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Behavioural anomalies in mice evoked by "Tokyo" disruption of the Vitamin D receptor gene

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Abstract

Vitamin D is a steroid hormone with many important functions in the brain, mediated through the nuclear Vitamin D receptor (VDR). Mounting clinical data link VDR mutations to various psychiatric phenotypes. We have reported previously that mutant mice lacking functional VDR ("Tokyo" VDR mutant mice) display several behavioural anomalies, including high anxiety and aberrant grooming. Given the important role of Vitamin D and VDR in brain development and functioning, we hypothesized that several other important behavioural domains may be affected by disruption of the VDR gene in mice. Here we report that VDR mutants display unaffected depressive-like behaviour, but show abnormal social behaviours, reduced social barbering and aggressiveness, impaired nest building and aberrant maternal (pup neglect, cannibalism) behaviours. Taken together, these findings confirm the important role postulated for the VDR in the regulation of behaviour, and suggest the mice lacking functional VDR may be a useful tool to model different brain disorders.

Keywords: Vitamin D receptors; Knockout mice; Social interaction; Nest building; Barbering; Aggressiveness; Maternal behaviour

1. Introduction

Neurosteroid hormone Vitamin D plays an important role in the nervous system including differentiation, regulation of Ca^{2+} homeostasis, modulation of neurotrophins release and activity of key brain genes and enzymes of neurotransmitter metabolism (Siegel et al., 1984; Carswell, 1997; Garcion et al., 2002; Brown et al., 2003; Kalueff et al., 2004a). The functions of Vitamin D are mediated through the nuclear Vitamin D receptor (VDR), a member of the nuclear receptors superfamily of ligand-activated transcription factors (Kamei et al., 1995; Kato et al., 1999; Malloy et al., 1999). VDR is a 50–60 kDa protein, consisting of several functional domains, typical for all steroid receptors, and responsible for ligand and DNA binding, heterodimerization, nuclear localization and transcriptional activation (Kato et al., 1999; Malloy et al., 1999). In both animals and humans, VDR are widespread in the brain and the spinal cord including the areas involved in the regulation of motor activity and behaviour (Prufer et al., 1999; Langub et al., 2001; Walbert et al., 2001; Eyles et al., 2005), collectively supporting the possible role of Vitamin D and VDR in the regulation of behaviours (Altemus et al., 1987; Carswell, 1997; Kalueff et al., 2004b,c,d; Becker et al., 2005; Burne et al., 2005).

Genetically targeted animals provide a powerful tool for neuroscience research (Crawley and Paylor, 1997; Crawley, 1999, 2000). Several groups have generated mutant mice lacking functional VDR (VDR knockout mice, KO). Yoshizawa et al. (1997) ablated exon 2 encoding the first zinc finger of the DNA-binding domain of the VRD gene (Tokyo mice), while Li et al. (1997) ablated a fragment spanning exons 3–5 and encoding the second zinc finger (Boston mice). In addition, Erben et al. (2002) have recently generated mice expressing non-functional VDR without the first zinc finger (Munich mice), while Van Cromphaut et al. (2001) deleted a fragment encompassing exons 1 and 2 (Leuven mice). Some behavioural abnormalities have been recently reported for "Tokyo" mutant mice, expressing truncated VDR lacking DNA-binding domain

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(Bula et al., 2005), such as high anxiety, altered pre-pulse inhibition, poor swimming and aberrant grooming (Kalueff et al., 2004b,c,d, 2005; Burne et al., 2005).

Given the importance of Vitamin D and VDR in brain development and functioning (Burne et al., 2004a,b; Mackay-Sim et al., 2004; McGrath et al., 2004; Ko et al., 2004; Eyles et al., 2005; Feron et al., 2005), it is possible to expect that several other behavioural domains may be affected in VDR KO mice, justifying their further ethological analyses. In the present study, we performed expanded behavioural phenotyping of ''Tokyo'' VDR KO mice and ethologically dissected different behavioural domains, not assessed previously, including their social interaction, nest building, maternal care, depressive-like behaviours, barbering (hair-removal) and aggression.

2. Materials and methods

2.1. Animals

VDR KO were bred in the University of Tampere from the line initially generated in the University of Tokyo (Yoshizawa et al., 1997). Subjects were adult homozygous (−/−) KO mice and homozygous (+/+) wild type (WT) 129S1 male or female mice. Mice of both genotypes were littermates produced by 4–5 heterozygous (HZ) crosses. Tail clips were taken for genotyping performed using polymerase chain reaction (PCR) on DNA prepared from tail tissue. Four primers were used to amplify a 130 bp VDR band and a 450 bp Neo band from the targeted gene (Burne et al., 2005). On Day 21 postpartum, pups were weaned and assigned to different cages based on their genotype and gender. Adult mice used in the present study were 3.5–4 months old, maintained in a virus/parasite-free facility and exposed to a 12 h light, 12 h dark cycle. Lights were turned off at 18.00 pm and on at 6.00 am. The animals were experimentally naïve and housed individually in transparent cages (13 cm × 12 cm × 14 cm), with food and water freely available. In barbering and aggression studies, VDR KO males were socially housed (3 mice per cage) since weaning, or housed in pairs (WT + KO) for 4 weeks. In all experiments, the VDR KO mice were fed a special diet containing 2% Ca, 1.25% P and 20% lactose (Lactamin AB, Sweden), to normalize their mineral metabolism.

