitoneal Healthcare, Vélizy, France) following provider's recommendations for preparation and quality control. Mice were anesthetized by intraperitoneal injection of 10 $\mu I. g^{-1}$ of a solution made with ketamine hydrochloride 10% quality control. Mice were anesthetized by intraperitoneal injection of 10 μ l.g⁻ of a
solution made with ketamine hydrochloride 10% (Imalgene, Centravet, Velaine en H
France) and Xylazine 5% (Rompun, Centravet, Velai France) and Xylazine 5% (Rompun, Centravet, Velaine en Haye, France) and placed in
prone position in the animal holder after injection in the tail vein. μ SPECT imaging was
performed 4 minutes post-intravenous injection prone position in the animal holder after injection in the tail vein. μ SPECT imaging wa
performed 4 minutes post-intravenous injection of 30 MBq (in mean) of 99mTc-HMPA
injected in the tail vein. The μ SPECT system c performed 4 minutes post-intravenous injection of 30 MBq (in mean) of 99mTc-HMPAC
injected in the tail vein. The μ SPECT system consists of a four heads detection gamma
camera. Each head comprises five-separated detecti performance in the tail vein. The μ SPECT system consists of a four heads detection gamma
camera. Each head comprises five-separated detection modules arranged along a circle
of 58 mm with the pinhole as the center. A d camera. Each head comprises five-separated detection modules arranged along a circlum of 58 mm with the pinhole as the center. A detection module consists of a YAP:Ce mat of 8 x 8 scintillating crystals 2.3 x 2.3 x 28 mm of 58 mm with the pinhole as the center. A detection module consists of a YAP:Ce matri
of 8 x 8 scintillating crystals 2.3 x 2.3 x 28 mm³ each coupled to a multi-anode
photomultiplier 8 x 8 (Hamamatsu H 8804). The dista of 8 x 8 scintillating crystals 2.3 x 2.3 x 28 mm³ each coupled to a multi-anode
photomultiplier 8 x 8 (Hamamatsu H 8804). The distance from the pinhole to the axis of
rotation is 28 mm and the distance between the pinh of 8 x 8 scintillating crystals 2.3 x 2.3 x 28 mm³
photomultiplier 8 x 8 (Hamamatsu H 8804). Th
rotation is 28 mm and the distance between th
results in a magnification factor of 2.07. Image
(Ordered Subset Expectation M e distance from the pinhole to t
ne pinhole and the crystal is 58 r
s were reconstructed using the
rative algorithm adapted for pin
sing the Anatomist freeware
p the four following stens: (i) bra photomultiplier and the distance between the pinhole and the crystal is 58 mm, which
results in a magnification factor of 2.07. Images were reconstructed using the OSEM
(Ordered Subset Expectation Maximisation) iterative a results in a magnification factor of 2.07. Images were reconstructed using the OSEM
(Ordered Subset Expectation Maximisation) iterative algorithm adapted for pinhole
imaging. Images were viewed and quantified using the Ana

(Ordered Subset Expectation Maximisation) iterative algorithm adapted for pinhole
imaging. Images were viewed and quantified using the Anatomist freeware
(http://brainvisa.info/index f.html).
2. SPECT image analysis
SPEC imaging. Images were viewed and quantified using the Anatomist freeware

(http://brainvisa.info/index f.html).
 2. SPECT image analysis

SPECT image analysis is conducted according to the four following steps: (i) brain
 International Chinagonal Chinagonal Chinagonal Chinagonal Chinagonal Chinage analysis

SPECT image analysis is conducted according to the four following steps: (i)

extraction, (ii) asymmetry map calculation, (iii) registr 2. SPECT image analysis
SPECT image analysis is conducted acextraction, (ii) asymmetry map calcul
voxel-based statistical analysis. The f
software (https://piiv.u-strasbg.fr/tranalysis is conducted using SPM (http
2.1. Bra **2. SPECT image analysis**
SPECT image analysis is conducted according to the four following steps: (i) brain extraction, (ii) asymmetry map calculation, (iii) registration in a common space an
voxel-based statistical analysis. The first three steps are performed using medipy
software (https://piiv.u-strasbg.fr/traitement-images/m voxel-based statistical analysis. The first three steps are performed using medipy
software (https://piiv.u-strasbg.fr/traitement-images/medipy/). Voxel-based statistical
analysis is conducted using SPM (http://www.fil.ion software (<u>https://piiv.u-strasbg.fr/traitement-images/medipy/</u>). Voxel-based stat
analysis is conducted using SPM (<u>http://www.fil.ion.ucl.ac.uk/spm/</u>).
2.1. Brain extraction software (https://pii.u-strasbe.jp/traitement-images/medipy/). Voltation analysis is conducted using SPM (http://www.fil.ion.ucl.ac.uk/spm/).
2.1. Brain extraction

