The molecular, temporal and region-specific requirements of the beta isoform of Calcium/Calmodulin-dependent protein kinase type 2 (CAMK2B) in mouse locomotion

Martijn J. Kool1,2, Jolet E. van de Bree1,2, Hanna E. Bodde1, Ype Elgersma1,2 & Geeske M. van Woerden1,2

Genetic approaches using temporal and brain region-specific restricted gene deletions have provided a wealth of insight in the brain regions and temporal aspects underlying spatial and associative learning. However, for locomotion such extensive studies are still scarce. Previous studies demonstrated that Camk2b−/− mice, which lack the β isoform of Calcium/Calmodulin-dependent protein kinase 2 (CAMK2B), show very severe locomotion deficits. However, where these locomotion deficits originate is unknown. Here we made use of novel Camk2b mutants (Camk2bβ/β and Camk2bT287A), to explore the molecular, temporal and brain region-specific requirements of CAMK2B for locomotion. At the molecular level we found that normal locomotion requires Calcium/Calmodulin mediated activation of CAMK2B, but CAMK2B autonomous activity is largely dispensable. At a systems level, we found that global deletion of Camk2b in the adult mouse causes only mild locomotion deficits, suggesting that the severe locomotion deficits of Camk2b−/− mice are largely of developmental origin. However, early onset deletion of Camk2b in cerebellum, striatum or forebrain did not recapitulate the locomotion deficits, suggesting that these deficits cannot be attributed to a single brain area. Taken together, these results provide the first insights into the molecular, temporal and region-specific role of CAMK2B in locomotion.

The Calcium/Calmodulin-dependent protein kinase II (CaMKII, from hereon called CAMK2) family members are pivotal to normal synaptic plasticity and learning in the hippocampus, cortex and cerebellum. The family consists of four different isoforms, (alpha, beta, gamma and delta) of which CAMK2A is the most studied (for review, see1–3). In the last decade, the role of CAMK2B in the brain has gained attention largely due to the generation of new mutant mice4–7.

In sharp contrast to CAMK2A, CAMK2B has been shown to play an important role in locomotion, since two independent Camk2b mutants both show severe locomotion deficits4,6. Like CAMK2A, CAMK2B is highly expressed in the brain4 and it has been shown that CAMK2B plays an enzymatic as well as a structural role in both hippocampal and cerebellar plasticity4,5. This structural role of CAMK2B comes from an additional domain within CAMK2B, the F-actin binding domain, which enables CAMK2B to cluster CAMK2A to the actin cytoskeleton5,9,10. Indeed, most hippocampal phenotypes observed in Camk2b−/− mutants require CAMK2B protein, but not its enzymatic function, since the Camk2bA303R mutants, in which CAMK2B cannot be activated, do not show an overt hippocampal phenotype5. At the cerebellar parallel fiber–Purkinje cell synapse, CAMK2B appears to

1Department of Neuroscience, Erasmus University Medical Center, 3015 CN, Rotterdam, The Netherlands. 2ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus University Medical Center, 3015 CN, Rotterdam, The Netherlands. Correspondence and requests for materials should be addressed to Y.E. (email: y.elgersma@erasmusmc.nl) or G.M.V.W. (email: g.vanwoerden@erasmusmc.nl)
have both a structural role as an enzymatic role but it is unknown to what extend the severe locomotion deficits seen in Camk2b<sup>−/−</sup> mice, arise from loss of the enzymatic or the structural role of CAMK2B.

Expression of CAMK2B starts around E12.5, whereas expression of CAMK2A starts postnatal, around P1<sup>11</sup>. According to this difference in temporal expression, one might expect that CAMK2B is involved in embryonic development, whereas CAMK2A is not. Indeed, it has been shown that deletion of CAMK2A in an adult brain gives a similar phenotype to germ-line deletion of CAMK2A with respect to learning and synaptic plasticity<sup>12</sup>. Similar studies have not been performed for CAMK2B, but considering the early onset of expression, it is conceivable that germ-line deletion of CAMK2B results in a more severe phenotype compared to deletion in adult animals.

Since CAMK2B has been shown to play an important role in controlling the direction of synaptic plasticity at the parallel fiber–Purkinje cell synapse<sup>4</sup>, the Purkinje cells are a likely candidate for being responsible for the locomotion deficits observed in the Camk2b<sup>−/−</sup> mice. However, there are several other CAMK2B-expressing brain areas involved in motor control, such as motor cortex, several nuclei in brainstem, spinal cord and basal ganglia (e.g. striatum and subthalamic nucleus). Besides the hippocampus<sup>5,13</sup>, cerebellum<sup>6,14</sup> and later habenula<sup>15</sup>, CAMK2B function in different specific motor control areas has not been studied. For this study we generated an autophosphorylation-deficient CAMK2B mutant (Camk2b<sup>T287A</sup>) and made use of the previously described CAMK2B mutant which can no longer bind Calcium/Calmodulin (Camk2b<sup>Δex2</sup>)<sup>4</sup> to study the structural and enzymatic role of CAMK2B in locomotion using the accelerating rotarod. Additionally, we generated a floxed Camk2b mutant to study the temporal and brain region-specific role of CAMK2B in motor behaviour. We show that Calcium/Calmodulin-dependent activation of CAMK2B is essential for normal locomotion, but surprisingly, CAMK2B autonomous activity is largely dispensable. Additionally, we found that normal locomotion requires CAMK2B to be present during development, and that the locomotion deficits observed in the Camk2b<sup>−/−</sup> mutant cannot be assigned to a single brain area.

**Results**

The role of Calcium/Calmodulin-dependent and autonomous activity of CAMK2B in locomotion.

We have previously shown that Camk2b<sup>−/−</sup> mice (in which exon 11 was deleted) show severe locomotion deficits on the rotarod and the balance beam<sup>4</sup>. Since CAMK2B can have both structural and enzymatic functions in the brain, we first set out to understand which molecular aspects of CAMK2B are important for normal locomotion.

Mutating Alanine 303 to Arginine (A303R) in CAMK2B, interferes with Calcium/Calmodulin binding and activation of CAMK2B, and renders CAMK2B into a persistently F-actin bound state<sup>5</sup>. To investigate whether this mutation has any effect on motor behaviour, we tested the Camk2b<sup>A303R/A303R</sup> mice on the rotarod. Even though this mutant has normal hippocampal learning and plasticity<sup>6</sup>, Camk2b<sup>A303R/A303R</sup> mice showed a severe locomotion deficit, not being able to stay on the rod for more than 5–10 seconds (effect of genotype: F<sub>1,18</sub> = 39.05, p < 0.001; effect of time: F<sub>1,16</sub> = 2.48, p = 0.05; interaction: F<sub>1,14</sub> = 2.44, p = 0.05, repeated measures ANOVA; Fig. 1a).

