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Multiple behavioral anomalies in *GluR2* mutant mice exhibiting enhanced LTP

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Abstract

We have previously disrupted the ionotropic glutamate receptor type 2 gene (*GluR2*) using gene targeting in embryonic stem cells and generated mice which lacked the GluR2 gene product. Neurophysiological analyses of these mice showed a markedly enhanced long-term potentiation (LTP) and a 9-fold increase in kainate induced Ca^{2+} permeability in the hippocampus. Here, we analyze the behavioral and neuroanatomical consequences of GluR2 deficiency in homozygous null mutant and age-matched littermate control mice. We show that despite unaltered gross brain morphology, several aspects of behavior were abnormal in the mutants. Object exploration, rearing, grooming and locomotion were altered in the novel arena. Eye-closure reflex, motor performance on the rotating rod and spatial and non-spatial learning performance in the water maze were also abnormal in the mutants. These abnormalities together with the widespread expression pattern of *GluR2* in most excitatory CNS pathways suggest that the absence of GluR2 leads to neurological phenotypes associated with not only the hippocampus but several other brain regions potentially including the cortex and cerebellum. We speculate that GluR2 mutant mice suffer from an overall non-specifically increased excitability that may alter cognitive functions ranging from stimulus processing to motivation and learning. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Gene targeting; GluR2; Calcium; Neural processing; Long-term potentiation; Hippocampus

1. Introduction

Gene targeting in embryonic stem (ES) cells allows the investigator to generate mutations within a single gene in mice [32]. Such mutants may be valuable tools to study in vivo roles particular genes may play in the mammalian central nervous system (CNS) [25]. Metabotropic and ionotropic glutamate receptors have recently become a target of molecular genetic studies [1,12,31,50,53,34,7] since glutamate is one of the major excitatory neurotransmitters of the CNS and its receptors are postulated to play roles in neural plasticity [7,47,50]. A subclass of glutamate receptors (GluR1, 2, 3, and 4) is defined by their high affinity for α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) but low affinity for kainate [28]. GluRs form heteromeric receptors which are involved in fast excitatory synaptic transmission throughout the CNS. Fast synaptic transmission may have an important role in neural processing and thus in behavior. The electrophysiological characteristics of these glutamate receptors may be different depending on their subunit constitution which in turn may determine Ca^{2+} permeability and gating in the given neuronal cell type [18]. Using gene targeting, Jia et al. [31]. have shown that the GluR2 subtype plays a crucial role in these mechanisms. The lack of GluR2 in null mutant mice led to a 9-fold increase in Ca^{2+} permeability in hippocampal CA1 pyramidal neurons in response to kainate. Although passive membrane properties, inhibitory synaptic components, and pre- and postsynaptic responses to baseline stimulation were

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found to be unaltered in GluR2 null mutant mice, these mice exhibited a significantly elevated, NMDA receptor independent hippocampal long-term potentiation (LTP) in response to tetanic stimulation [31]. Furthermore, LTP appeared to be unsaturable and could be further increased with repeated tetanic stimulation in the mutants but not in control mice.

Synaptic strengthening such as LTP has been the focus of attention in behavioral and molecular neuroscience studies [43,55]. LTP has been an attractive hypothesis to explain the cellular mechanisms underlying certain forms of learning and memory [5,43,46]. Both pharmacological [45] and genetic [25,55] manipulations of LTP appear to be associated with hippocampal dependent behavioral alterations that may be measured as impaired relational (spatial) learning [43,4]. Although the evidence for the importance of LTP in hippocampal function is clearly substantial, LTP is not an exclusive property of the hippocampus. It can be observed in other brain regions including the somatosensory [8,9], visual [35,36], auditory [37], olfactory [11,52], motor [2], piriform [33], entorhinal [17], cingulate [27] cortices, the amygdala [42,10] and thalamo-cortical synapses [13]. Furthermore, a higher elevation of intracellular Ca^{2+} levels is associated with LTP and a smaller increase of Ca^{2+} is seen when long-term depression (LTD) is induced [3,41]. LTD is known to play a prominent role not only in the hippocampus but also in the cerebellum where it is thought to be involved in motor learning and in the fine tuning of motor responses [1,30,39,40]. Because changes in Ca^{2+} permeability in GluR2 null mutant mice [31] may alter synaptic processes including LTP and LTD, the mutants may posses functional abnormalities that involve several brain regions. Therefore, a thorough analysis of brain function is warranted.

In the present paper we investigate brain function using behavioral tests, an approach that has been suggested informative [20] in the analysis of null mutant or transgenic mice. We compare several behavioral traits of the GluR2 null mutant mice with age-matched control littermates. Since indirect neurophysiological evidence [31] suggested decreased dendritic arborization in hippocampal neurons we also report results of a survey for potential neuroanatomical defects.

