

# Reduced aggression in AMPA-type glutamate receptor GluR-A subunit-deficient mice

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**The importance of AMPA-type glutamate receptors has been demonstrated in neuronal plasticity and in adaptation to drugs of abuse. We studied the involvement of AMPA receptors in social interaction and anxiety and found that in several paradigms of agonistic behavior naive male mice deficient for the GluR-A subunit-containing AMPA receptors are less aggressive than wild-type littermates. GluR-A deficient mice and wild-type littermates exhibited similar basic behavior and reflexes as monitored by observational Irwin's test, but they tended to be less anxious in elevated plus-maze and light-dark tests. Maternal aggression or male-female encounters were not affected which suggests that male hormones are involved in the expression of suppressed aggressiveness. However, testosterone levels and brain monoamines can be excluded and found to be similar between GluR-A deficient and wild-type littermates. The reduced AMPA receptor levels caused by the lack of the GluR-A subunit, and measured by a 30% reduction in hippocampal [<sup>3</sup>H]-S-AMPA binding, seem to be the reason for suppressed male aggressiveness. When we analyzed mice with reduced number of functional AMPA receptors mediated by the genomic introduced GluR-A(Q582R) channel mutation, we observed again male-specific suppressed aggression, providing additional evidence for GluR-A subunit-containing AMPA receptor involvement in aggression.**

Keywords: agonistic behavior, AMPA receptors, glutamate receptors, knockout mice, social interaction

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Glutamate receptors are widely expressed in brain structures controlling emotions and behavioral patterns such as agonistic behavior (Davidson *et al.* 2000; Dingledine *et al.* 1999; Krishnan 1999; Schoepfer *et al.* 1994), suggesting a role for them in these behaviors. Both N-methyl-D-aspartate (NMDA) and L- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors (GluRs) are involved in making up the hormonal status (Carbone *et al.* 1996), and feedback by gonadal steroids to regulate GluR subunits has been shown (Diano *et al.* 1997). Interactions between hormonal status and aggressiveness in male mice are also well documented (Martinez-Sanchis *et al.* 1998; Sandnabba *et al.* 1994). The shift of the excitatory-inhibitory neurotransmission ratio in favour of the excitatory one leads to augmentation of agonistic behavior in many species (Bullock & Rogers 1986; Munoz-Blanco *et al.* 1986; Munoz-Blanco & Porras Castillo 1987). Thus, in regulation of aggressive behavior, the glutamate system might well have an important but still unclear significance (Siegel *et al.* 1999). However, there are little experimental data on how glutamate agonists and antagonists affect social behavior. The NMDA receptor antagonist dizocilpine (MK-801) has a biphasic effect (enhancement at low doses and inhibition at higher doses) on apomorphine-induced agonistic behavior (Lang *et al.* 1995) and on the play behavior in rats (Siviy *et al.* 1995). The social behavioral aspect of AMPA receptors have not been investigated at all, although these receptors are involved in emotional regulation, the antagonists often producing anxiolysis (Czlonkowska *et al.* 1997; Kotlinska & Liljequist 1998; Matheus & Guimaraes 1997).

The lack of the AMPA receptor subunit- or subtype-selective antagonists available to study the role of AMPA receptors in agonistic behavior supports the use of mice with targeted deletion of specific genes. These mutant animals provide a new approach to establish the molecular basis of behavior (Nelson & Young 1998). In the present study, we examined the aggressive profile of a mouse line lacking the GluR-A subunit-containing AMPA receptors (GluR-A<sup>-/-</sup> mice) (Zamanillo *et al.* 1999). These mice show normal spatial learning (Zamanillo *et al.* 1999) except when spatial choices must change flexibly in response to trial-specific memories (Reisel *et al.* 2002). Then their spatial memory performance becomes impaired. They are hyperactive but they habituate normally to

novel environment (Vekovischeva *et al.* 2001). Furthermore, the GluR-A deficient mice have impairment in learning cued conditioning responses (Mead & Stephens 2003).

To substantiate our findings and to exclude phenotypes mediated by genetic background or developmental effects we employed a second mouse line which has a strong reduction in functional GluR-A containing AMPA receptors (Vekovischeva *et al.* 2001). In these mice the reduction of functional GluR-A containing AMPA receptors is achieved by replacing a glutamine (Q) codon by an arginine (R) codon in the gene segment encoding the glutamate receptor channel pore. AMPA receptor channels made of subunits with the amino acid residue R582 are very low in conductance and practically calcium-impermeable (Burnashev *et al.* 1992). In fact, in principal neurons of these mice (GluR-A<sup>R/R</sup>) the pool of functional GluR-A subunit-containing AMPA receptors is strongly reduced and comparable to the pool of remaining functional AMPA receptors in GluR-A subunit-deficient mice (Rolf Sprengel, unpublished data).

The immediate impetus for the present study was the reduced aggressive behavior of GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice in our pilot experiments (not shown). To better assess the role of GluR-A subunit-containing AMPA receptors in behavior we compared GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice and their littermates in tests of agonistic behavior in situations provoked by male attacks (Brain & Haug 1992; Vekovischeva *et al.* 2000), of the male sexual behavior towards a female (Hilakivi *et al.* 1989a) and of the maternal agonistic behavior towards a male intruder during lactation period (Parmigiani *et al.* 1998). Furthermore, parts of Irwin's observational test and elevated plus-maze and light/dark tests of anxiety were carried out to establish the behavioral phenotype of the GluR-A<sup>-/-</sup> mice. Brain levels of monoamines and blood testosterone concentrations were also determined and ligand autoradiography with [<sup>3</sup>H]S-AMPA to image the brain GluR distribution was performed to compare the mouse lines.

## Materials and methods

### Animals

The GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice were constructed as described (Vekovischeva *et al.* 2001; Zamanillo *et al.* 1999) using the embryonic stem cell line R1 (Nagy *et al.* 1993), that derived from F1 embryos of males from 129S1/Sv-p<sup>+</sup>Tyr<sup>+</sup>Kitl<sup>S<sup>-</sup>J</sup>/+ substrain crossed to females from 129 × 1/SvJ substrain. The RI cells did not receive the Kitl<sup>S<sup>-</sup>J</sup> mutation (Laura Trepanier, Jackson Laboratories, Bar Harbor, ME). The mutant mice were backcrossed for more than five times in C57BL/6J strain in Germany. For our experiments heterozygous breeding pairs were produced with C57BL/6J mice by a balanced breeding schedule to maintain genetic components equally from males and females of both homozygous mutants and wild-types and transferred to Finland. The experimental mice (mutants and littermates) were produced

by heterozygous mating, and raised by heterozygous mothers to exclude possible emotional influence of mutant parents on pups' development (Winslow *et al.* 2000). When tested, the male mice had no sexual experiences with females, except that the males cohabited with females for 5 days and later tested in a resident-intruder paradigm (see below). The genotype of mice was determined at the age of one month by PCR analysis of tail-tip DNA (Vekovischeva *et al.* 2001; Zamanillo *et al.* 1999), and the mutant and wild-type animals were thereafter maintained in separate cages. At the age of 3–5 months adult male mice living in 5–7 male groups were transferred into individual polypropylene cages (20 × 10 × 15 cm) in a facility artificially illuminated from 07:00 to 19:00 with air-conditioning (21 °C) and relative humidity of 50–60%. Tap water and rodent pellets (Special Diet Service, Witham, UK) were available *ad libitum*. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Turku.