The Camk2b<sup>A303R</sup> mutation interferes with the Calcium/Calmodulin dependent activity, as well as the autonomous (Calcium/Calmodulin independent) activity of CAMK2B. To specifically investigate the role of autonomous activity in locomotion, we generated an autophosphorylation-deficient CAMK2B mouse mutant in which Threonine 287 is substituted by an Alanine, thus blocking autonomous CAMK2B activity (Camk2b<sup>T287A</sup>)(Fig. 1b,c). Protein quantification using western blot indeed revealed a near complete absence of T287-phosphorylated CAMK2B in Camk2b<sup>T287A/T287A</sup> mice without changes in T286-phosphorylated CAMK2A or total levels of both CAMK2A and CAMK2B (Fig. 1d; for quantification see Table 1).

Camk2b<sup>T287A/T287A</sup> mice showed a trend towards reduced locomotion compared to their wildtype littermates, however this difference was not significant (effect of genotype: F<sub>1,13</sub> = 3.72, p = 0.07; effect of time: F<sub>1,21</sub> = 6.47, p < 0.001; interaction: F<sub>1,21</sub> = 0.71, p = 0.59, repeated measures ANOVA; Fig. 1e). These results indicate that, in contrast to the Calcium/Calmodulin-dependent activation, the contribution of CAMK2B autonomous activity in normal locomotion is marginal.

Temporal involvement of CAMK2B in locomotion.

Expression of CAMK2B starts during early development (E12.5)<sup>4</sup>, hence the motor deficits seen in the Camk2b<sup>−/−</sup> mice could very well be due to a crucial role for CAMK2B during development. To assess temporal contribution of CAMK2B in the severe locomotion deficits, we generated a novel floxed allele of Camk2b<sup>−/−</sup>, with LoxP sites around exon 2 (containing the catalytic site) of the Camk2b gene (Camk2b<sup>Δex2</sup>), which was crossed into the C57Bl/6 background (see Methods and Fig. 2a). To ensure that the locomotion deficits are also present in the C57Bl/6 background (the previous mutants were tested in an F2 129P2-C57Bl/6 hybrid background), we crossed the Camk2b<sup>Δex2</sup> mice with a Cag-cre transgene, deleting exon 2 from germline (Camk2b<sup>Δex2/Δex2</sup>Δ<sub>Δex2</sub>). Using immunocytochemistry and western blots, we confirmed the global deletion of CAMK2B in this line. As expected, Camk2b<sup>Δex2/Δex2</sup> showed loss of CAMK2B without changes in the expression of CAMK2A (Fig. 2b,c; Table 1). Furthermore, Camk2b<sup>Δex2/Δex2</sup> mice showed a severe locomotion deficit compared to their wildtype littermates (effect of genotype: F<sub>1,14</sub> = 48.01, p < 0.001, repeated measures ANOVA; Fig. 2d), which indicates that the motor deficits are present in both F2 129P2-C57Bl/6 hybrid mice<sup>4</sup> as well as congenic (16 backcrosses) C57Bl/6 mice.

After having shown that our novel Camk2b mutant indeed recapitulates the severe locomotion deficit described before, we continued determining the developmental component of CAMK2B in the locomotion deficit. Therefore Camk2b<sup>Δex2</sup> mice were crossed with CAG-Cre<sup>ER</sup>, giving us Tamoxifen-dependent temporal control over gene deletion. As expected, 12–14 week old Camk2b<sup>Δex2/CAG-Cre<sup>ER</sup></sup> mice showed loss of CAMK2B 4 weeks after 4 daily consecutive injections with Tamoxifen and showed no changes in levels of CAMK2A (Fig. 2b,c; Table 1).
We tested 8–10 week old mice prior to Tamoxifen-induced deletion on the rotarod and found no difference between genotypes (effect of genotype: $F_{1,38} = 0.53$, $p = 0.47$, repeated measure ANOVA), indicating that before Tamoxifen mediated gene deletion, the Camk2bf/f;CAG-CreER mice do not have a locomotion deficit (Fig. 2e, left).

When tested 28 days after Tamoxifen-induced deletion, Camk2bf/f;CAG-CreER mice showed a clear locomotion deficit on the accelerating rotarod (effect of genotype: $F_{1,38} = 24.25$, $p < 0.001$, repeated measures ANOVA; Fig. 2e, right), however, this phenotype was not as severe as upon germ-line deletion. To make sure that the milder phenotype was not caused by the initial rotarod testing before gene deletion, we induced Camk2b gene deletion in a naïve cohort of 8–10 week old mice, and tested the mice 4 weeks after gene deletion. Although these Camk2bf/f;CAG-CreER mice showed no improvement of locomotion over time compared to their Camk2bf/f littermates without expression of CRE (overall effect of genotype: $F_{1,14} = 4.01$, $p = 0.06$; effect of time: $F_{1,14} = 24.25$, $p < 0.001$; interaction: $F_{1,14} = 3.02$, $p < 0.05$ Fig. 2f), performance at the first two trials was indistinguishable from control mice, which is markedly different from Camk2bΔex2/Δex2 mice (Fig. 2d).

Taken together, deletion of CAMK2B in adulthood resulted in a much milder locomotion deficit compared to germline deletion of CAMK2B, indicating a significant developmental origin for the locomotion deficits seen in Camk2bΔex2/Δex2 mice.

**Brain areas contributing to the CAMK2B dependent rotarod deficits.** Lesion studies in rodents have indicated that several brain areas are involved in locomotion, the most important being the cerebellum,
striatum and motor cortex (for review see48). To our knowledge, the specific contribution of each of these brain areas to rotarod motor behaviour has not been systematically assessed through genetic lesions. CAMK2B is expressed in all these brain regions, therefore we used our conditional mutant to assess which brain area, if not all, is responsible for the severe rotarod phenotype seen in Camk2bΔex2/Δex2 mice. Knowing that loss of CAMK2B in the cerebellum reverses plasticity at the parallel fiber–Purkinje cell synapse, we started with assessing the importance of the cerebellum in the rotarod phenotype. Camk2bΔex2/Δex2 mice showed no deficits compared to their controls (Fig. 4d,f; Table 2). Interestingly, the training paradigm (Fig. 4c,e; for statistics see Table 2). When tested for 5 consecutive days these region-specific mice even showed significantly enhanced locomotion (Fig. 4f; Table 2).