2.2. Procedures

Behavioural testing was always conducted between 14.00 and 18.00 h. On the days of the experiments, animals were transported to the dimly lit room and left undisturbed for 1 h prior to testing. In all experiments, the animals were observed by an experienced investigator (intra-rater reliability >0.9). During the testing sessions, the experimenter remained standing in front of (and 2 m away from) the testing boxes scoring mouse behaviours using a specially designed register.

In Experiment 1, we subjected the mice to the tail suspension test (Cryan et al., 2005). Each mouse was suspended from its tail, scoring the latency (s) to immobility, the number and total duration (s) of immobility episodes, as conventional measures of behavioural despair (depressiveness), for 5 min. Defecation boli were also assessed in this test. Two weeks later, the mice were tested in the social interaction test. For this, WT or KO males were placed individually in the open field test (50 cm × 50 cm × 50 cm plastic box), and exposed to an unfamiliar male of another strain, introduced in the diagonally opposite corner. To minimize harm to our mice due to inter-male fighting, we used relatively non-aggressive NMRI mice as the partners. In this test, we assessed the number of initiated sniffing, follows, fighting, vocalizations, hetero-grooming episodes as well as vertical rears for both genotypes for 5 min.

Three days later, we performed a similar test, termed ''social confrontation test'' (to avoid confusion with the social interaction test described earlier). For this, two single-housed male mice (1 WT, 1 KO) were confronted in the plastic actimeter box (30 cm × 30 cm × 30 cm) for 5 min, scoring the number of initiated sniffing, follows, vocalizations, self- and hetero-grooming episodes as well as vertical rears for each genotype. Between subjects, each apparatus was thoroughly cleaned (wet and dry cloths); to remove olfactory stimuli, each apparatus was cleaned with a 70% ethanol solution and dried with paper towelling.

Since mice of both sexes show nest building activity, its assessment may be a useful tool in behavioural phenotyping of mutant mice (Sieber et al., 1980; Bulloch et al., 1982; Moretti et al., 2005). For this, in Experiment 2, male and female mice of both genotypes were housed individually for at least 3 weeks in small plastic cages with standard bedding (aspen chips 4 mm × 4 mm × 1 mm, Tapwei Oy, Finland). A standard piece of paper towel (23 cm × 23 cm) was provided 3 days prior to inspection, and the nests were assessed by two observers (inter-rater reliability >0.9), using the following scoring system: 0—no nests, 1—primitive flat nests (pad-shaped, consist of a flat paper tissue which slightly elevates a mouse above the bedding), 2—more complex nests (including warping and biting the paper towel), 3—complex accurate cup-shaped nests (with shredded paper interwoven to form the walls of the cup), 4—complex hooded nests, with walls forming a ceiling so the nest becomes a hollow sphere with one opening. In addition, the working out activity (''paper-work''), including biting and shredding the paper, was assessed here. For this, each nest was unwrapped, fixed by four corners, and glued to a black paper. The amount of paperwork was assessed by the same observers, using the following scoring system: 0—intact paper of little damage (<5% paper destroyed), 1—some paper damage (5–20%), 2—pronounced paper damage (20–40%), 3—severe paper damage (>40%). In all these experiments, all nests were photographed, and then assessed by two raters blind to the genotypes, to avoid any possible biases in scoring the nest completeness and ''paperwork''.

Phenotyping of maternal behaviour, categorized into direct (direct contact with the offspring) and indirect (maternal nest building), also represents an important area in behavioural neurogenetics (Libhaber and Eilam, 2002; Caldji et al., 2004; Jin et al., 2005). To assess maternal nest building, we provided pregnant females (separated from their male partners and kept in big plastic cages for 5–7 days) with a standard 20 cm × 60 cm paper tissue, and assessed their nest quality on the Day 1 postpartum, using the scoring system as described previously. The litter size was also examined for each mouse.

Since mutant mice often demonstrate marked differences in their aggression, in Experiment 3 we studied aggressiveness in 15 male mice of each genotype, housed socially (3 mice per cage) since weaning, or in pairs (WT + KO) for 4 weeks. Inter-male aggression was assessed by analysing scarring on the hind limbs, base of the tail and rear flanks (Garner et al., 2004a,b). Each mouse was assessed individually for 2–4 min by the same experienced observer, and a score of 0 (intact skin) or a score of 1 (scarred/scubbed skin) were given for each of these areas. Total score was obtained from the sum of the score of each areas.