### Agonistic behavior

Numerous behavioral situations were chosen to provoke agonistic behavior in mice (Brain & Haug 1992). Most of the tests were carried out as resident-intruder paradigms, in which each male interacted in his individual home cage (20 × 10 × 15 cm) with an unfamiliar intruder male for 4 min. Mouse encounters were videotaped and analyzed subsequently using computer-assisted data acquisition software (Etholograph 2.06, Ritec, St. Petersburg, Russia) (Nyberg *et al.* 2003; Poshivalov *et al.* 1988). Mouse social behavior was subdivided in categories such as consummate aggression (throws, fighting, bites, boxing), ambivalent aggression (tail rattling, threats, digging of aspen chips, spinning around the partner, aggressive intensive partner grooming), defence (defence pose on the back, vertical upright and lateral sideways stances, freezing, pushing away, flight) and exploration of the partner (sniffing partners' body and its anogenital area, light grooming of the partner). The division of aggression to consummate and ambivalent aggressions made it possible to separate the execution of aggression (consummate) from the demonstration of intentions to attack (ambivalent). The body cleaning (self-groom), rearing, locomotion (all horizontal movements including chasing, to minimize subjective evaluation) and sitting with sniffing were monitored separately. Other behavioral elements were pooled in a category 'other behavior' (e.g. mounting on a partner, creeping under a partner, rotation, jumping, recumbent pose). The intruder was selected from nonaggressive group-housed males of the same strain as the resident, except for the interline encounters. When the resident-intruder paradigm was repeated after various periods of isolation, the intruder for each isolated mouse remained the same for all tests. The behavioral profile was determined as relative durations of separate elements and categories in a 240 second test period. In addition, for some tests latencies and frequencies of the behavioral elements were also recorded.

Because the differences in agonistic behaviors between the mutants and their wildtypes were so clear, the tests were scored openly by OYV and OE. However, to decrease subjectivity in scoring, intermale encounters were also rated by TA, who did not know the experimental details and was blind in 50% of the tests in regard to the genotype of the individual mice. Spearman correlation coefficients ( $>0.66$ ) between the two scored durations of each behavioral element for 11 GluR-A<sup>-/-</sup> and 11 littermate mice were highly significant ( $>0.66$ ,  $P < 0.0008$ ), except for the duration of 'other behaviors' (coefficient 0.37, NS) that was estimated longer and more variably by TA. Means of both scorings are used to describe these data in Table 2 and Fig. 2.

#### **Isolation-induced agonistic behavior analysed by home-cage resident-intruder test**

The test mice were individually housed for 21 days. The encounters of each test male with an unfamiliar group-housed intruder were carried out on the 1st, 5th and 21st days of isolation, on the resident territory of the test mouse. The experiment involved 11 isolated GluR-A<sup>-/-</sup> and 11 littermates and 8 GluR-A<sup>R/R</sup> mutants and 8 littermates.

#### **Agonistic behavior in male mice with sexual experience**

Adult male mice were first pair-housed with fertile females for 5 days and their sexual potential was tested first as described below in *Sexual behavior*. Testing using the home-cage resident-intruder paradigm started when the female was removed and the intruder of the same strain entered the cage. Seven GluR-A<sup>-/-</sup> mice with 7 littermates and 9 GluR-A<sup>R/R</sup> with 9 littermates were tested.

#### **Interline agonistic behavior on neutral territory**

Encounters between male GluR-A<sup>-/-</sup> and littermates or between male GluR-A<sup>R/R</sup> and littermates were analysed in neutral territory (fresh aspen chips), that was similar in size to that of home cages. Two 3-week isolated males, chosen randomly from different families, were put on neutral territory simultaneously. The number of encounters of GluR-A<sup>-/-</sup> vs. wild-type littermates was 11, and eight for GluR-A<sup>R/R</sup> vs. wild-type littermates.

#### **Agonistic behavior within the group**

Within each mouse genotype, individual mice isolated for 30 days were exposed to each other. For GluR-A<sup>-/-</sup> mice and their controls we used 11 mice per line, and for GluR-A<sup>R/R</sup> and their controls eight mice. These mice were experienced with social contacts from previous three resident-intruder examinations. All mice of one GluR-A genotype (e.g. GluR-A<sup>-/-</sup>) were put simultaneously in a novel large cage (25 × 45 × 15 cm) with fresh aspen chips-covered floor and the number of aggressive interactions was monitored for 10 min. The test was repeated daily and males that had shown aggression were excluded from the encounters

on the following day(s). This procedure was stopped as soon as all aggressive males were identified. The relative durations of consummate aggression between individuals were calculated.

#### **Sexual behavior**

The sexual potential of sexually naïve males was tested in male-female encounters during 10 min. The last four min were analyzed statistically. The standard group-housed naïve female of the same strain and age (3–5 months) was put with a one-week isolated male. The sniffing of female anogenital area, pursuit of female and category of sexual behavior (attempt of mount, female capture, mounting with intromission, ejaculation) were added to behavioral profile used in the resident-intruder paradigm. Seven males of GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice and seven and eight males of their littermates were used, respectively.

#### **Maternal agonistic behavior**

The females co-housed with a male for five days were isolated during the period of gestation. The female's behavioral reaction towards an unfamiliar male intruder of the same genotype was tested for 10 min on 10th postpartum day (Gammie & Nelson 1999) immediately after the newborn mice had been removed. Because of problems by GluR-A<sup>-/-</sup> females with baby rearing, the test was carried out only for GluR-A<sup>R/R</sup> females ( $n = 5$ ) and their littermates ( $n = 5$ ).

#### **Observational behavioral testing**

Behavioral properties of the male GluR-A<sup>-/-</sup> mice and their littermates (16 GluR-A<sup>-/-</sup> mice and eight littermates) were tested using parts of the primary screen of SHIRPA protocol ([www.mgu.har.mrc.ac.uk/mutabase/shirpa\\_1.html](http://www.mgu.har.mrc.ac.uk/mutabase/shirpa_1.html)), originally based on Irwin's systematic examinations (Irwin 1968; Rogers *et al.* 1999) in the manner described below in detail. Mice were observed with the naked eye in a viewing glass jar, in an open arena and on a grid floor. The viewing glass jar was a transparent Plexiglas cylinder (15 × 11 cm), where a mouse was evaluated for 45 seconds and evaluated for body position, spontaneous activity, respiratory rate and presence of tremor. The open arena (55 × 33 × 18 cm) floor was covered with a plastic sheet marked with 15 equal squares. The first behavioral reaction of a mouse placed at the centre of the arena (transfer arousal), number of crossed squares, gait, pelvic and tail elevations were assessed for 30 seconds. The grid floor (40 × 20 cm with 12-mm mesh) was used as a support for the viewing jar and to measure tail suspension, grip strength and negative geotaxis reflex. Negative geotaxis reflex was assessed as a possibility of a mouse to turn around and climb up on the grid floor quickly raised to the vertical plane. The sensorimotor responses consisted of startle, pinna, corneal and toe pinch reflexes. A click box generating a sudden tone at 90 dB was held 30 cm above the mouse to measure startle reflex. Pinna and corneal reflexes were provoked by a stainless steel wire (15 mm in

length and 0.15 mm in diameter) with a gentle touch on ear lobe or cornea of the eye. The toe pinch reaction was measured by pinching the central toe of the right hind paw with forceps. The struggle-escape behavior combined the touch-escape response and positional passivity scores as well as agonistic behavior toward an experimenter and mouse vocalization provoked during handling. Touch-escape behavior was determined as a response to a finger stroke of the back.