2. Materials and methods

2.1. Animals and housing

We used an isogenic (129 strain derived) targeting vector to electroporate embyonic stem (ES) cells of 129 mouse strain origine [31]. ES cell clones containing the desired targeting event were aggregated with CD1 morulae [57] to develop chimeras. One male chimera transmitted the mutation through the germ line as determined by Southern blotting of tail DNA from the offspring of this male. The heterozygous mutant offspring were intercrossed to produce a segregating F_2 generation with homozygous mutant and control mice. Homozygous females and males were fertile, but poor breeders. Homozygous mutants produced no detectable GluR2 protein by Western blots or immunocytochemistry [31]. In the behavioral and neuroanatomical experiments homozygous GluR2 null mutant mice ($-/-$) were compared with heterozygous control animals ($+/-$) which appeared to be indistingusihable from $+/+$ wild type. All mice were tested at 5–8 weeks, an age by which physical characterisics of homozygous and control animals were indistinguishable. All animals (males, females separated) were housed in groups of 3–4 in plastic cages ($25 \times 18 \times 12$ cm) on sawdust bedding in the same room ($20 + 1$°C, 45% relative humidity, food and water ad lib, 12/12 h light/dark cycle the light being turned on at 07:00 h).

2.2. Neuroanatomical analysis

To examine GluR2 null mutant mice for potential neuroanatomical defects, 30 μm serial sections were taken through the entire extent of the cranium in both the sagittal and coronal orientations (three mice per genotype group). Mice were anesthetized with sodium pentobarbital (Somnitol 80 mg/kg) and perfused transcardially with 15 ml of 100 mM phosphate buffered saline (PBS, pH 7.4), followed by 50 ml of freshly prepared 4% paraformaldehyde in PBS at 4°C. Samples were then postfixed for 2 h in 4% paraformaldehyde in PBS at 4°C and the brain or spinal cord were further dissected and subsequently processed in a blind manner for either cryostat, paraffin, or thin sections.

For paraffin sections samples were first dehydrated in ethanol/water baths and embedded into paraffin blocks. Sections (10 μm) were cut on a Reichert-Jung microtome, mounted on $2\times$ gelatin coated slides and heated to 60°C for 90 min. Slides were subsequently dewaxed and stained with thionin. To examine the optic nerves of the spinal dorsal root (L4), specimens were postfixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.4) for 4 h at 4°C, rinsed free of glutaraldehyde, fixed in 1% osmium tetroxide buffered in PBS for 1 h, and then dehydrated in a series of water/ethanol, ethanol/propylene oxide baths. Following removal of propylene oxide, samples were embedded in Spurr resin and baked at 50°C for 36 h. A series of 1-μm-thick cross-sections were cut through the entire nerve and stained with 1% toluidine blue.

Cryostat brain sections were obtained following 3 h post-fixation in 4% paraformaldehyde and equilibrated overnight in a solution of 30% sucrose, 0.1 M PBS (pH 7.4) at 4°C, followed by freezing in 2-methyl butane at

−20°C. Serial sections were obtained using a Reichardt-Jung Fridgocut cryostat. Sections were then thaw-mounted and either stained with thionin or examined by interference contrast microscopy. In addition to the above, the activity of cytochrome oxidase, a mitochondrial enzyme located primarily in the dendrites and somata of post synpatic cells, was assessed on 50 μm serial coronal sections obtained at intervals of 200 μm throughout the brain.

2.3. Behavioral apparatus and procedure

The behavioral tests applied included the novel arena, eye-closure test, rotorod and Morris water maze. For the novel arena [23], mice were placed singly in a plastic box (46 × 25 × 15 cm) illuminated by three pairs of 40-W fluorescent light tubes from the ceiling, and their motor and posture patterns were video-recorded for a 5-min session and later analyzed. The frequency (*f*) and duration (*d*, relative to the session length) of spontaneous motor and posture patterns [15,16,23,56] appearing upon exposure to the test situation were measured using an event-recorder computer program [24]. The following elements were analyzed: leaning (*f*), leaning against the wall with one or both forepaws while standing on the hindlegs; rearing (*f*), standing upright on the hindlegs; passivity (*d*), remaining motionless; locomotion score, number of squares crossed were counted using a square grid (5 × 5 cm); a cross was counted when the mouse entered a new square with both of its forepaws; grooming (*d*), stereotypical fur licking or face cleaning movements; object exploration (*f*), exhibiting typical whisker movements (sniffing) while holding the nose close to a novel object placed in the open field before the session. In the eye-closure test, mice were approached in their home-cage from above by a rubber rod slowly which was stopped in front of them 1 cm away from their eyes. The behavioral responses were videotaped and scored later (score 2, both eyes closed fully; score 1, partial eye closure or only one eye closed; score 0, both eyes open). For the rotorod test [21], after a habituation session, each mouse was placed on a rod (3 cm in diameter) rotating at 15 rpm. Falling latency (seconds) of each mouse was measured six times and the three best results (longest latencies) were averaged and analyzed statistically.