### Table 1. Overview of western blot quantification performed on the Camk2b mutants in percentage of controls. WT = wildtype; Het = heterozygous knockout; KO = knockout. Cre− = Cre-negative Camk2b mutant; Cre + = Cre-positive Camk2b mutant. Number of samples is depicted in brackets.

| Antibody | Cerebellum | Cortex | Hippocampus | L7-Cre |
|----------|------------|--------|-------------|--------|
| WT (4)   | 100 ± 22  | 100 ± 16 | 100 ± 15 | 100 ± 16 |
| Cre− (3) | 84 ± 22   | 0 ± 0   | 0.2 ± 0.1 | 0 ± 0  |
| Cre+ (5) | 100 ± 15  | 100 ± 15 | 100 ± 13 | 0 ± 0  |
| Camk2bΔex2/Δex2 | 100 ± 22 | 100 ± 16 | 100 ± 15 | 100 ± 16 |

For the rotarod experiments with the Camk2bΔex2/Δex2 mutants in percentage of their wildtype offspring, we used two different genotypes: L7-cre mice showed selective loss of CAMK2B in cerebellar Purkinje cells (Fig. 3a–c; Table 1) and Camk2bΔex2/Δex2; Gabaa6-cre mice showed selective loss of CAMK2B in cerebellar granule cells. Surprisingly, Camk2bΔex2/Δex2; L7-cre mice lacked CRE recombinase (expression (Table 1). Interestingly, both Cre− and Cre+ lines express the CRE protein within the first postnatal week7,18.

Camk2bΔex2/Δex2; L7-cre mice showed selective loss of CAMK2B in cerebellar Purkinje cells (Fig. 3a–c; Table 1) and Camk2bΔex2/Δex2; Gabaa6-cre mice showed selective loss of CAMK2B in cerebellar granule cells. Surprisingly, Camk2bΔex2/Δex2; L7-cre mice also showed a decrease in levels of CAMK2A in the cerebellum, whereas in Camk2bΔex2/Δex2; Gabaa6-cre mice Camk2A levels were unaffected (Fig. 3a,c; Table 1). Importantly, Camk2bΔex2/Δex2; Gabaa6-cre mice showed impaired motor learning (Fig. 3e; Table 2), whereas the granule cell specific mutants still showed no hint of any deficit compared to their littermate control mice (Fig. 3g; Table 2).

Since the loss of CAMK2B in cerebellar granule cells or Purkinje cells does not appear to contribute to the locomotion deficit as seen in Camk2bΔex2/Δex2 mice, we then focused on striatum and motor cortex. Camk2bΔex2/Δex2 mice were crossed with Rgs9-cre (deletion specifically in medium spiny projection neurons of the striatum) and Emx-cre (deletion in glutamatergic pyramidal neurons in cortex and hippocampus) transgenic mice to test respectively striatal and cortical involvement in the rotarod phenotype. Rgs9-cre is expressed from P8 onwards20 and Emx-cre is expressed from E10.521. Camk2bΔex2/Δex2; Rgs9-cre mice showed selective loss of CAMK2B in striatum (Fig. 4a,b; Table 1) and Camk2bΔex2/Δex2; Emx-cre mice showed selective loss of CAMK2B in glutamatergic pyramidal neurons in hippocampus and cortex with no changes in levels of CAMK2A in both mutants (Fig. 4a,b; Table 1). Importantly, none of the specific lines showed notable off-target deletion in other brain areas (data not shown). Notably, neither the striatal nor forebrain-specific Camk2b mutants showed a rotarod deficit on the one-day training paradigm (Fig. 4d,e; for statistics see Table 2). When tested for 5 consecutive days these region-specific mutants still showed no deficits compared to their controls (Fig. 4d,f; Table 2). Interestingly, the Camk2bΔex2/Δex2; Emx-cre mice even showed significantly enhanced locomotion (Fig. 4f; Table 2).
Figure 2. **Temporal requirement of CAMK2B in locomotion.** (a) Schematic overview of the generation of the floxed Camk2b and Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) mice. Camk2b locus and targeting construct with Exons 1, 2 and 3 depicted in black boxes. LoxP sites are indicated by the black triangles and the Frt sites are indicated by the grey ovals. The Diphtheria Toxin Cassette (DTA) was inserted for positive selection. Recombined depicts the mutant Camk2b locus after homologous recombination. Floxed depicts the Camk2bf/f mutant locus after transient expression of the Flp recombinase, resulting in a floxed locus without the neomycin cassette. \(\Delta\text{Ex}2\) depicts the Camk2b\(\Delta\text{Ex}2\) mutant locus after Cre-mediated deletion. (b) Immunohistochemistry stainings of CAMK2B, showing (Top to bottom, left to right): normal expression in WT mice and no expression in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) mice; normal expression in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) mice and deletion throughout the brain in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\), Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\); CAG-CreER mice (a). (c) Western blots using an antibody specific for CAMK2A and CAMK2B in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) and Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\); CAG-CreER mice and their control littermates. Actin levels are shown as loading control. Decreased levels of CAMK2B in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) mice and no detection of CAMK2B in Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) and Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\); CAG-CreER mice in hippocampus, cortex and cerebellum with no changes in levels of CAMK2A. (d) Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\); CAG-CreER mice show increased latency to fall over the trials compared to Camk2b\(\Delta\text{Ex}2/\Delta\text{Ex}2\) mice.
mice (n = 8) show a significant impairment in locomotion compared to wildtype littermates (n = 8). (e) 8–10 week old Camk2b<sup>+/+</sup>;CAG-Cre<sup>ex2</sup> mice (n = 13) and their Camk2b<sup>+/+</sup> control littermates (n = 17) were trained on the rotarod before (Day 1) and 4 weeks after Tamoxifen injections (Day 28). Before deletion, both genotypes performed equally. After deletion, Camk2b<sup>+/+</sup>;CAG-Cre<sup>ex2</sup> mice showed a significant impairment of locomotion compared to Camk2b<sup>+/+</sup> control littermates as shown in the 5 trials given 28 days after the first injection. (f) 8–10 week old Camk2b<sup>+/+</sup>;CAG-Cre<sup>ex2</sup> mice (n = 8) show no impairment in locomotion compared to Camk2b<sup>+/+</sup> control littermates (n = 8) 4 weeks after Tamoxifen injections with no prior training. Error bars indicate SEM.