All these tests were scored as described in the SHIRPA protocol. Because all the scores for the mutant mice were statistically not different from those for their wildtype littermates, the data are not shown.

### **Anxiety behavior**

To evaluate the anxiety profile of GluR-A<sup>-/-</sup> mice and littermates, the elevated plus-maze and light-dark tests were used with naïve mice for 5 min sessions. Fourteen GluR-A<sup>-/-</sup> males and 12 littermates were tested in the plus-maze. The maze, made of dark-brown Plexiglas, comprised two open (40 × 10 cm) and two closed (40 × 10 × 45 cm) arms, which extended from a common central platform (10 × 10 cm). The entire maze was elevated to a height of 50 cm above floor level. The test started with gently placing the mouse on the central platform facing an open arm. Arm entries were defined as entry of all four paws into an arm. The behavior occurring in closed arms and central platform was indicated as 'protected', while the behavior in open arms was regarded as 'unprotected'. The main behavioral categories were chosen as described (Rodgers & Dalvi 1997).

In the light-dark test, five male GluR-A<sup>-/-</sup> and littermate mice were used. The test was started by placing the mouse into the light compartment of the two-compartment box divided into a dark and lit area, 30 × 30 × 35 cm each with an open door (12 × 9 cm) in between (Crawley 1981). The latency until the first entry into the dark compartment, number of crossings between the compartments and the time spent in the light compartment (minus the latency time) were recorded (Nyberg *et al.* 2003).

### **Tissue samples**

The levels of testosterone in blood and monoamines in several brain areas such as dorsal and ventral striatum, amygdala, hippocampus, olfactory bulb and cerebral cortex were determined in seven male GluR-A<sup>-/-</sup> and eight littermates. To be sure that the biochemical measures could reflect the behavioral differences between the mouse lines, we used mice that had been tested previously in resident-intruder paradigm after 5 days of isolation. The mice showed representative line differences in agonistic behaviors similar to the data in Table 1. Five days later the behavioral testing age-matched male mice were anaesthetised with CO<sub>2</sub> and decapitated. The time schedule should be short enough to prevent any strong isolation-induced alterations in brain monoamines (Hilakivi *et al.* 1989b), and long enough for the animals to recover from possible stress-effects at the begin-

ning of isolation (Rilke *et al.* 1998). The trunk blood was collected into heparinized tubes, centrifuged and plasma stored for analysis of testosterone. The brain areas were dissected with a brain mould (for reference see the mouse brain atlas Paxinos & Franklin 2001), frozen on dry ice and stored at -80 °C until the determination of monoamine concentrations.

Whole brains from three male GluR-A<sup>-/-</sup> mice and three littermates were dissected, frozen on dry ice and stored at -80 °C until sectioned for autoradiography with a cryostat. The animals were three months old and naïve to experimental manipulations.

### **Determination of testosterone in blood plasma**

Plasma samples were purified using diethylether extraction and testosterone concentrations were determined using a radioimmunoassay as described (Huhtaniemi *et al.* 1985). The intra-assay and interassay coefficients of variation of the method are <6% and <12%, respectively.

### **Determination of brain monoamine levels**

The brain monoamines dopamine and serotonin (5-hydroxytryptamine or 5-HT) and their metabolites [3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) for dopamine and 5-hydroxyindoleacetic acid (5-HIAA) for 5-HT] were measured using HPLC with electrochemical detection as described earlier (Kankaanpää *et al.* 2001), with the following necessary modifications for processing of brain tissue samples. The frozen tissues were weighed and placed in ninefold quantity of antioxidative solution (1.0 mM oxalic acid, 3.0 mM L-cysteine and 0.1 M acetic acid), using a Vibra-Cell VC 600 high intensity ultrasonic processor (Sonic and Materials Inc., Danbury, CT) equipped with a tapered microtip. The processing time was 1.5 seconds and the amplitude was set at 40% of the maximum value. After centrifugation, the supernatants were transferred to clean vials, and injected to HPLC in a volume of 10 µl.

### **Glutamate receptor autoradiography**

Coronal brain sections of 14 µm thickness were cut in a cryostat and were thaw-mounted onto gelatine-coated glass slides. Slides were dried at room temperature and stored at -70 °C until time for experiments. The autoradiographic procedure for the AMPA-type glutamate receptor ligand α-[5-methyl-<sup>3</sup>H]-(*S*)-AMPA [(*S*)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (NEN Life Science Products, Boston, MA)] was modified from (Nielsen *et al.* 1990), that for the kainate-type glutamate receptor ligand [<sup>3</sup>H]-(*2S*, 4*R*)-4-methylglutamate ([<sup>3</sup>H]SYM 2081, Tocris, Bristol, UK) was according to (Carroll *et al.* 1998), and that for the NMDA-type glutamate receptor ligand [<sup>3</sup>H]MK-801 (NEN) according to (Näkki *et al.* 1995). [<sup>3</sup>H]-(*S*)-AMPA procedure was started with 30-min preincubation of the thawed sections in ice-cold 50 mM Tris-citrate (pH 7.2), followed by

**Table 1:** Behavior of the GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mutants and littermates in home-cage resident-intruder encounters after various periods of social isolation

Behavioral categories and elements		WT (n = 11)			GluR-A <sup>-/-</sup> (n = 11)			WT (n = 8)			GluR-A <sup>R/R</sup> (n = 8)		
		1st	5th	21st	1st	5th	21st	1st	5th	21st	1st	5th	21st
Consummate aggression	l	148	155	122	232*	213*	230*	104	96	130	220*	215*	206*
	f	7	5	12	0.1*	0.3*	0.2*	10	14	11	1*	3	3
	d	3	7	12	0	0*	0*	26	19	19	0*	7	3*
	n	45	55	82	9	18	9*	75	75	88	13*	13*	38
Ambivalent aggression	f	10	7	5	0.3*	0.4*	0.4*	7	7	7	0.2*	1.5*	1.2*
	d	19	22	10	0*	0*	3*	12	24	12	0*	3*	3*
Partner exploration	f	28	18	16	36	29*	37*	28	23	30	39	43*	45*
	d	40	36	40	79*	65	84*	45	38	57	91	101*	120*
Locomotion	f	26	32	37	33	33	34	23	24	21	28	28	27
	d	26	53	60	70*	67	72	46	36	31	44	54	56
Grooming	f	3.0	1.1	2.0	0.3	0.3	0.3	0.4	0.4	0.7	0.4	0.3	0.5
	d	17	5	0	3	0	3	5	3	5	0	3	3
Rearing	f	4	5	5	13*	14*	11*	4	4	6	18*	13*	12
	d	7	12	17	41*	38*	29	10	12	17	55*	36*	29
Sitting with sniffing	f	22	24	18	15	21	16	24	21	19	12*	10*	10*
	d	122	100	91	46*	63*	51*	91	102	89	48*	36*	24*
Other behaviors	f	2	9	4	3	2	0.4	4	7	6	1	1	2
	d	7	5	10	3	7	0	5	7	10	3	3	5

The data (number of animals shown in parentheses) are expressed as follows: l – latency (seconds) for the first consummate aggressive element (animals not showing the element were rated at 240 seconds); f – frequency of the behavioral element per 240 s-test time; d – average duration of separate behavioral elements (seconds); n – proportion of animals that showed aggression (%). \**P* < 0.05 (Duncan test) for the significance of the differences between GluR-A<sup>-/-</sup> or GluR-A<sup>R/R</sup> mice and their littermates.