In addition to the above tests, GluR2 null mutant and age-matched control mice were tested in two paradigms of the Morris water maze as described previously [18,22]: the hidden platform task (spatial learning test) and a visible platform task (non-spatial learning test). A white acrylic circular tank (diameter 120 cm, height 30 cm) was filled with water (23 ± 1°C) made opaque with a non-toxic white color 'tempera'. A white circular platform (diameter: 20, 15 or 10 cm) was submerged 5 mm below the water level. After two habituation sessions [22] mice received a 6-day-long hidden platform training followed by a probe trial, a 3-day resting period, and subsequently by a 3-day-long visible platform training. In the hidden platform training the mice, started from random locations of the water maze, were required to find the fixed location of a submerged platform in relation to external visual cues. They received two sessions (90-min intersession interval) of three trials (maximum trial length 60 s, 40-min intertrial interval) each day between 10:00 and 17:00 h. Latency to escape (seconds) onto the platform was recorded. The platform size was decreased between the 1st, 2nd and 3rd day (diameter being 20, 15, 10 cm, respectively) [22]. After the hidden platform training, a probe trial was administered during which the platform was removed from the tank and the swimming pattern of the mice was video-recorded for 60 s. The relative duration of time (%) the mice spent in four equal quadrants of the tank, the number of exact platform location crossings, average swimming speed (cm/s) during the first ten bouts of swimming, and the duration spent with floating motionless in the tank were measured with an event recorder computer program [24]. After the 3-day resting period that followed the probe trial the mice were exposed to the visible platform training which had similar motivational, perceptual and motor requirements to those of the hidden platform task but did not require learning multiple spatial cues. Mice, started from a fixed location in the tank, had to find randomized variable locations of a submerged platform marked by a single visible cue (15-cm-high colorful rod). External visual cues were obscured. The minimum daily distance the mice had to swim to the platform was equal in the hidden and visible platform tests. The size of the platform was decreased between days 1 and 3 as described in the hidden platform training. Other parameters of the test were also the same as in the hidden platform training.

2.4. Statistical analysis

SYSTAT 5.2 statistical software package [54] was used. Wherever possible, parametric statistical tests (multiway univariate ANOVA and multivariate ANOVA, *t*-test, and Tukey honestly significant distance (HSD) multiple comparison test) were applied. However, if the variance homogeneity criterion was not met (checked by Bartlett's test), non-parametric tests such as Mann Whitney (MW) were used. For the hidden platform water maze testing, individual trial data were averaged for each session (three trials per session) per mouse and the session data were analyzed. In all tests, males and females were found not significantly different, therefore their data are pooled.

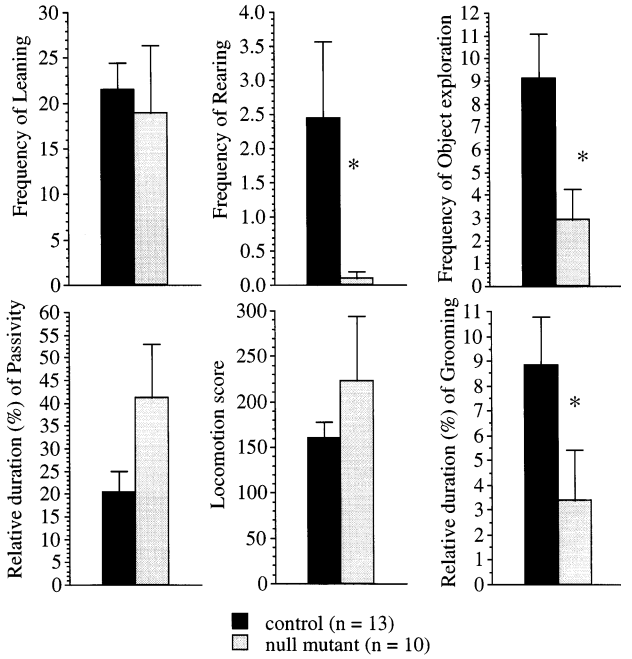

Fig. 1. Behavior of control and GluR2 homozygous null mutant mice in the novel arena. Bars represent mean, and error bars standard error (* $P < 0.05$). Sample sizes (n) are indicated. Note that although no differences were found in leaning, rearing frequency was significantly smaller in null mutants. Also note the apparent increase in the duration of passivity and, at the same time, the elevation of locomotion score in the null mutants.