Taken together these results show that selective deletion of CAMK2B in brain areas supporting locomotion, is not sufficient to recapitulate the locomotion deficits observed in either the Camk2b<sup>lox/lox;Δex2</sup> or the Camk2b<sup>+/+</sup>;CAG-Cre<sup>ex2</sup> mice. This suggests that multiple brain areas are responsible for locomotion deficits observed in Camk2b<sup>lox/lox;Δex2</sup> mice. The only mouse line showing a small but significant effect on motor learning (5 day paradigm), but not motor performance (1 day paradigm), was the Purkinje cell specific knockout (Camk2b<sup>+/+</sup>;L7-cre). However, this effect is still marginal compared to Camk2b<sup>lox/lox;Δex2</sup> mice.

Discussion

Using several novel as well as previously described Camk2b mouse mutants, we dissected the molecular, temporal and systems requirements for CAMK2B in locomotion. At the molecular level we showed that even though the autonomous activity of CAMK2B in locomotion is dispensable, the ability of CAMK2B to bind Calcium/Calmodulin is crucial for normal locomotion. At the developmental level we found that deletion of Camk2b in the adult mouse causes a much milder locomotion deficit compared to germline Camk2b deletion. Finally, at a systems level we showed that early onset deletion of Camk2b in any of the major motor areas of the brain (cerebellum, striatum or forebrain) did not recapitulate the locomotion deficit of global Camk2b deletion. Taken together these results suggest that the locomotion deficits are mainly of a developmental origin, as well as a result of interplay between multiple regions (systems level).

We showed here that loss of autophosphorylation of CAMK2B at the T287 site does not significantly affect rotarod behaviour, although a small trend could be seen. In contrast, mutating the Calcium/Calmodulin binding site of CAMK2B, such that it cannot bind Calcium/Calmodulin and rendering CAMK2B bound to actin (Camk2b<sup>ΔA303R/A303R</sup>) led to a severe locomotion deficit. Whether the inability to bind Calcium/Calmodulin and becoming active, or the inability to be released from actin causes the deficits observed, remains to be investigated. Camk2b forms heteromers with CAMK2A<sup>22,23</sup> and ratios of CAMK2A/CAMK2B can differ between cell types<sup>22,23</sup>. It has been shown for the hippocampus (in dissociated neuronal cultures and using Camk2b<sup>ΔA303R/A303R</sup> mice) that CAMK2B kinase activity is not important for synaptic plasticity or hippocampal learning, but that CAMK2B is required for regulating the subcellular location of CAMK2A<sup>23</sup>. However, in Purkinje cells, CAMK2B not only regulates the subcellular localization of CAMK2A, but also plays an important enzymatic role in regulating the direction of plasticity at the parallel fiber–Purkinje cell synapse<sup>1</sup>. These different requirements likely reflect the differences in the CAMK2A/CAMK2B ratio in the different brain areas. Thus, when the relative expression level of CAMK2A is low, both the enzymatic and the non-enzymatic function of CAMK2B may be important, whereas in areas where CAMK2A is abundant, CAMK2B serves mainly to regulate the subcellular localization of CAMK2A. It would be interesting to investigate the CAMK2A/CAMK2B ratio in the specific motor areas of the brain to get more insight in which brain areas are involved in the severe locomotion deficits seen in the Camk2b<sup>–/–</sup> and Camk2b<sup>ΔA303R/A303R</sup> mice. Notably, Camk2b<sup>+/+</sup>;L7-cre mice showed a significant decrease in CAMK2A in western blot when only CAMK2B was deleted. A similar trend could be found in the cerebellum of Camk2b<sup>+/+</sup>;CAG-Cre<sup>ex2</sup> and in Camk2b<sup>–/–</sup> mice<sup>1</sup>. These results imply that specifically in Purkinje cells, which are the only cells in the cerebellum expressing CAMK2A, CAMK2B stabilizes CAMK2A, and that absence of CAMK2B therefore results in reduced levels of CAMK2A. However, the exact mechanism behind this regulation requires further research.

From a developmental point of view, we found that deleting most of Camk2b in the adult mice resulted in a significant locomotion impairment showing its necessity for normal motor behaviour. However, the phenotype was rather mild compared to the germ-line knock out mouse, indicating that acute deletion of Camk2b does not dramatically affect neuronal function. These results indicate that the severe locomotion deficits of Camk2b<sup>–/–</sup> mice largely arise during development of the nervous system. Unfortunately, little is known about the role of both the Camk2 isoforms in brain development. For Camk2A, which starts to be expressed around P1, we recently showed that deletion of Camk2a in adulthood is as detrimental as a germline deletion of Nmdar1 in the adult mouse causes a much milder locomotion deficit compared to germline Camk2b deletion. Finally, for hippocampal learning and plasticity<sup>12,25</sup>, indicating an important post-developmental role. CAMK2B however, starts to be expressed already during early embryonic development (around E12.5)<sup>11,24</sup>. Therefore it is likely that CAMK2B plays a significant role during development. Previous studies looking at the developmental role of CAMK2B have compared to the Purkinje cell specific knockout (Camk2b<sup>+/+</sup>;L7-cre). However, this effect is still marginal compared to Camk2b<sup>lox/lox;Δex2</sup> mice.
Figure 3. Requirement of cerebellar CAMK2B in locomotion. Motor performance (1-day paradigm; (d,f)) and learning (5-day paradigm; (e,g)) was tested using the accelerating rotarod. (a) Immunohistochemistry stainings of CAMK2B, showing (left to right): normal expression in Camk2b^{+/+} mice; specific deletion in cerebellar Purkinje cells in Camk2b^{+/+};L7-cre mice and specific deletion in the cerebellar granule cells of Camk2b^{+/+};Gabaa6-cre mice. (b) Immunofluorescent and immunohistochemical zoomed-in picture of the cerebellum of Camk2b^{+/+} mice, Camk2b^{+/+};L7-cre and Camk2b^{+/+};Gabaa6-cre mice showing specific deletion in cerebellar Purkinje Cells in the Camk2b^{+/+};L7-cre mice and deletion in the granule cell layer (GCL) of Camk2b^{+/+};Gabaa6-cre mice, but not in Purkinje cells of the molecular layer (ML). (c) Western blots using an antibody specific for CAMK2A and CAMK2B in the different Camk2b mutants and their control littermates. (Left) Decreased levels of CAMK2A and CAMK2B in the cerebellum of Camk2b^{+/+};L7-cre mice. LoxP sites do not affect CAMK2A and CAMK2B levels. (Right) Decreased levels of CAMK2B in the cerebellum of Camk2b^{+/+};Gabaa6-cre mice with no changes in CAMK2A levels. Actin levels are shown as loading control. (d) Camk2b^{+/+};L7-cre mice (n = 12) show no difference in performance compared to Camk2b^{+/+} control littermates (n = 12). Camk2b^{+/+};L7-cre (n = 12) and Camk2b^{+/+} mice (n = 15) do not differ in performance compared to Camk2b^{+/+};L7-cre and Camk2b^{+/+} mice implying no effect of CRE or the flanked LoxP sites on rotarod performance. (e) Camk2b^{+/+};L7-cre mice (n = 10) show an impairment in learning compared to Camk2b^{+/+} control littermates (n = 12). Camk2b^{+/+};L7-cre (n = 12) and Camk2b^{+/+} mice (n = 15) do not differ in performance compared to Camk2b^{+/+};L7-cre and Camk2b^{+/+} mice implying no effect of CRE or the flanked LoxP sites on rotarod learning. (f) Camk2b^{+/+};Gabaa6-cre mice (n = 8) show no impairment in performance compared to Camk2b^{+/+} control littermates (n = 8). (g) Camk2b^{+/+};Gabaa6-cre mice (n = 8) and Camk2b^{+/+} control littermates (n = 8) show normal learning. Error bars indicate SEM.
rotarod20. Taken together, these studies all indicate that, depending on the lesion or genetic change, specific brain areas could potentially be responsible for deficits in motor performance as measured with the rotarod.