60-min incubation in ice-cold Tris-citrate supplemented with 20 nM [<sup>3</sup>H]-S)-AMPA. The sections were briefly (3 × 2 seconds) washed ice-cold Tris-citrate, dipped in ice-cold H<sub>2</sub>O and dried under a fan at room temperature. [<sup>3</sup>H]SYM 2081 procedure was started with 30-min preincubation in ice-cold 50 mM Tris-citrate – 100 mM KCl (pH 7.4), followed by 45-min incubation in ice-cold buffer supplemented with 5 nM [<sup>3</sup>H]SYM 2081. The sections were washed 3 × 5 seconds in ice-cold buffer, and treated as above. In both of these assays, the non-specific binding in the presence of 1 mM glutamate was negligible at the background level. [<sup>3</sup>H]MK-801 procedure was started with 30-min preincubation at room temperature in 50 mM Tris-acetate (pH 7.4), followed by 120-min incubation at room temperature in the same buffer supplemented with 10 nM [<sup>3</sup>H]MK-801, 100 μM glutamate and 10 μM glycine. After incubation, the sections were rinsed in ice-cold buffer, washed for 80 min in the same conditions, and then treated as above. The non-specific binding was assessed in the presence of 10 μM unlabelled MK-801. The dried sections were then exposed to BioMax MR films (Eastman Kodak, Rochester, NY) together with plastic [<sup>3</sup>H]microscales standards (Amersham Biosciences, Piscataway, NJ). The films were developed, and the binding densities on the films were measured with the M5 program (Imaging Research Inc., St. Catharine’s, Ontario, Canada) with standards as a reference. Representative images of the

[<sup>3</sup>H]-S)-AMPA binding were scanned and processed with identical settings using an HP scanner and the CorelDraw 10 program.

**Statistical analyses**

Statistical analysis was conducted using SAS-STAT software (release 6.12, SAS Institute, Cary, NC). If the data were not distributed normally by Shapiro Wilk’s test, the rank statistical procedure was used to normalize them. The comparison of behavioral and physiological measurements between the knockout mice and their littermates was carried out by one-way ANOVA with appropriate *post hoc* Duncan test for measurement of group differences. Additionally, the role of the genotype (knockout or their littermates) and repeated confrontations (1st, 5th and 21st days) in behavior after isolation was evaluated by two-way repeated measures ANOVA. Null hypothesis was rejected at *P* < 0.05 level. The percentage of animals showing aggressive behavior in each test was analyzed by Fisher’s test (*P* < 0.05).

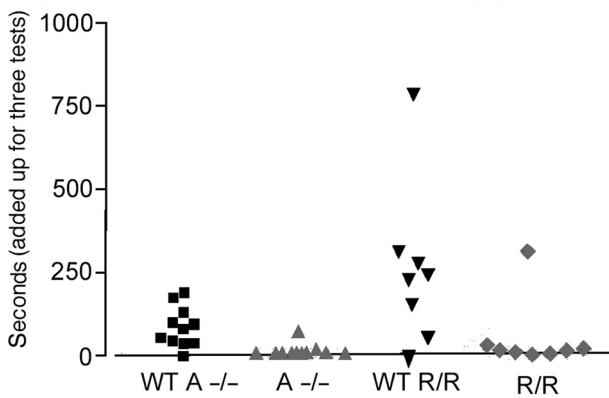
**Results**

**Agonistic behavior in GluR-A deficient mice**

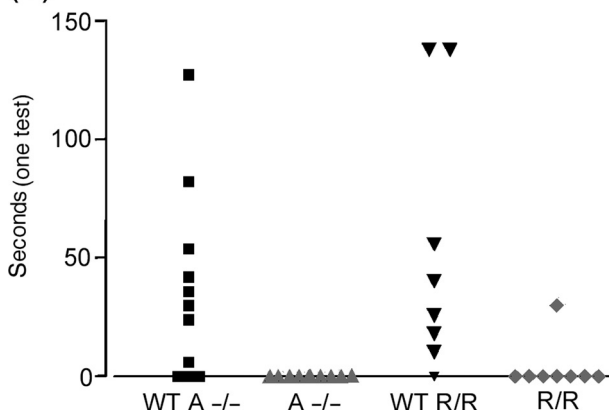
In the first experiments, we kept the mice isolated in single cages for 1, 5 and 21 days and studied their behavior in the resident-intruder paradigm in the home cage. Higher levels of

agonistic behavior, both in duration, frequency and shorter latency of aggressive elements and in number of mice expressing them, were found in the wild-type compared to GluR-A deficient mice, especially as resident's isolation time was getting longer. The summary of the all three time points tested showed that the GluR-A<sup>-/-</sup> mice exhibit significantly reduced frequency and duration of consummate [ $F_{1,66} = 16.69, P < 0.01$ ] and ambivalent aggression [ $F_{1,66} = 34.79, P < 0.0001$ ] (Fig. 1a). Duration, but not frequency [ $F_{1,66} = 2.07, NS$ ], of passive exploration of environment (sitting with sniffing) was reduced [ $F_{1,66} = 23.75, P < 0.0001$ ]. Frequency and duration of partner exploration [ $F_{1,66} = 7.45, P < 0.05$ ] and rearing [ $F_{1,66} = 24.25, P < 0.0001$ ] were increased and the duration, but not frequency, of locomotion was slightly higher [ $F_{1,66} = 12.34, P < 0.005$ ] in GluR-A<sup>-/-</sup> mice than in their controls (Table 1).

(a) Duration of consummate aggression



(b)



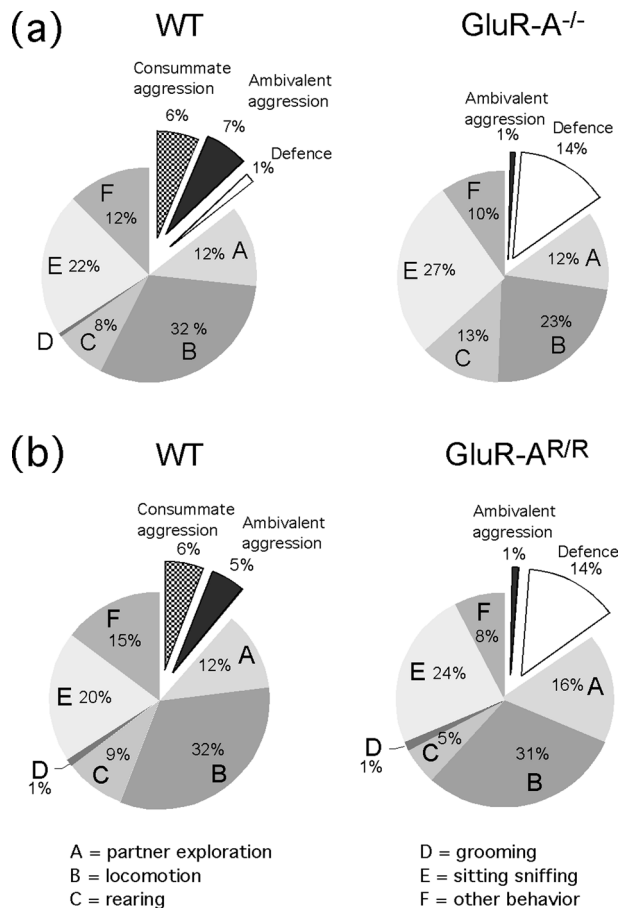
**Figure 1: Aggression of GluR-A subunit-deficient and control animals in resident-intruder and group tests.** (a) Sums of durations of consummate aggression for individual mice from three resident-intruder tests with the total test time of 12 min (3 × 4 min) after different periods of isolation. (b) Duration of consummate aggression during a group encounter for individual male mice after 4-week isolation. Testing time was 10 min. Symbols on the x-axis denote nonaggressive animals.