3. Results

The results obtained in the novel arena are presented in Fig. 1. This situation evokes novelty induced exploratory behaviors [14–16] and is regarded as a test for exploratory activities, motor responses as well as curiosity [23]. GluR2 null mutant mice exhibited normal frequency of leaning but significantly decreased frequency of rearing (MW, $P < 0.05$), object exploration (t-test, $P < 0.03$) and decreased duration spent with grooming (MW, $P < 0.01$). Interestingly, mutant mice appeared to move more (increased locomotion score) and at the same time to stay motionless longer (increased passivity duration), nonsignificant changes compared to control (MW, $P > 0.95$; $P > 0.33$). Although nonsignificant in the mean difference, the peculiar locomotory pattern of GluR2 mutants (periods of complete passivity interrupted by bouts of highly elevated activity) translated into a high betwen individual variance in locomotion score (12-fold elevation compared to control, Bartlett test, $P < 0.0001$) and in passivity (Bartlett test, $P = 0.013$). In addition to the univeriate analyses, a multivariate ANOVA using all the above behavioral measures also showed a highly significant difference between GluR2 mutant and control mice (MANOVA $F_{12,30} = 74.035$, $P < 0.0001$) confirming a clear behavioral alteration.

The peculiar locomotory behavior of the GluR2 mice prompted us to study their motor performance (see Fig. 2B) on the rotating rod, an apparatus for testing motor coordination and balance with no requirement for extensive muscle strength or endurance. In this test GluR2 null mutants were significantly impaired (MW, $P < 0.0001$). Another test also confirmed the abnormalities. GluR2 mutant mice were significantly unresponsive to an approaching object (rubber rod) in the eye-closure test (see Fig. 2A) compared to control (MW, $P < 0.001$). The eye-closure response was associated with increased whisker movements and may imply that normal mice switched perceptual modality from visual to olfactory to explore the rod when it was placed close to them. The lack of response by GluR2 mutants may imply sensory impairment in either modality or impairment in processing such stimuli.

In the hidden platform task of the water maze (Fig. 3) GluR2 mice were highly passive and exhibited no improvement throughout the training, whereas their control counterparts decreased their escape latency dra-

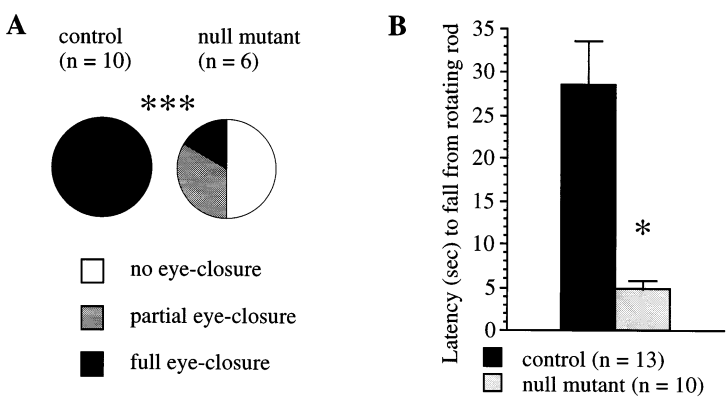

Fig. 2. A. The eye-closure test. The percent of mice responding to an approaching rod with full, partial, and lack of eye-closure is shown for control and GluR2 null mutant mice. Sample sizes (n) are also shown. B. The rotating rod test. The latency to fall from a rod rotating at 15 rpm is shown (mean + S.E.). Sample sizes (n) are also indicated. Note the significant differences (* $P < 0.05$; *** $P < 0.001$) between mutant and control mice.

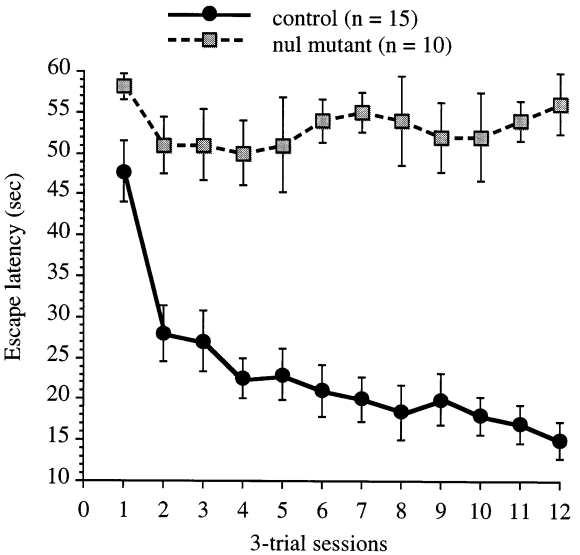

Fig. 3. The hidden platform (spatial version) of the Morris water maze. Latency to escape onto the platform from the water is measured in seconds. Error bars represent standard error. Sample sizes (n) are indicated. Note that mutant mice are significantly impaired compared to control in all sessions except the first.