analysis is conducted using SPM (<u>http://www.fil.ion.ucl.ac.uk/spm/)</u>.
2.1. Brain extraction $\frac{1}{2}$ 2.1. Brain extraction

this end, we use the anatomical information carried out by the CT image, which is in the same physical space as the SPECT image. The skull is segmented from the CT image by using an intensity thresholding technique (Otsu's same physical space as the SPECT image. The skull is segmented from the CT image by
using an intensity thresholding technique (Otsu's method) and by affinely registering a
probabilistic prior to remove bones that do not be using an intensity thresholding technique (Otsu's method) and by affinely registering a
probabilistic prior to remove bones that do not belong to the skull. Then, a mask of the
brain is built from the skull using morpholog probabilistic prior to remove bones that do not belong to the skull. Then, a mask of the
brain is built from the skull using morphological operators dedicated to cavity filling.
This brain mask is then applied on the SPECT

presenting probability the remove and the strong of the studies of the shain is built from the skull using morphological operators dedicated to cavity filling.
This brain mask is then applied on the SPECT image to extract This brain mask is then applied on the SPECT image to extract the brain perfusion ma
2.2. Asymmetry map calculation
The inter-hemispheric symmetry plane is then estimated from each brain perfusion r
by minimizing the squar 2.2. Asymmetry map calculation
The inter-hemispheric symmetry plane is then estimated from each brain perfusion map
by minimizing the squared intensity differences between the original and the swapped
images. Then, an asym - 1
- 1
- 1
- 1
- 1 2.2. Asymmetry map calculation
The inter-hemispheric symmetry plane is then estimated from each brain perfusion map The inter-hemispheric symmetry differences between the original and the swapped
images. Then, an asymmetry map is computed as the difference between the original
and swapped images divided by the sum of the original and sw by minimizing the squared intensity in accomputed as the difference between the original
and swapped images divided by the sum of the original and swapped images. By this
way, the values of the asymmetry map range from -1

images. Then, an asymmetry map is computed as the original and swapped images. By this
way, the values of the asymmetry map range from -1 to 1 and are made independent
from the perfusion amplitude.
2.3. Registration in a c way, the values of the asymmetry map range from -1 to 1 and are made independent
from the perfusion amplitude.
2.3. Registration in a common space
To conduct voxelwise analysis of these asymmetry maps across a population, From the perfusion amplitude.

2.3. Registration in a common space

To conduct voxelwise analysis of these asymmetry maps across a population, they

should be registered in a common space. This is done by affinely registe 2.3. *Registration in a common*:
To conduct voxelwise analysis of
should be registered in a comm
image on a study specific temp
corresponding asymmetry map
and *Rere^{+/om}* images. This is doi
chosen image. Then, an averag 2.3. Registration in a common space
To conduct voxelwise analysis of these asymmetry maps across a population, they should be registered in a common space. This is done by affinely registering each S
image on a study specific template, and by applying the estimated transformation
corresponding asymmetry map. The study specific template image on a study specific template, and by applying the estimated transformation to the corresponding asymmetry map. The study specific template is built from both wild-type and *Rere^{+/om}* images. This is done by affine corresponding asymmetry map. The study specific template is built from both wild-type
and *Rere^{+/om}* images. This is done by affinely registering all SPECT images on an arbitrary
chosen image. Then, an average image is and *Rere^{t/om}* images. This is done by affinely registering all SPECT images on an arbitrary
chosen image. Then, an average image is computed from all registered images. Then, all
images are registered again on the avera and *Rere^{-/om}* images. This is done by attinely registering all SPECT images on an arbitrary
chosen image. Then, an average image is computed from all registered images. Then, all
images are registered again on the aver

chosen image. Then, an average image is computed in an average image is built. This
is done until the process converges to obtain the final template.
2.4. Voxel-based statistical analysis
All registered asymmetry maps are is done until the process converges to obtain the final template.