The reduced aggression observed in the GluR-A subunit-deficient mice might be the result of the lack of GluR-A containing AMPA receptors in the brain of these mice, because we observed a very similar phenotype when we monitored mice which express the GluR-A(R582Q) mutation (GluR-A<sup>R/R</sup> mice). This mutation inhibits the expression of functional GluR-A containing AMPA receptors. Mice homozygous for this mutation should show a very similar phenotype to GluR-A 'knockout' mice, if this phenotype is mediated by the lack of GluR-A containing AMPA receptors. When we repeated the home-cage resident-intruder test which we did for the GluR-A deficient mice with single resident mice kept in isolated cages for 1, 5 and 21 days but now used the GluR-A<sup>R/R</sup> mutants, we found that the mutants showed a very similar phenotype to the GluR-A<sup>-/-</sup> mice (Table 1, Fig. 1a). GluR-A<sup>R/R</sup> mutants showed lower frequency and shorter duration of consummate [ $F_{1,48} = 11.24, P < 0.005$ ] and ambivalent aggression [ $F_{1,48} = 23.38, P < 0.0001$ ] and sitting with sniffing [ $F_{1,48} = 24.42, P < 0.0001$ ], but more frequent and enduring partner exploration [ $F_{1,48} = 6.05, P < 0.05$ ] and rearing [ $F_{1,48} = 7.82, P < 0.05$ ] than their wild-type littermates. These matching phenotypes in agonistic behavior of the two GluR-A mutant lines strongly suggest an involvement of GluR-A containing receptors in the expression of agonistic behavioral elements in wild-type mice.

**Other agonistic behaviors in GluR-A mutant mice**

Agonistic behavior was further analysed by interline male encounters on a new, neutral territory between GluR-A<sup>-/-</sup> and wild-type littermates and between GluR-A<sup>R/R</sup> mice and wild-type littermates, respectively (Fig. 2, Table 2). This analysis showed that the wild-types always dominated the mutants by attacking or by immediately suppressing any rare attack by the mutants, as is evident from the durations of consummate aggression. The frequencies of consummate aggression were also significantly higher in the wild-type controls than in GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice [ $F(1,16-22) = 6.86, P < 0.05$ ]. The mutants either sometimes showed defence in response to an attack or did not show any agonistic behavior. In this test, the durations of defence behavior were longer in GluR-A<sup>-/-</sup> [ $F_{1,22} = 4.35, P < 0.05$ ] and GluR-A<sup>R/R</sup> mice [ $F_{1,16} = 8.09, P < 0.05$ ] than in their littermates. The frequency of the defence elements was unchanged in GluR-A<sup>-/-</sup> mice [ $F_{1,22} = 0.24, NS$ ], but increased in GluR-A<sup>R/R</sup> mice [ $F_{1,16} = 20.1, P < 0.0001$ ] as compared to wild-types. The defence level predominated over aggression in the mutants [ $F_{1,22} = 12.86, P < 0.01$  and  $F_{1,16} = 8.47, P < 0.05$  for the difference between the durations of defence and consummate aggression in GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice, respectively], confirming the reduced aggression of GluR-A mutant mice.

Next we investigated whether reduced aggression of GluR-A mutant mice is challenged by prolonged isolation of male mice. Now resident-intruder experienced mice of each genotype group (GluR-A<sup>-/-</sup>, GluR-A<sup>R/R</sup>, wild-type littermates



**Figure 2: Behavioral elements during interline encounters in a new territory in GluR-A subunit-deficient and control animals after 3 weeks of social isolation.** The pie-graphs depict relative durations of various behavioral elements in the GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice confronted by wild-type controls from different litters. Testing time was 4 min. Codes for the behavioral elements not spelled out in the panels are shown at the bottom of the figure.

of GluR-A<sup>-/-</sup> and wild-type littermates of GluR-A<sup>R/R</sup>) were kept isolated for 30 days and then all mice of one group were simultaneously placed in a novel environment. The aggressive interactions were monitored for 10 min. The test was repeated daily and males that had shown aggression were excluded from the encounters on the following days. This procedure was stopped as soon as all aggressive males were identified. When we analysed the relative duration of individual consummate aggression from all aggressive mice of one group, we found that mice of both wild-type groups exhibited high level of aggression (73 and 88%, respectively; Fig. 1b). This was in contrast to GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mutant mice, which showed modest aggression, if at all (0 and 13%, respectively) with significant differences from the wild-type groups (Fisher's test  $P < 0.05$ ).

The failure of challenged aggression in GluR-A mutant mice was confirmed when we tried to induce aggression by individual social experience in non-isolated mice. Now the resident-intruder paradigm was used on male mice, which were pair-housed with a female for 5-days. After this social experience the wild-type males exhibited aggressive behavior towards intruder males, but the GluR-A mutants still showed less ambivalent (and tended to show less consummatory) aggression towards the intruder (Table 2).

**Basic behavioral profiles**

The altered agonistic behavior of GluR-A mutant mice appears to be a very specific phenotype, that involves higher control centres in the brain, as the overall behavior showed no significant abnormalities. In the open arena, GluR-A<sup>-/-</sup> mice and their littermates showed similar behavioral reactions and responded similarly to challenges by the experimenter. Therefore, these mice do not have major defects in their basic behavioral strategies, reflexes and sensorimotor coordination. However, the locomotor activity in the open arena of both of the GluR-A mutants was significantly higher than the activity of the wild-types [ $F_{1,22} = 10.53$ ,  $P < 0.05$ ;  $F_{1,16} = 12.16$ ,  $P < 0.05$ , respectively], which is consistent with our earlier report (Vekovischeva *et al.* 2001) and was reported by others (Bannerman *et al.* 2003). The increased activity is not a result of increased anxiety of the mutants in the plus-maze test. In that test, GluR-A<sup>-/-</sup> mice failed to reveal any significant difference in anxiety ( $P > 0.05$ ) compared to wild-type littermates, although they tended to show shorter latencies of the first entry to the open arms, less freezing and more arm entries to the open arms than the littermates (Fig. 3). In the light-dark test, the GluR-A<sup>-/-</sup> mice spent more time in the lit compartment than did the littermates [ $F_{1,10} = 5.84$ ,  $P < 0.05$ ], suggesting that the mutants are somewhat less anxious. In this test, the mutants also tended to have more shifts between the light and dark compartments than the littermates. Furthermore, the sensing of sensory information, e.g. recognition of pheromones, seems to be normal in GluR-A mutant mice. No drastic differences between the lines in sexual behavior could be observed in male-female encounters. Sexual behavior towards females was seen in male mice from each line (Table 3). The GluR-A<sup>-/-</sup> mice sniffed the body of a female less [ $F_{1,14} = 5.39$ ,  $P < 0.05$ ] than their controls. The GluR-A<sup>R/R</sup> mutants chased females [ $F_{1,15} = 24.80$ ,  $P < 0.0001$ ] and sniffed their anogenital regions [ $F_{1,15} = 37.40$ ,  $P < 0.0001$ ] more than their wild-types.