matically (repeated measure ANOVA: mutation, $F_{1,23} = 108.41$, $P < 0.0001$; session, $F_{11,253} = 4.392$, $P < 0.0001$; session × mutation interaction, $F_{11,253} = 2.929$, $P = 0.001$). Tukey HSD test confirmed that GluR2 mutant mice were impaired significantly ($P < 0.01$) compared to control throughout the training except at the first session. It also showed that while control mice improved significantly with training, GluR2 null mutant mice did not.

Analysis of the probe trial data (Fig. 4) showed that after having been trained in the hidden platform task, control mice (Fig. 4B) exhibited a significant spatial bias towards the target quadrant and spent more than 60% of their time in it, a significant difference (t-test, $P < 0.0001$) compared to GluR2. Interestingly, GluR2 mutant mice stayed in the release quadrant for almost 65% of the session (second bar on Fig. 4A) due to their increased passivity. Furthermore, control mice were able to cross the exact location of the previously present platform significantly more frequently (MW, $P = 0.001$) than their GluR2 mutant counterparts (Fig. 5A). The observed severe impairments may be due to motor factors. Motor anomalies were implied by the novel arena test results and the rotorod. In the water maze, abnormal motor performance was also evident: the mutant mice floated motionlessly for prolonged periods of time (Fig. 5B) whereas their control counterparts hardly ever floated, a significant difference (MW, $P < 0.0001$). Interestingly, this passivity was not due to an inability to swim. When active, GluR2 mutant mice could swim well, in fact they swam significantly (MW, $P < 0.0001$) faster than control (Fig. 5C). This implies

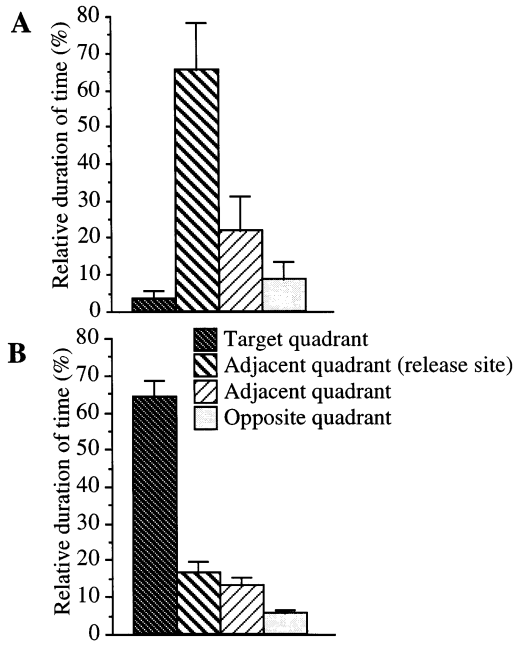

Fig. 4. Probe trial after completion of the hidden platform training. The relative duration of time (mean ± S.E.) the mice spent in each of four equal quadrants of the water maze is shown (A, GluR2 mutant; B, control). Sample sizes are as in Fig. 3. Note that control mice spent significantly more time in the target quadrant than mutant mice, whereas mutant mice spent most of their time near the release site (a quadrant adjacent to the target quadrant).

that impaired motor abilities per se did not play a major role in the abnormal learning performance of GluR2 null mutant mice in the hidden platform test, and perhaps other factors including sensitivity to sensory stimulation, motivational factors, or impaired spatial learning itself might have played roles. This suggestion is partially confirmed by the results of the

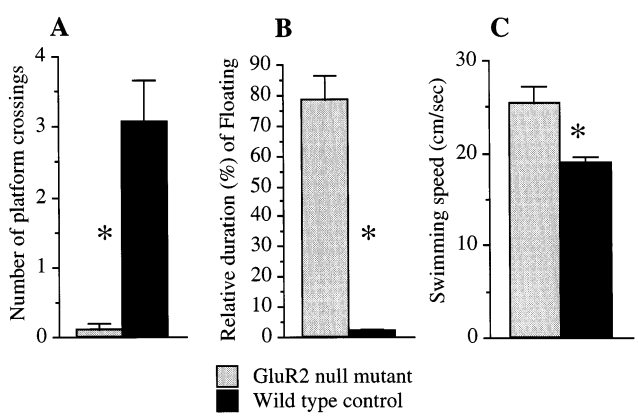

Fig. 5. The number of platform crossings, relative duration (%) of floating, and swimming speed (cm/s) during bouts of swimming recorded in the probe trial are shown (mean ± S.E., * $P < 0.05$). Sample sizes are as in Fig. 3. Note that control mice crossed the exact platform location more frequently and they floated less than the mutants. Also note that when active, mutant mice swam faster than control mice.