2.4. Voxel-based statistical analysis

All registered asymmetry maps are smoothed using a Gaussian kernel (FWHM: 1 mm)

and a One-sample t-test is conduct 2.4. *Voxel-based statistical analysis*
All registered asymmetry maps are smoothed using a Gaussian k
and a One-sample *t*-test is conducted voxelwise to find out the a
perfusion is asymmetric within each group. Statistica ミノミ ドリート All registered asymmetry maps are smoothed using a Gaussian kernel (FWHM: 1 mm) All registers are purely intered as the same of the duties and a One-sample t-test is conducted voxelwise to find out the areas where the brain perfusion is asymmetric within each group. Statistical maps are thresholded a

Histology and quantification of neuronal populations in mouse brain sections
P7 mouse brains were perfused with 4% paraformaldehyde, dehydrated in ethanol and

and a One-sample t-test is conducted voxelwise to find out the areas where the brain
perfusion is asymmetric within each group. Statistical maps are thresholded at $P < 0.0$
(uncorrected P-value).
Histology and quantificat perfusion is asymmetric within each group. Statistical maps are thresholded at $P \le 0.01$
(uncorrected P-value).
P7 mouse brains were perfused with 4% paraformaldehyde, dehydrated in ethanol and
embedded in paraffin prior Histology and quantifierty
P7 mouse brains were
embedded in paraffin p
were obtained and sub
and anti-CTIP2 antibod
animals identified by M
analysis software Fiji⁷³ embedded in paraffin prior to sectioning. 7 µm transverse sections of the entire brains
were obtained and subsequently processed for immunohistochemistry with anti-Tbr1
and anti-CTIP2 antibodies. The asymmetric region of embedded in paraffin prior to sectioning. 7 μ m transverse sections of the entire brains
were obtained and subsequently processed for immunohistochemistry with anti-Tbr1
and anti-CTIP2 antibodies. The asymmetric region and anti-CTIP2 antibodies. The asymmetric region of the sensorimotor cortex of dextra
animals identified by MRI was manually drawn on one hemisphere using the image
analysis software Fiji 73 . The symmetric region was animals identified by MRI was manually drawn on one hemisphere using the image
analysis software Fiji⁷³. The symmetric region was then drawn on the other hemisphere
and both regions were cropped and analyzed independent analysis software Fiji⁷³. The symmetric region was then drawn on the other hemisp
and both regions were cropped and analyzed independently. Nuclei were automatic
segmented on each channel using the image analysis softwa analysis software Fiji¹³. The symmetric region was then drawn on the other hemisphere
and both regions were cropped and analyzed independently. Nuclei were automatically
segmented on each channel using the image analysis segmented on each channel using the image analysis software CellProfiler⁷⁴. In short,
the intensity of each channel was normalized and the segmentation threshold to
distinguish positive and negative nuclei was manually s segmented on each channel using the image analysis software CellProfiler ⁷. In short,
the intensity of each channel was normalized and the segmentation threshold to
distinguish positive and negative nuclei was manually s distinguish positive and negative nuclei was manually set for each channel but ke
constant for every brain slice. The number of positive nuclei per channel was cou
for each hemisphere. The number of single and double-posit constant for every brain slice. The number of positive nuclei per channel was counte
for each hemisphere. The number of single and double-positive nuclei (CTIP2: Red+,
for each hemisphere. The number of single and double-p for each hemisphere. The number of single and double-positive nuclei (CTIP2: Red+,
for each hemisphere. The number of single and double-positive nuclei (CTIP2: Red+, for each hemisphere. The number of single and double-positive number of single-positive number \mathcal{R}^2

normalized excess between the two hemispheres ((right-left)/left) was then calculated
for all the brain slices. Similarly, the area difference between the two sides was
calculated using the same index. The significance of for all the brain slices. Similarly, the area difference between the two sides was
calculated using the same index. The significance of the difference between the wild
type and *Rere^{om}* heterozygotes excesses was assess calculated using the same index. The significance of the difference between the
type and $Rere^{om}$ heterozygotes excesses was assessed using the Mann-Whitney
we said $Rere^{om}$ heterozygotes excesses was assessed using the Man type and $Rere^{om}$ heterozygotes excesses was assessed using the Mann-Whitney test. type and *Rere^{om}* heterozygotes excesses was assessed using the Mann-Whitney test.

Anno 2012

The Mann-Whitney test.