However, it seems likely that the reduced agonistic behavior is sex specific. When pups were removed from females 10 days after being born and the female mouse was immediately exposed to a new male intruder, the GluR-A<sup>R/R</sup> females exhibited similar levels of agonistic behavior against the male intruder as did the wild-type littermates (Table 2).

**Table 2:** Summary of consummate and ambivalent aggression durations shown by GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mutant mice and their littermates in different tests

Line			Male-male interline aggression	Resident-intruder after pair-housing	Female-male aggression
WT	Consummate	d	15.4	15.1	nd
		n	90	86	nd
GluR-A <sup>-/-</sup>	Ambivalent	d	16.1	18	nd
		n	46	29	nd
WT	Consummate	d	1.3*	1.2*	nd
		n	50	78	60
GluR-A <sup>R/R</sup>	Ambivalent	d	12.7	16.8	10.1
		n	25	33	20
	Consummate	d	0.2*	9.8	2.4
		n	25	33	20
	Ambivalent	d	2.4*	1.4*	7.9
		n	25	33	20

The data for consummate and ambivalent aggression are expressed as average duration (d – seconds percentage of the total test time of 240 seconds) and the proportion of the animals (n) showing consummate aggression is given as a percentage (%). \*  $P < 0.05$  (Duncan test) for the significance of the differences between GluR-A<sup>-/-</sup> or GluR-A<sup>R/R</sup> mice and their littermates. nd – not done.

### Brain dopamine and serotonin and plasma testosterone

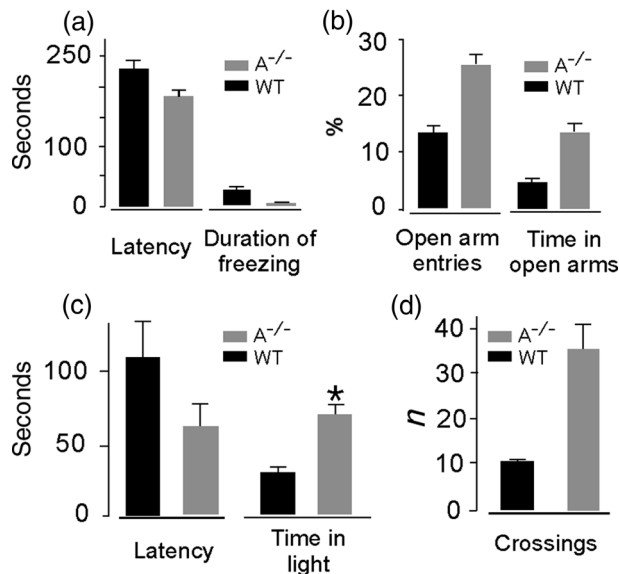
In emotional behavior and expression of aggression and impulsivity it is known that dopaminergic and serotonergic systems in the limbic areas are involved (Garris 2003; Gingrich & Hen 2001; Linnoila & Virkkunen 1992; Shih *et al.* 1999). Therefore, the levels of dopamine and serotonin might differ between brains of GluR-A mutant and wild-type mice. However, when dopamine and 5-hydroxytryptamine were analysed in various brain regions of GluR-A<sup>-/-</sup> and wild-type littermates after 10-day social isolation with one social interaction session on the fifth day, we found comparable monoamine levels in all brain regions analysed (Fig. 4a,c). Similarly, the ratios of acidic dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine and the ratios of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) to serotonin failed to demonstrate clear differences in the hormone levels (Fig. 4b,d). There was just a slight hint of minor differences from the ratio of 5-HIAA and 5-HT (serotonin), that in GluR-A deficient mice tended to be lower than that of wild-types in several brain regions, with the hippocampus achieving statistical significant lower ratios in the mutant ( $P < 0.01$ ).

In addition, we tested the testosterone levels in males of our different genotypes. We found that the plasma testosterone level was not different between GluR-A deficient and control mice [ $1.05 \pm 0.19$  (6) and  $0.81 \pm 0.25$  (7) ng/l (mean  $\pm$  SEM, number of animals)] nor between GluR-A<sup>R/R</sup> and control mice [ $0.64 \pm 0.21$  (8) and  $0.99 \pm 0.23$  (8) ng/L], which supports our notion that the reduced aggression in mice lacking functional GluR-A containing AMPA receptors is a behavioral phenotype mediated by altered function of higher CNS control centres of male mice.

### Brain regional ligand binding to glutamate receptors

In GluR-A subunit-depleted mice, hippocampal excitatory neurons are depleted in extrasynaptic AMPA receptors but show nearly normal synaptic AMPA receptor mediated synaptic transmission (Andrasfalvy *et al.* 2003; Jensen *et al.* 2003; Zamanillo *et al.* 1999). The overall reduction of AMPA receptor remains to be determined. Because subunit assembly of AMPA receptors is disturbed when the GluR-A subunit is missing (Zamanillo *et al.* 1999) traditional immunoblotting, immunocytochemistry or biochemical procedure have difficulties determining the exact level of AMPA receptor reduction. We studied the binding of glutamate receptor ligands to frontal brain sections from naïve GluR-A<sup>-/-</sup>, GluR-A<sup>+/-</sup> and wild-type mice and found that the hippocampal binding of [<sup>3</sup>H]-S-AMPA was significantly reduced (by about 30%) in brains of GluR-A<sup>-/-</sup> mice in comparison with brains of heterozygous and wild-type mice (Fig. 5). There was no significant difference in the [<sup>3</sup>H]-S-AMPA binding to other brain regions, such as the amygdala, cerebral cortex and caudate-putamen (data not shown). Nor was there any significant alteration in glutamate- and glycine-activated [<sup>3</sup>H]MK-801 binding to NMDA receptors and in [<sup>3</sup>H]SYM 2081 binding to kainate receptors in the hippocampus or other brain regions between the GluR-A<sup>-/-</sup> and GluR-A<sup>+/-</sup> or wild-type mice (see Fig. 5 for the hippocampal data).

In the brains of hetero- and homozygous GluR-A<sup>R/R</sup> mutants this reduction in [<sup>3</sup>H]-S-AMPA binding was not observed (data not shown). Thus, the GluR-A(Q582R) mutation seems to be incorporated into AMPA receptors which can be monitored by [<sup>3</sup>H]-S-AMPA binding. However, those channels are low in conductance, which leads to reduced number of functional AMPA receptors (Rolf Sprengel, unpublished results), similar to that of GluR-A deficient mice.



**Figure 3: Anxiety-related behaviors of the GluR-A<sup>-/-</sup> and control (WT) mice in elevated plus-maze and light-dark test.** Elevated plus-maze test: (a) The latency (seconds) to the first open arm entry and total duration of freezing, and (b) the percentage of the open arm entries and the percentage of time spent on the open arms during 5-min sessions. ‘Arm entries’ depict the number of entries into open or closed arms of the elevated plus-maze. The data are means ± SEM for 8–16 animals. No significant differences between GluR-A<sup>-/-</sup> mutants and littermates (Duncan test, *P* > 0.05). Light-dark test: (c) The latency (seconds) to the entry into the dark compartment, the time (seconds) spent in the white compartment, and (d) the number of crossings between the compartments during the 5-min test period. The data are means ± SEM for 5 animals. \*Significance of the difference from wildtype result (Duncan test, *P* < 0.05).