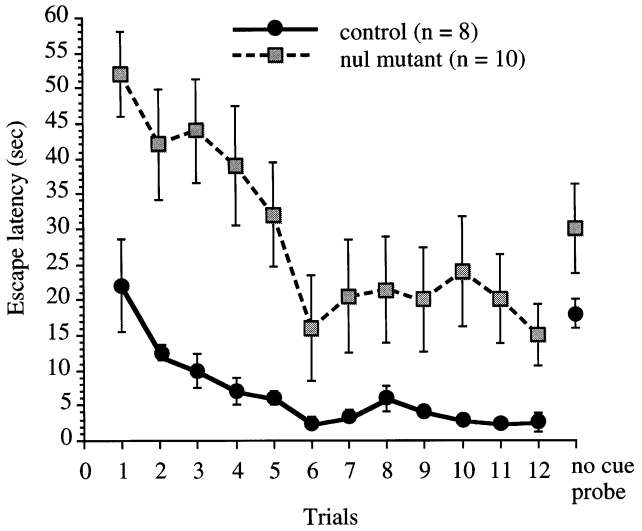

Fig. 6. Visible platform (non-spatial) task of the Morris water maze. Latency to escape (s) onto the platform from the water is measured. Error bars represent standard error. Sample sizes (n) are indicated. Separate points represent last trial with no visible cue marking the platform. Note that although GluR2 null mutant mice significantly improved with training, they were impaired compared to control.

non-spatial learning task in which, although impaired, GluR2 mutant mice showed significant improvement. In this latter task (visible platform) GluR2 null mutant mice were able to improve (Fig. 6) and perform three times better by the end of the training compared to the start. Nevertheless, the mutant mice continued to be more passive and exhibited greater escape latency values than control throughout the training (ANOVA mutation $F_{1,16} = 30.64$, $P < 0.0001$; trial $F_{11,176} = 4.73$, $P < 0.0001$; interaction term $F_{11,176} = 1.49$, $P > 0.10$).

After the last trial of the visible platform training a probe trial was administered during which the visible cue marking the platform was removed (separate points on Fig. 6). Comparison of performance during this probe trial and the last trial of the training shows that both the GluR2 mutant and the control mice needed more time (increased escape latencies) when no visible cue marked the location of the platform. However, GluR2 mice were impaired compared to control even in the no-visible cue situation (ANOVA mutation, $F_{1,15} = 6.80$, $P = 0.020$; cue, $F_{1,15} = 14.40$, $P < 0.01$; interaction term, $F_{1,15} = 0.003$, $P > 0.95$).

Gross neuroanatomical analysis of the GluR2 null mutant mice demonstrated that their brain possessed all major neuroanatomical loci and fiber pathways in normal proportion. As shown in Fig. 7A and C, the hippocampi of GluR2 mutant mice exhibited normal cytoarchitecture in the CA1-3 region and dentate gyrus and possessed all major fibre tracts of this structure. The perforant pathway connecting the etorhinal cortex with dentate gyrus was also normal (data not shown). Tracts such as the stria medullaris, habenulo-interpeduncular tract, and segments of the anterior commissure and corpus callosum seemed also unaltered in GluR2 mutants (Fig. 7A). As expected, regions which express lower levels of GluR2 (e.g. hindbrain and brainstem) were also without neuroanatomical defects (Fig. 7B). Also note that GluR2 mutant mice exhibited a normal pattern of collicular layers. The tracts of the cerebellar peduncles and the pontine nuclei were present in normal proportions (Fig. 7B). A representative cross-section of the cerebellum of a GluR2 mutant mouse (Fig. 7D) demonstrates that the mutants possess all the cerebellar lobes. Examination of the laminae of the dorsal spinal cord (a region of strong GluR2 expression) also revealed no major defects (data not shown). In addition, coronal serial sections examined for cytochrome-c oxidase activity showed no alterations in GluR2 mutants compared to control. This mitochondrial enzyme is located primarily on the dendrites and somata of post-synaptic cells and may reflect the level of pre-synaptic input. Finally, 10 μm thionin stained coronal sections suggested no differences in the morphology or cytostructural properties of GluR2 mutant and control somatosensory cortices or hippocampi (not shown).