Surprisingly, restricted deletion of CAMK2B in the major motor areas of the brain did not reveal a specific brain area or cell type to be responsible for the severe rotarod phenotype observed in the Camk2b–/– mice. In these groups we not only assessed motor performance, but also motor learning by testing the mice over 5 consecutive days. Even though there are small variations in performance and learning in the control groups between the different experiments, the effects of Camk2b deletion on locomotion was only marginal compared to the profound phenotypes seen in Camk2bΔex2/Δex2 and Camk2bA303R/A303R mice. There are three possible explanations for our findings. First, onset of Camk2b gene deletion in our mutants is likely to be too late. Second, the cre-lines used in this study start cre expression after onset of CAMK2B expression (L7-cre starts expression at E18, Gabaa6-cre starts expression at P5-P717, and Rgs9-cre expression is seen as early as P820). Thus, these lines offer a normal embryonic CAMK2B expression. Since we do not know the critical time window for CAMK2B in rotarod behaviour, it could be that in these mice CAMK2B is deleted too late in these separate brain regions or cell types to cause a significant effect on rotarod behaviour, comparable to Camk2bΔex2/Δex2 mice, where adult deletion resulted only in a mild phenotype when CAMK2B was deleted throughout the brain. Only the L7-cre/cn line, which expresses cre from E10.521, induces deletion of Camk2b before onset of CAMK2B expression (E12.5). Interestingly, deletion in this cre-line resulted in enhanced, instead of impaired performance. Second, Camk2b deletion in thalamus, brainstem and the deep cerebellar nuclei is only obtained in the Camk2bΔex2/Δex2 mice. Since it has been shown that these areas are involved in locomotion22, it is possible that the motor deficits arise from these areas. Third, the severity of the locomotion deficits observed in the Camk2b–/– mice could indicate a distributed role for CAMK2B in several brain areas and cell types, each one of them contributing a little to the deficit. For example, we cannot exclude that deleting Camk2b in Purkinje-cells and cerebellar granule cells simultaneously would have an effect on locomotion. Additionally, even though the different cre-lines used in this study were very effective at deleting Camk2b throughout multiple brain areas and cell types (as judged by immunohistochemistry and Western blot), we cannot exclude the possibility that a small number of cells did not undergo deletion, and prevent the lack of a severe phenotype.

Taken together we conclude that the nature of severe the locomotion deficits observed in the Camk2bΔex2/Δex2 is predominantly of developmental origin and cannot be attributed to one specific motor area or cell type in the brain. Moreover we conclude that the Calcium/Calmodulin-dependent activation of CAMK2B is essential for normal locomotion, but that the autonomous activity of CAMK2B is largely dispensable for normal locomotion. These findings are in sharp contrast to the role of CAMK2A in spatial learning, where loss of CAMK2A in adult mice recapitulates the phenotype of Camk2a−/− mice, where selective loss of CAMK2A in the hippocampus causes spatial learning deficits, and where loss of autonomous CAMK2A activity causes severe spatial learning deficits11,31.