**Discussion**

Detailed analyses of the behavioral profile of the GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> males in resident-intruder paradigms showed

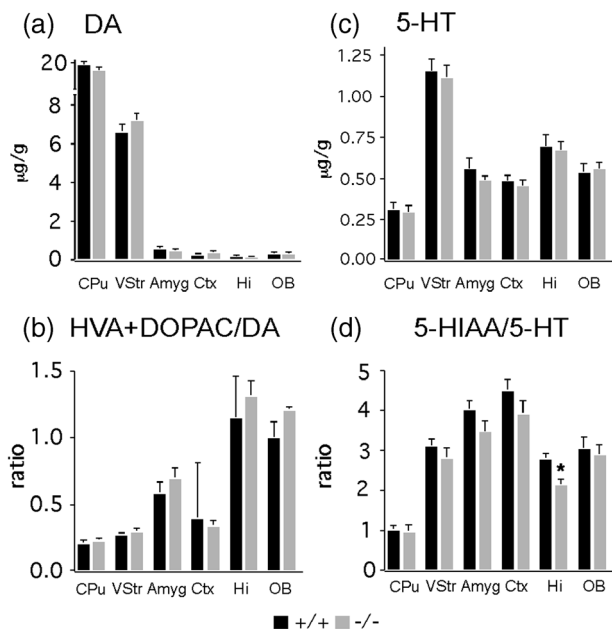
absence of overt aggressiveness in the presence of increased exploration of the opponent and surroundings, contrasting with that of the littermates showing higher levels of agonistic behavior towards a partner and a preference for exploring passively (by sitting with sniffing). The mutants exhibited little agonistic behavior towards control opponents on a new territory, independent of the aggressiveness of the control opponent. The agonistic behavior of wild-type littermates increased during the isolation period, but even 30 days of isolation failed to provoke agonism in most of the GluR-A mutants.

These strong effects of the GluR-A subunit deficiency on social behavior were not accompanied by any drastic alterations in general behaviors and reflexes as assessed in Irwin’s test. Elevated plus-maze and light-dark tests indicated that the GluR-A<sup>-/-</sup> mice were slightly less anxious than their controls, which might explain their reduced aggression in resident-intruder tests (Guillot & Chapouthier 1996). The lower anxiety is consistent with the reported actions of AMPA receptor antagonists (Kotlinska & Liljequist 1998; Matheus & Guimaraes 1997). The relationship between anxiety and aggression may be a complex one, because for example, genetically highly aggressive mice exhibit a low level of anxiety in elevated plus-maze and light-dark tests (Nyberg *et al.* 2003). Furthermore, the anxiety scores may be confounded by the hyperactivity of the GluR-A<sup>-/-</sup> mutants. It is well known that odors are important in the behavior of the resident towards the partner [mice usually sniff the opponent prior to an attack (Nowell *et al.* 1980)] and that elimination of the olfactory sense reduces the aggressiveness in mice (Brain & Haug 1992). Glutamate is the major excitatory neurotransmitter at synapses between the olfactory nerve to mitral and tufted cells that play an important role in the process of odor discrimination (Giustetto *et al.* 1997), making it possible that GluR-A mutants have a low level of male aggression and increased partner exploration due to impaired perception of partners’ odor. However, their sexual behavior was not impaired at all, suggesting that their olfactory responses are largely intact. Similar agonistic behavior of the

**Table 3:** Behavioral characteristics of male GluR-A<sup>-/-</sup> and GluR-A<sup>R/R</sup> mice and their male littermates during male-female encounters

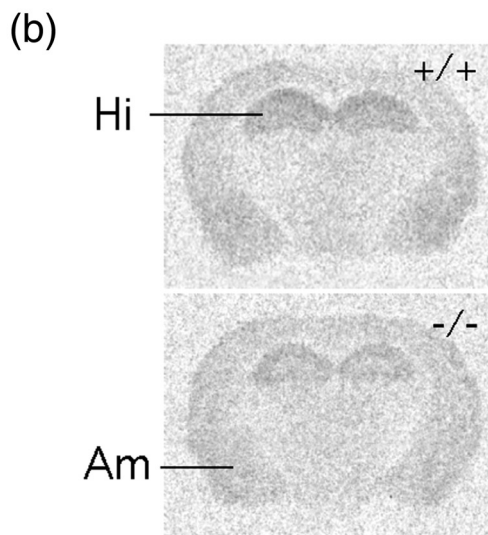
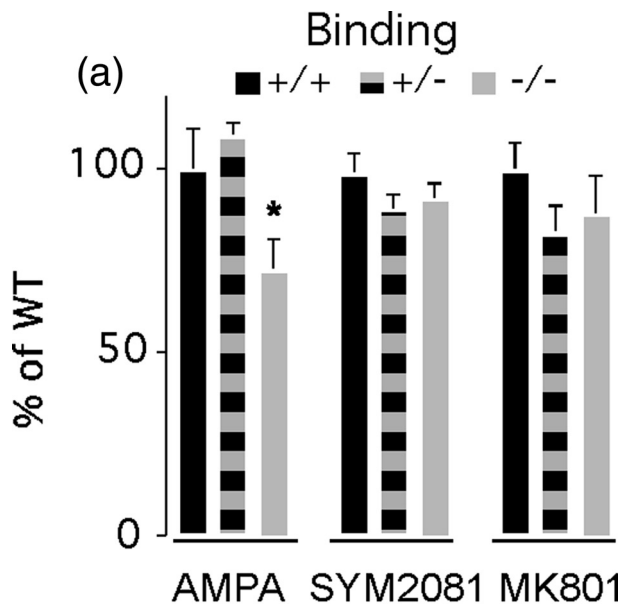
Behavioral categories and elements	WT (n=7)	GluR-A <sup>-/-</sup> (n=7)	WT (n=8)	GluR-A <sup>R/R</sup> (n=7)
Sexual behavior	19	31	5	22
Pursuit of female	9	29	7	43*
Anogenital sniffing	126	132	168	300*
Sniffing of female’s body	109	49*	91	83
Locomotion	72	120	126	66*
Rearing	53	65	45	13*
Sitting with sniffing	144	162	120	55
Other behaviors	68	12	38	18

The data are expressed as the average duration (seconds) of separate behavioral elements (of the total time of 10 min). \* *P* < 0.05 (Duncan test) for the significance of the differences between GluR-A<sup>-/-</sup> or GluR-A<sup>R/R</sup> mice and their littermates.



**Figure 4: Concentrations of brain monoamines in GluR-A subunit-deficient and control animals.** The concentrations of dopamine (DA) (a) and serotonin (5-HT) (c) in various brain regions of the GluR-A<sup>-/-</sup> mice and wild-type (WT) littermates after 4-week social isolation. (b) The ratios of the concentrations of the acidic dopamine metabolites homovanillic acid (HVA)+3,4-dihydroxyphenylacetic acid (DOPAC) to that of DA and (d) the ratios of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT. Cpu, caudate-putamen; VStr, ventral striatum; Amyg, amygdala; Ctx, cerebral cortex; Hi, hippocampus; OB, olfactory bulb. The concentrations are given as means ± SEM for 6–8 replicates in ng/g tissue wet weight. \* Significance of the difference from the corresponding wild-type control value ( $P < 0.01$ ).