4. Discussion

No gross neuroanatomical alterations were found in the GluR2 null mutant mice. The behavioral deficits and the expression pattern of GluR2, however, suggest the involvement of multiple brain regions. This implies that the significant neurophysiological abnormalities previously shown in the hippocampus [31] may in fact be characteristic of neural processing throughout the brain. Furthermore, it is unlikely that the deficits are due to background genes [20] because comparison of the founder strains 129 and CD1 and their F_2 segregating hybrid generation did not reveal alterations similar to those seen in the mutants.

Although the overall muscle strength of mutants appeared to be unaffected (healthy appearance, unchanged leaning frequency and locomotion score, etc.), a number of abnormalities were noted. Motorically not demanding behaviors, including grooming or object exploration were significantly altered. Also, activity of the mutants was distributed in a variable manner: they were either hyperactive or lethargically passive. Furthermore, although rearing and leaning are both associated with good motor function only rearing frequency was reduced in the GluR2 mutant mice. A study of genetic correlations have suggested different biological mechanisms underlying rearing and leaning [15]. Thus the above results imply that the abnormalities of the mutant mice are not simply due to altered motor function but perhaps to impaired motivational or stimulus

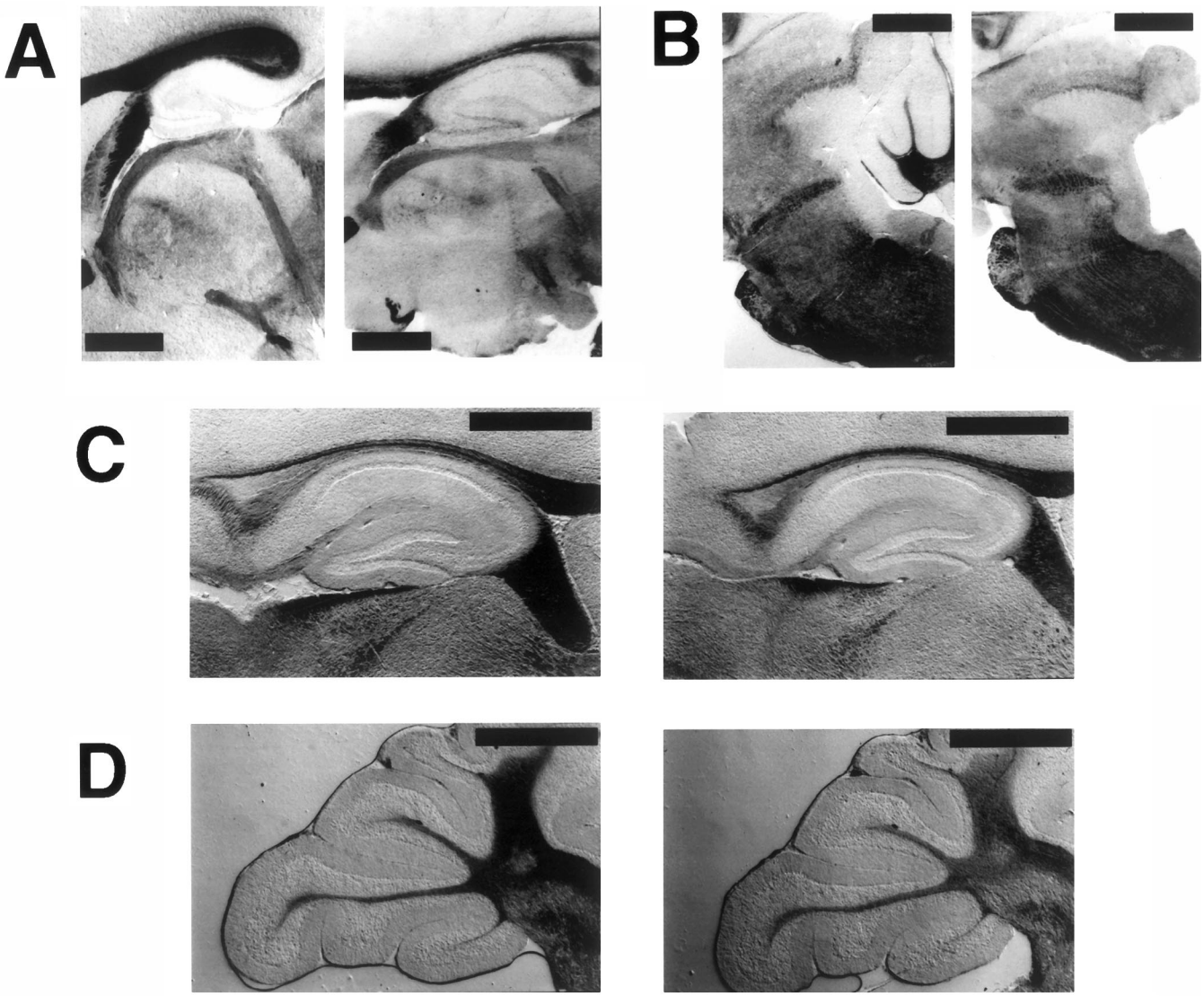

Fig. 7. Pairwise sagittal comparisons of GluR2 −/− (left) and +/− (right) littermates at 6.5 weeks of age. Sections were mounted in aqueous glycerol media and visualized by interference contrast microscopy. A. Hippocampal formation and thalamus (approximately 200 μm from midline). B. Hindbrain and brainstem structures (350 μm from midline). C. Higher power view of the hippocampus (900 μm from midline). D. Cerebellum (900 μm from the midline). Scale bar = 1 mm. Note that no differences could be detected at the level of light microscopy between mutant and control mice.

processing properties. For example, it is known that leaning can be seen in novel situations within the first minute of the test whereas rearing appears later suggesting different motivational backgrounds underlying these behaviors.

Sensitivity to sensory stimulation is clearly changed in GluR2 mutants: they exhibited decreased eye-closure responses. Performance at a rotorod task was also impaired in GluR2 null mutants, a finding that may suggest altered motor function but may also be compatible with impaired sensory abilities. Spatial and non-spatial learning performance was also severly disrupted in the GluR2 null mutant mice despite the fact that their swimming ability (speed) was not impaired. Notably, however, non-spatial learning performance was less impaired than spatial suggesting a potential involvement of a hippocampal component [51]. A hippocampal dysfunction, however, is unlikely to explain all observed defects. But perhaps hyposensitivity to stimuli is an underlying