Exterioed data Figure 1: Inhibition of NA signaling disrupts bilateral symmetry of the
hindbrain.
(a-d) 8-10 somite zebrafish embryos hybridized *in situ* with a *krox20* probe labeling r
and r5. a. 8-10s control embryo tr (a-d) 8-10
and r5. a. i
with 10 µM
bars: 100 µ
(e-f) 18-19
and r5. (e) (a a) 8 10 somice zebrarish embryos hybridized in situ with a krox20 probe labeling rs
and r5. a. 8-10s control embryo treated with 1% DMSO. b-d. 8-10s embryos treated
with 10 µM BMS showing different types of abnormal *kr*

with 10 µM BMS showing different types of abnormal *krox20* expression patterns. Sca
bars: 100 µm
(e-f) 18-19 somite zebrafish embryos hybridized *in situ* with a *krox20* probe labeling r.
and r5. (e) Control embryo treat with 10 μM BMS showing different types of abnormal krox20 expression patterns. Scale
bars: 100 μm
(e-f) 18-19 somite zebrafish embryos hybridized *in situ* with a *krox20* probe labeling r3
and r5. (e) Control embryo trea bars: 100 μm
(e-f) 18-19 so
and r5. (e) Co
BMS showing
Dorsal views,)
3
|} (e-f) 18-19 somite zebrafish embryos hybridized *in situ* with a krox20 probe labeling rs
and r5. (e) Control embryo treated with 1% DMSO. (f) Embryo treated with 10 μ M
BMS showing an asymmetric *krox20* expression pat BMS showing an asymmetric *krox20* expression pattern. Scale bars: 100 μ m
Dorsal views, anterior to the top.
 BMS showing an asymmetric *krox20* expression pattern. Scale bars: 100 μ m
Dorsal views, anterior to the top.
 μ

 \overline{a} Dorsal views, anterior to the top.

A. Circular Swim Tank with Infrared Transparent Bottom

B. Overhead screen shot of 5 tank automated system with infrared illumination and auto-tracking

C. Examples of robust rotational swimming behaviour

Exterior data Figure 2: Behavioral setup for lateralization determination in adult
zebrafish
A. Example of the "donut"-shaped swimming chambers used during the test.

A. Exampl
I
 A. Example of the "donut"-shaped swimming chambers used during the test.

tracking of 5 fish.
C. Examples showing individual adult fish with consistent directional swimming
preferences from day to day over four consecutive days. Het = $bab^{tb210}/+$; WT =
type +/+. Mean L.I. = Laterality Index aver C. Examples show
preferences from
type +/+. Mean L
Clockwise; CCW =
indicate the numk
during a single 25 preferences from day to day over four consecutive days. Het = $bab^{tb210}/+$; WT =
type +/+. Mean L.I. = Laterality Index averaged over all four days of the assay. C
Clockwise; CCW = counterclockwise. Numbers under the column type $+$ /+. Mean L.I. = Laterality Index averaged over all four days of the assay. CW = Clockwise; CCW = counterclockwise. Numbers under the column headings CW and CCW indicate the number of clockwise versus counter-clock Clockwise; CCW = counterclockwise. Numbers under the column headings CW and CCW Considerable with the number of clockwise versus counter-clockwise circles that a given fish swam
during a single 25 minute assay period on a single day.

Survey is that a given fish swam

during a single 25 minute assay p during a single 25 minute assay period on a single day.

The number of counter-clockwise counter-clockwise counter-clockwise counter-clockwise counter-clockwise counter-

The matter of counter-clockwise counter-clockwise c during a single 25 minute assay period on a single day.

Extended data Figure 3: Swimming speed and swimming distance in *bab* heterozygotes $(bab^{tb210}/+)$ and wild type $(+/+)$ adult zebrafish
(a-b) Mean swimming speed during the rotation assays (25 min recording), for the female (a-b) Mean swimming speed during the rotation assays (25 min recording), for the (and the response of the relation assays for the males (b, WT n=40 and bab H
n=40).
(c-d) Mean total distance during the rotation assays for the females (c, WT n=41 a
Het n=40) and for the males (d, WT n=40 and *bab* Het

females (a, WT n= 41 and bab Het n= 40) and for the males (b, WT n=40 and bab Het n=40).
n=40).
(c-d) Mean total distance during the rotation assays for the females (c, WT n=41 and Het n=40) and for the males (d, WT n=40 $(c-d)$ M
Het n=4
P value (c-d) Mean total distance d'armig the rotation assays for the remains (c, WT n=41 and babelet n=40) and for the males (d, WT n=40 and *bab* Het n=40).
P values from Mann-Whitney test.

Het n=40) and for the males (d, WT n=40 and *bab* Het n=40).
P values from Mann-Whitney test. P values from Mann-Whitney test.

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