wild-type littermates in within-line and interline tests suggests that the intruder effects by the mutants do not cause their aggressiveness. Furthermore, the effect of background strains (C57BL/6J and hybrid 129S1/Sv-p<sup>+</sup>Tyr<sup>+</sup>Kitl<sup>S<sup>-</sup>J</sup>/x 129 × 1/SvJ strains) used to construct the GluR-A subunit-deficient mouse lines is unlikely to explain the differences found, as the littermates have the identical genepool and two independent lines with different GluR-A subunit mutations (Vekovischeva *et al.* 2001; Zamanillo *et al.* 1999) show similar low aggressiveness towards a partner. Our data thus shows that GluR-A subunit-deficient male mice have normal explorative activity towards the environment or a partner, increased locomotor activity, slightly reduced anxiety and normal sexual activity, whereas aggression was associated with the gene changes. Although the GluR-A subunit-deficient mutants showed increased defence in interline encounters, they did not display any agonistic behavior in within-line encounters. This suggests that their behavioral strategy was not shifted towards defence, but that they largely lacked agonistic behaviors unless provoked by aggressive wild-type



**Figure 5: Autoradiography of glutamate receptor binding sites in brain sections of GluR-A subunit-deficient and control animals.** (a) Binding densities in the hippocampus quantified from film autoradiographs for [<sup>3</sup>H]-S-AMPA, [<sup>3</sup>H]SYM 2081 and glutamate-and glycine-activated [<sup>3</sup>H]MK-801 binding to brain sections from wild-type (WT), GluR-A<sup>+/-</sup> heterozygous and GluR-A<sup>-/-</sup> homozygous mutants. Values are means ± SEM ( $n = 3$ ) and expressed in relation to the wild-type control values. The wild-type values for hippocampal [<sup>3</sup>H]-S-AMPA, [<sup>3</sup>H]SYM 2081 and [<sup>3</sup>H]MK-801 bindings were  $6.5 \pm 0.7$ ,  $7.7 \pm 0.4$  and  $33 \pm 2$  nCi/mg, respectively, in reference to radioactivity standards. \* $P < 0.05$  for the significance from the other groups (ANOVA). (b) Representative images of [<sup>3</sup>H]-S-AMPA binding to brain sections from wild-type mice and GluR-A<sup>+/-</sup>, showing reduced binding in the hippocampus (Hi) of the mutants. Am, amygdala.

intruders. Because the GluR-A<sup>R/R</sup> homozygous mutant mice showed similar phenotype in social interaction tests as the null mutants, and both have reduced number of functional AMPA receptors, it is likely that GluR-A subunit-containing AMPA receptors are the critical neurochemical activator of neuronal pathways needed in male aggression. The females of GluR-A<sup>R/R</sup> exhibited normal maternal aggression, indicating male specificity of reduced aggression in the mutants.

Aggressiveness/impulsive behavior may correlate with high dopaminergic activity and/or low serotonergic activity in the brain (Hadfield & Milio 1988; Olivier *et al.* 1995). We found no differences in DA and 5-HT concentrations in various brain regions between the GluR-A<sup>-/-</sup> and control mice, and only minor changes in the metabolites or the ratios of the metabolites and parent amines. Both low 5-HIAA/5-HT ratio and high DOPAC concentrations of the mutants could actually be interpreted to increase rather than decrease aggressiveness (Allikmets & Rago 1983). It should be noted that, to verify the behavioral phenotypes of the test mice, the monoamine levels were determined in mice after short isolation and one-time experience in resident-intruder test, which might have affected brain dopamine and serotonin. Anyway, the monoamine data indicate that the GluR-A<sup>-/-</sup> mice are close to normal in their brain neurochemistry.

GluR-A<sup>-/-</sup> mice have been reported to show impaired memory in the T-maze test for non-matching-to-place (Reisel *et al.* 2002). Spatial memory required in the T-maze can be abolished by hippocampal lesions. The GluR-A<sup>-/-</sup> mice are also impaired in learning cue-induced conditioned responses (Mead & Stephens 2003), whose deficit can be found in rats after lesions of basolateral amygdala (Everitt *et al.* 2000). Further work will be needed to relate the learning deficits with altered agonistic behavior. GluR-A subunits are abundant in hippocampal and amygdaloid neurons, that in the absence of functional GluR-A subunits are depleted in extrasynaptic AMPA receptors pools as indicated by the subcellular distribution of GluR-B subunit in GluR-A<sup>-/-</sup> mice (Mead & Stephens 2003; Zamanillo *et al.* 1999). A similar defect in subcellular distribution is apparently also in the basolateral amygdala, but not in the central amygdaloid nucleus (Mead & Stephens 2003), suggesting that in the absence of GluR-A subunit some of the remaining AMPA receptors are not properly targeted in all neurons. Our reduction of [<sup>3</sup>H]AMPA binding in the brain sections of the GluR-A<sup>-/-</sup> mice confirms reduced glutamate-induced soma-patch currents in the GluR-A<sup>-/-</sup> animals. However, the poor resolution of the autoradiographic technique with such a rapidly dissociating ligand as [<sup>3</sup>H]AMPA prevents more detailed analysis of somal and dendritic AMPA binding sites. Because [<sup>3</sup>H]AMPA binding is distributed more widely than on the cell soma layer (Fig. 5), where robust increase in GluR-B subunit immunoreactivity has been observed in young mouse (Jensen *et al.* 2003), most of the aberrantly located GluR-B subunit must be non-functional. Furthermore, our binding data indicate that

the distributions of kainate and NMDA receptors are normal in the GluR-A<sup>-/-</sup> mice. In summary, the effects of the missing GluR-A subunits on the mouse brain development and function seem too subtle, although most brain regions have not been studied carefully enough.

Further study on physiology and neurochemistry, e.g. using brain microdialysis, needs to be focussed on various hypothalamic nuclei, some of which elicit certain types of agonistic behaviors in rats (Siegel *et al.* 1999). Hypothalamic neurons with receptors for androgenic steroids also express AMPA receptors (Diano *et al.* 1997). Any reduction of the AMPA responses in these neurons might prevent the steroids from influencing social interaction and agonistic behavior and/or the normal aggression circuitry to be activated. We found no deficits in blood testosterone levels in the mutants. This indicates that reduced agonistic behavior is not due to lack of androgens, which are known to regulate this behavior (Brain & Haug 1992).

Usual social organization of laboratory mice in groups involves several aggressive males within the group in addition to a dominant mouse (Benton & Brain 1979). Those males exhibit agonistic contacts if the dominant mouse is removed from the group or if several of these males unfamiliar to each other are put together into one cage (Benton & Brain 1979; Diaz & Asai 1990; Vekovishcheva *et al.* 2000). In the present study, littermate male mice introduced after prolonged isolation into one cage displayed numerous aggressive acts, whereas the GluR-A<sup>-/-</sup> mice remained non-aggressive in spite of repeated tests. From the GluR-A<sup>R/R</sup> mutants tested only one male displayed aggression in this situation. It seems that GluR-A subunit-deficient mice fail to construct hierarchical structure based on agonistic contacts in a similar way to normal mouse groups (Benton & Brain 1979).

Quantitative trait loci mapping of aggression in mice using a behavioral paradigm different from our resident-intruder paradigm; a dangle paradigm, has not implicated any of the AMPA receptor subunits (Brodkin *et al.* 2002), and our present data cannot be explain whether the GluR-A containing AMPA receptors are directly responsible for activation of brain aggression pathways. Genetic regulation of agonistic behavior is most likely a polygenic event with a strong environmental component (Miczek *et al.* 2001). However, as suggested by the large general analysis of the significance of phenotype of knockout mouse lines for establishing drug indications and targets (Zambrowicz & Sands 2003), our data might warrant further efforts to develop subtype-selective AMPA receptor antagonists to test for antiaggressive efficacy.

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