factor. It could explain abnormal responses to novelty, the absence of eye-closure reflex, the inability to stay on the rotating rod, and the lack of motivation to escape from the cold water of the water-maze. Hyposensitivity to stimuli is unlikely to be due to simple sensory problems since the various tests applied involved highly different perceptual requirements. Since simple motor impairment is also unlikely, we suggest that the observed multiple abnormalities may be due to central neural processing of stimuli, and potentially to abnormal motivation or arousal levels.

The neurobiological substrate of these abnormalities is only speculative at this point. Neurophysiological analysis of the GluR2 null mutant mice [31] showed that kainate induced Ca^{2+} permeability was increased to approximately 9-fold in isolated CA1 pyramidal neurons of the mutant GluR2 hippocampus in comparison with control responses. This was accompanied by 2 fold elevated LTP responses evoked by tetanic stimulation in the mutants compared to control animals. Furthermore, the abnormal elevation of LTP in the GluR2 mutant hippocampus was NMDA receptor independent and appeared to be nonsaturable [31]. Based on these and other neurophysiological experiments, we suggested [31] that calcium permeable AMPA-receptors can induce LTP and the absence of GluR2 (an AMPA receptor subtype) leads to abnormally elevated LTP via increased Ca^{2+} permeability in the hippocampal neuron.

These results may account for the observed behavioral abnormalities if one postulates that increased Ca^{2+} permeability can lead to abnormally elevated or altered synaptic strengthening. Since in the mutant mice Ca^{2+} permeability may be increased in all neurons that would normally express GluR2 (mostly pyramidal and granule cells [19]), the absence of this protein may lead to non-specific synaptic strengthening throughout the brain. Our present behavioral findings therefore are significant in that they suggest the involvement of multiple brain areas and not only the hippocampus, the target of a previous study. Since several aspects of brain function are thought to depend upon input specificity or synapse specific strength changes [26], an overall non-specific synaptic potentiation could plauslibly lead to a variety of problems in the GluR2 mutant mice. For example, constitutively elevated Ca^{2+} permeability may cause hyposensitivity (sensory and somatosensory cortices), motor dysfunction (cerebellum), emotional and motivational (amygdala), and learning impairments (association cortices and hippocampus). It is also possible that increased calcium permeability of the AMPA channel may lead to other abnormalities, including altered neuronal survival [44], accelerated aging [48,38] and vulnerability to excitotoxic stimulation [45], etc. Therefore, the GluR2 mutant mouse, the first mammalian model with abnormally elevated LTP and neuronal Ca^{2+} permeability, will be a valuable tool for the study of calcium related neurobiological processes.

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