Materials and Methods

Animals. Camk2bA303R/A303R and Camk2bΔex2/Δex2 mice were tested in a 129P2-C57Bl/6OlaHsd F2 hybrid background. Camk2bΔex2/Δex2 mice were backcrossed >16 times into C57Bl/6OlaHsd background. All conditional mice used in this study were backcrossed 10–12 times into the C57BL/6JolaHsd background and crossed with cre lines maintained in the C57BL/6JolaHsd background. Mice were genotyped when they were 7–10 days old, and re-genotyped after the mice were sacrificed. Genotyping records were obtained and kept by a technician not involved in the experimental design, performance and analysis. All mice were tested between 2–3 months of age, except the Camk2bΔex2/Rgs9-cre group, which was tested between 3–5 months of age. All mice were kept group-housed in IVC cages (Sealsafe 1145 T, Tecniplast) with bedding material (Lignocel BK 8/15 from Rettenmayer) on a 12/12 h light/dark cycle (22°C ±1°C), humidity at 40–70% and with food pellets (801727CRM(P) from Special Dietary Service) and water available ad libitum. For all experiments mutants were compared to WT or cre-negative homozygous floxed littermates. All groups were matched for age and sex and all experiments were
done during daytime. Experimenters were blind for genotype throughout experiments and data analysis. All research was performed in accordance with and approved by a Dutch Animal Ethical Committee (DEC) for animal research.
Generation of Camk2b<sup>A303R/A303R</sup> and Camk2b<sup>T287A/T287A</sup> mice. Generation of the Camk2b<sup>A303R</sup>/A303R mice has been described previously<sup>3</sup>. The Camk2b<sup>T287A</sup> targeting construct to generate Camk2b<sup>T287A/T287A</sup> mice was generated as follows. The Camk2b genomic sequence (ENSMUSG00000057897) was obtained from a public database (Ensembl) and used to design the primers for the targeting constructs. PCR fragments encompassing exon 6–11 using 5′ primer: 5′-GGTACCTGAGGAAGTCCAGCTGTGCCC-3′ and 3′ primer: 5′-GTGGCAGGTTAGTCAAGGTGGTC-3′ (5.3 Kb; exon denotation according to ENSMUST00000019133) and exon 11–12 using 5′ primer: 5′-GCCGCCGCTTTAAGGATAGTTCTC-3′ and 3′ primer: 5′-ATGACATCAAGGAGGAGTATGATG-3′ (6 Kb) were amplified using High Fidelity Taq Polymerase (Roche) on ES cell genomic DNA and cloned on either site of a PGK-Neomycin selection cassette. All exons were verified by sequencing. Site directed mutagenesis was used to introduce the Thr287Ala point mutation, which induced a TspRI restriction site (Fig. 1). For counter selection, a gene encoding Diphtheria toxin chain A (DTA) was inserted at the 5′ end of the targeting construct. The targeting construct was linearized and electroporated into E14 ES cells (derived from 129P2 mice). Cells were cultured in BRL cell conditioned medium in the presence of Leukaemia inhibitory factor (LIF). After selection with G418 (200 μg/ml), targeted clones were identified by PCR (long-range PCR from Neomycin resistance gene to the region flanking the targeted sequence). A clone with normal karyotype was injected into blastocysts of C57Bl/6 mice. Male chimeras were crossed with female C57Bl/6 mice (Harlan). The resulting F1 heterozygous mice (in the 129P2-C57Bl/6 background) were subsequently inter-crossed to obtain F2 129P2-C57Bl/6 hybrid mice to generate homozygous mutants and wild-type littermate controls.

Generation of floxed Camk2b and Camk2b<sup>Δex2/Δex2</sup> mice. The floxed Camk2b targeting construct was generated as follows. The Camk2b genomic sequence (ENSMUSG00000057897) was obtained from a public database (Ensembl) and used to design the primers for the targeting constructs. PCR fragments encompassing intron 1 using 5′ primer: 5′-TTTGGTACCGGATTGTTGGGATCTATGAG-3′ and 3′ primer: 5′-AAAGGATCCGAGCTGGAATGACAGTG-3′ (2.6 Kb; intron and exon denotation according to ENSMUST00000019133), exon 2 using 5′ primer: 5′-TTTATGATGATAGCTGGTGTACACAG-3′ and 3′ primer: 5′-AAAGTCGACCTCTGCTGGACAGAAG-3′ (583 bp) and intron 2 using 5′ primer: 5′-TTTGGCAGCCGAGTCTCTCATTGAGGAGG-3′ and 3′ primer: 5′-AAAAACGGTGTACTCCTATTGACGAGACC-3′ (4 Kb) were amplified using High Fidelity Taq Polymerase (Roche) on ES cell genomic DNA. The 5′ fragment containing part of intron 1 was inserted before the first LoxP site whereas exon 2 and part of intron 2 were cloned into a site of a PGK-Neomycin selection cassette, which is flanked by frt and loxp sites (for the schematics see Fig. 2). Exon 2 and flanking intronic sequences were sequenced to verify that the absence of secondary mutations. For counter selection, a gene encoding Diphtheria toxin chain A (DTA) was inserted at the 5′ end of the targeting construct. The targeting construct was linearized and electroporated into E14 ES cells (derived from 129P2 mice). Cells were cultured in BRL cell conditioned medium in the presence of Leukaemia inhibitory factor (LIF). After selection with G418 (200 μg/ml), targeted clones were identified by PCR (long-range PCR from Neomycin resistance gene to the region flanking the targeted sequence). To delete the Neomycin resistance cassette and obtain the conditional construct, the correctly targeted clones were transiently transfected with an fpt recombine (Fig. 2). Finally, a clone with correct karyotype was injected into blastocysts of C57Bl/6 mice. Male chimeras were crossed with female C57Bl/6/OlaHsd mice (Harlan). The resulting F1 heterozygous Camk2b<sup>Δex2/+</sup> mice, which were backcrossed 10–12 times with C57Bl/6/OlaHsd mice before generating the brain region and temporally restricted mutants as described below. To obtain the Camk2b<sup>Δex2/Δex2</sup> mice, the Camk2b<sup>Δex2/+</sup> mice were crossed with transgenic Cag-cre mice<sup>32</sup>. The Camk2b<sup>Δex2/Δex2</sup> heterozygous offspring were backcrossed >16 times in C57Bl/6/OlaHsd to obtain a congenic line.

Generation of Camk2b<sup>β/β</sup> conditional mutants. For the generation of conditional mutants, female Camk2b<sup>β/β</sup> mice (backcrossed 10–12 times with C57Bl/6/OlaHsd) were crossed with male transgenic Cre lines maintained in C57Bl/6/OlaHsd. To obtain temporal control, Camk2b<sup>β/β</sup> mice were crossed with CAG-Cre<sup>β/β</sup> mice (RRID:IMSR_JAX:00468233). Adult Camk2b<sup>β/β</sup> mice were 8–10 weeks of age) were injected with Tamoxifen intraperitoneally (Sigma-Aldrich) (0.1mg/g of bodyweight) for 4 consecutive days. To keep the dose of Tamoxifen constant throughout injection days we kept a tight injection scheme, injecting mice 24±1 hour after the previous injection. Tamoxifen was dissolved in sunflower oil (20 mg/ml). Behavioural testing was assessed 4 weeks after the first injection. Even though Tamoxifen does not have an effect on emotional reactivity, neurological functioning or learning<sup>30</sup> we injected both Camk2b<sup>β/β</sup> and Camk2b<sup>β/β/CAG-Cre</sup> mice to control for any possible effects of Tamoxifen.

Tamoxifen injections. Adult Camk2b<sup>β/β</sup> and Camk2b<sup>β/β/CAG-Cre</sup> mice were 8–10 weeks of age) were injected with Tamoxifen intraperitoneally (Sigma-Aldrich) (0.1mg/g of bodyweight) for 4 consecutive days. To keep the dose of Tamoxifen constant throughout injection days we kept a tight injection scheme, injecting mice 24±1 hour after the previous injection. Tamoxifen was dissolved in sunflower oil (20 mg/ml). Behavioural testing was assessed 4 weeks after the first injection. Even though Tamoxifen does not have an effect on emotional reactivity, neurological functioning or learning<sup>30</sup> we injected both Camk2b<sup>β/β</sup> and Camk2b<sup>β/β/CAG-Cre</sup> mice to control for any possible effects of Tamoxifen.

Rotarod. The accelerating rotarod (Ugo Basile, Comerio Varese, Italy, 7650) contains a cylinder 3 cm in diameter and can train 5 mice at the same time. Rotarod speed starts at 4 r.p.m., which increases to 40 r.p.m. at 270 seconds. The experiment stopped at 300 seconds. Latency to fall was measured in seconds after a mouse (i) fell off, (ii) clung to the rod for 3 consecutive rotations or (iii) clung to the rod for 2 rotations twice within 10 seconds.
Mice were trained with an inter-trial interval of 45 minutes. We used two different paradigms on the accelerating rotarod. For motor performance (which we define as locomotion throughout the text) we used 5 consecutive trials with naïve mice (1 day paradigm). To assess motor learning we took the average of the first two trials of the first day and continued training the mice for another 4 consecutive days with 2 trials per day (5 day paradigm).

**Immunohistochemistry and immunofluorescence.** Mice were anaesthetized with pentobarbital and perfused transcardially with PBS followed by freshly prepared 4% paraformaldehyde solution (PFA, Sigma). Brains were taken out after perfusion and post-fixed for 1.5 hours in PFA and afterwards kept in 30% sucrose solution overnight. Immunohistochemistry was performed on free-floating 40 μm thick sagittal cryostat sections. Sections were washed in PBS once and afterwards primary antibodies were added (anti-CAMK2B, 1:2000, #ab34703, Abcam) diluted in PBS containing 2% NHS, 0.5% Triton-X 100 and 150 mM bovine serum albumin (BSA) and kept at 4 °C overnight for 48 hours. Two days later sections were washed 3 times with PBS and then secondary antibodies were added (biotinylated goat anti-rabbit IgG antibody, Vector Laboratories, Burlingame, CA; 1:200) diluted in PBS containing 2% NHS, 0.5% Triton-X 100 and 150 mM bovine serum albumin (BSA) for 1 to 2 hours on room temperature. For diaminobenzidine (DAB) staining, sections were processed using a standard avidin-biotin-immunoperoxidase complex method (ABC, Vector Laboratories, USA) and 0.05% DAB for 1 to 2 hours incubation of the secondary antibody at room temperature. Sections were washed four times in PB (0.05 M) and mounted on slices using chrome(3) potassiumsulfatedodecahydrate and left to dry. Finally, sections were covered using Mowiol (Sigma-Aldrich). For immunofluorescence, the same CAMK2B was used as in immunohistochemistry (1:1000) and we used Cy3 rabbit (1:200) as a secondary antibody. After 1–2 hours incubation of the secondary antibody we washed slices 4 times in PB (0.05 M) and mounted on slides using chrome(3) potassiumsulfatedodecahydrate and left to dry. Finally, sections were covered using Mowiol (Sigma-Aldrich).

**Western blot.** Mice were anaesthetized using isoflurane and sacrificed by decapitation. Brain samples were taken out quickly and stored in liquid nitrogen. Upon protein determination lysates were first prepared and brain samples were homogenized in lysis buffer (10 mM Tris-HCl 6.8, 2.5% SDS, 2 mM EDTA). Protein concentration in the samples was determined and then lystate concentrations were adjusted to 1 mg/ml. Western blots were probed with primary antibodies against either CAMK2A (6G9, 1:40.000, Abcam), CAMK2B (CB-31, 1:10.000, Invitrogen), Actin (MAB1501R, 1:20.000, Chemicon) or Ph-T286/T287 (autophosphorylated α CAMK2A and CAMK2B antibody; #06–881; 1:5000; Upstate Cell Signaling Solutions) and secondary antibodies (goat anti-mouse and/or goat anti-rabbit, both 1:3000, AffiniPure #115-007-003 and #111-007-003). Blots were stained with Enhanced ChemoLuminescence (ECL) (#32106, Pierce) or stained and quantified using LI-COR Odyssey Scanner and Odyssey 3.0 software. Quantification of western blot in ECL was done using ImageJ.

**Data analysis and statistics.** All experiments and analyses were performed blind to genotype. All behavioural tests were analysed using a 2-WAY repeated measures ANOVA to determine the performance and learning of the genotypes, with genotype as the between subjects factor and the repeated measures as within subject factor. α was set at 0.05. All values represent average ± SEM. Group sizes for each genotype are depicted in the figure legends. All statistics were performed in Graphpad Prism. *p < 0.05, ***p < 0.001.

**References**

1. Lisman, J., Schulman, H. & Cline, H. The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci.*, 3, 175–190 (2002).
2. Lisman, J., Yasuda, R. & Raghavachari, S. Mechanisms of CaMKII action in long-term potentiation. *Nat. Rev. Neurosci.*, 13, 169–182 (2012).
3. Hell, J. W. CaMKII: Claiming Center Stage in Postsynaptic Function and Organization. *Neuron*, 81, 249–265 (2014).
4. Van Woerden, G. M. et al. betaCaMKII controls the direction of plasticity at parallel fiber-Purkinje cell synapses. *Nature Publishing Group*, 12, 823–825 (2009).
5. Borgesius, N. Z. et al. CaMKII plays a nonenzymatic role in hippocampal synaptic plasticity and learning by targeting oCaMKII to synapses. *Journal of Neuroscience*, 31, 10141–10148 (2011).
6. Bachstetter, A. D. et al. Generation and Behavior Characterization of CaMKIIβ Knockout Mice. *PLos one*, 9, e105191 (2014).
7. Gao, Z., Van Woerden, G. M., Elgersma, Y., De Zeeuw, C. I. & Hoebeck, F. E. Distinct roles of α- and βCaMKII in controlling long-term potentiation of GABAA-receptor mediated transmission in murine Purkinje cells. *Front Cell Neurosci*, 8, 16 (2014).
8. Cheng, D. et al. Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. *Mol. Cell Proteomics*, 5, 1158–1170 (2006).
9. Shen, K., Teruel, M. N., Subramanian, K. & Meyer, T. CaMKIIβ functions as an F-actin targeting module that localizes CaMKIIα/β to dendritic spines. *Neuron*, 21, 593–606 (1998).
10. Shen, K. & Meyer, T. Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science*, 284, 162–166 (1999).
11. Bayer, K. U., Löhler, J., Schulman, H. & Harbers, K. Developmental expression of the CaM kinase II isoforms: ubiquitous gamma- and delta-CaM kinase II are the early isoforms and most abundant in the developing nervous system. *Brain Res. Mol. Brain Res.*, 70, 147–154 (1999).
12. Achterberg, K. G. et al. Temporal and Region-Specific Requirements of CaMKIIα in Spatial and Contextual Learning. *Journal of Neuroscience*, 34, 11180–11187 (2014).
13. Okuno, H. et al. Inverse Synaptic Tagging of Inactive Synapses via Dynamic Interaction of Arc/Arg3.1 with CaMKIIβ. *Cell*, 149, 886–898 (2012).
14. Nagasaki, N., Hirano, T. & Kawaguchi, S.-Y. Opposite Regulation of Inhibitory Synaptic Plasticity by α and β Subunits of Ca2+/-calmodulin-dependent Protein Kinase II. *The Journal of Physiology* (2014), doi: 10.1113/jphysiol.2014.280230.
15. Li, K. et al. CaMKII in Lateral Habenula Mediates Core Symptoms of Depression. *Science*, 341, 1016–1020 (2013).
16. Lalonde, R. & Strazielle, C. Brain regions and genes affecting postural control in rats. *Neuroscience*, 81, 45–60 (2007).
17. Aller, M. L. et al. Cerebellar granule cell Cre recombinase expression. *Genesis*, 36, 97–103 (2003).
18. Barski, J. J., Dethleffsen, K. & Meyer, M. Cre recombinase expression in cerebellar Purkinje cells. *Genesis*, 28, 93–98 (2000).
19. Rahman, Z. et al. Cloning and characterization of RGS9-2: a striatal-enriched alternatively spliced product of the RGS9 gene. *J Neurosci*, 19, 2016–2026 (1999).
20. Dang, M. T. et al. Disrupted motor learning and long-term synaptic plasticity in mice lacking NMDAR1 in the striatum. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 15254–15259 (2006).

21. Iwasato, T. et al. Dorsal telencephalon-specific expression of Cre recombinase in PAC transgenic mice. *Genes* **38**, 130–138 (2004).

22. Brocke, L., Chiang, L. W., Wagner, P. D. & Schulman, H. Functional implications of the subunit composition of neuronal CaM kinase II. *J. Biol. Chem.* **274**, 22713–22722 (1999).

23. Miller, S. G. & Kennedy, M. B. Distinct forebrain and cerebellar isoforms of type II Ca2+/calmodulin-dependent protein kinase associate differently with the postsynaptic density fraction. *J. Biol. Chem.* **260**, 9039–9046 (1985).

24. Karls, U. et al. Structure, expression, and chromosome location of the gene for the beta subunit of brain-specific Ca2+/calmodulin-dependent protein kinase II identified by transgene integration in an embryonic lethal mouse mutant. *Mol. Cell. Biol.* **12**, 3644–3652 (1992).

25. Funk, C. C. et al. Selective regulation of neurite extension and synapse formation by the beta but not the alpha isoform of CaMKII. *Neuron* **39**, 283–297 (2003).

26. Caston, J., Jones, N. & Stelz, T. Role of preoperative and postoperative sensorimotor training on restoration of the equilibrium behavior in adult mice following cerebellectomy. *Neurobiology of Learning and Memory* **64**, 195–202 (1995).

27. Jeljeli, M., Strazielle, C., Caston, J. & Lalonde, R. Effects of centrolateral or medial thalamic lesions on motor coordination and spatial orientation in rats. *Neurosci. Res.* **38**, 155–164 (2000).

28. Jeljeli, M., Strazielle, C., Caston, J. & Lalonde, R. Effects of ventrolateral-ventromedial thalamic lesions on motor coordination and spatial orientation in rats. *Neurosci. Res.* **47**, 309–316 (2003).

29. Thullier, F., Lalonde, R., Mahler, P., Joyal, C. C. & Lestienne, F. Dorsal striatal lesions in rats: 1. Effects on exploration and motor coordination. *Arch. Physiol. Biochem.* **104**, 300–306 (1996).

30. Guimaraes, I. M., Carvalho, T. G., Ferguson, S. S., Pereira, G. S. & Ribeiro, F. M. The metabotropic glutamate receptor 5 role on motor behavior involves specific neural substrates. *Mol Brain* **8**, 24 (2015).

31. Giese, K. P., Fedorow, N. B., Filipkowski, R. K. & Silva, A. J. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* **279**, 870–873 (1998).

32. A transgenic mouse line that retains Cre recombinase activity in mature oocytes irrespective of the cre transgene transmission. *237*, 318–324 (1997).

33. Hayashi, S. & McMahon, A. P. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Dev. Biol.* **244**, 305–318 (2002).

34. Oberdick, J., Smeyne, R. J., Mann, J. R., Zackson, S. & Morgan, J. I. A promoter that drives transgene expression in cerebellar Purkinje and retinal bipolar neurons. *Science* **248**, 223–226 (1990).

35. Vogt, M. A. et al. Suitability of tamoxifen-induced mutagenesis for behavioral phenotyping. *Exp. Neurol.* **211**, 25–33 (2008).

Acknowledgements
We thank Erika Goedknegt, Minetta Elgersma and Mehrnoush Aghadavoud Jolfaei for technical support. This research was supported by the Netherlands Organization for Scientific Research (ALW-Veni project to G.v.W.).

Author Contributions
Y.E. and G.M.v.W. designed experiments. M.J.K., G.M.v.W., J.E.v.d.B. and H.E.B. performed experiments and analysed the data. M.J.K., Y.E. and G.M.v.W. wrote the paper.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kool, M. J. et al. The molecular, temporal and region-specific requirements of the beta isoform of Calcium/Calmodulin-dependent protein kinase type 2 (CAMK2B) in mouse locomotion. *Sci. Rep.* **6**, 26989; doi: 10.1038/srep26989 (2016). This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/