In the same experiment, we assessed the mouse barbering activity. Behaviour-associated hair loss (barbering) is common in mice and includes removal of fur or whiskers from cage-mates by a single unbarbered dominant animal (Strozik and Festing, 1981; Sarna et al., 2000; Kalueff et al., 2006). Barbering may be specifically affected by various mutations, and its analysis represents an important part of behavioural phenotyping (Crawley, 2000; Kalueff and Tuohimaa, 2005). Hair loss was recorded by a highly experienced observer (intra-rater reliability >0.9) using a custom-made register. Each mouse was visually inspected on both the dorsal and ventral surfaces (Garner et al., 2004a,b) for at least 2 min. The following 5-point scale was used in the present study: 0—no barbering, 1—whisker removal, 2—snout/face denuding, 3—individual barbered patches on head and body, 4—multiple barbered areas on head and/or body, 5—severe behaviour-associated alopecia including complete snout denuding and large pronounced denuded areas on head and body (Kalueff and Tuohimaa, 2005; Kalueff et al., 2006). Hair loss was scored as barbering if the hair lesion was non-puritic, there was no scarring or scabbing around the lesion, and the animal was otherwise in good health and the fur (where present) was in good conditions (Garner et al., 2004a,b). Since 3–4 months old VDR KO mice start to develop alopecia due to impaired VDR-mediated hair growth (Yoshizawa et al., 1997), we assessed only denuded (''clean-shaven'') areas and complete whisker removal (due to barbering), markedly different from alopecia due to ablated VDR (balding patches with rare hairs and several visible whiskers, Fig. 2). For each group, we analysed the number (%) of cages in which the barbering occurred, and the average severity of barbering in each cage. In addition, we analysed the percentages of barbers and barbered animals (of total animals of each strain). Barber animals were identified as the single intact mouse in the cage, according to Sarna et al. (2000) and Garner et al. (2004a,b).

All experimental procedures were conducted in accordance with the European legislation (86/609/EEC) and the guidelines of the National Institutes of Health. All animal experiments reported here were approved by the Ethical Committee of the University of Tampere.

2.3. Statistics

All results are expressed as mean ± S.E.M. Behavioural data were analysed by the Mann–Whitney *U*-test for independent samples. To evaluate genotype and sex differences between groups, analysis of variance (two-way ANOVA, factors: genotype, sex) was performed with the post-hoc *U*-test for independent samples. A probability of less than 0.05 was considered statistically significant in all tests.

3. Results

3.1. Experiment 1

In the tail suspension test, using two-way ANOVA, we found significant sex and genotype effects on the latency to immobility (genotype: $F(1,35) = 4.32$, $P = 0.045$, sex: $F(1,35) = 12.76$, $P = 0.0011$, sex × genotype: $F(1,35) = 3.65$, $P = 0.064$ (trend)), with the VDR KO males displaying significantly longer latency (55 ± 8 s) compared to their WT counterparts (31 ± 6 s, $P < 0.05$, *U*-test) and both female groups (WT: 21 ± 5, KO: 22 ± 4, $P < 0.05$, *U*-test). In addition, there were significant sex ($F(1,35) = 6.59$, $P = 0.01$), but not genotype or genotype × sex ($F(1,35) = 0.05$–0.07, $P = 0.8$, NS) effects on the duration of immobility (males: 104 ± 22 s WT, 95 ± 10 s KO; females: 146 ± 18 s WT, 147 ± 18 s KO, $P < 0.05$ versus KO males, *U*-test). The number of immobility episodes (8–14) and defecation boli (1–3.2) were similar in all four groups (NS) in this test.

As can be seen in Table 1, compared to their WT controls, the VDR KO males also showed reduced vertical rears in social interaction test, and robust differences in social confrontation test (increased self-grooming, reduced hetero-grooming, sniffing, follows and vertical rears).

Table 1
Social behaviours of VDR knockout (KO) and wild type (WT) male mice

Tests and behaviours	WT ($n = 10$)	VDR KO ($n = 10$)
Social interaction test, 5 min		
Following episodes	2 ± 2	2 ± 0.2
Sniffing episodes	6 ± 6	6 ± 1
Hetero-grooming frequency	0.4 ± 0.4	0.4 ± 0.2
Fighting frequency	0.4 ± 0.4	0.4 ± 0
Vocalization	0.1 ± 0.1	0.6 ± 0.2
Vertical rears	12 ± 3	$4 \pm 1^{*}$
Social confrontation test, 5 min		
Initiated sniffing	9 ± 1	$4 \pm 1^{**}$
Initiated follows	4 ± 0.8	$0.7 \pm 0.3^{**}$
Vocalizations	0 ± 0	0.7 ± 0.3
Self-grooming bouts	0.7 ± 0.3	$4 \pm 0.8^{**}$
Initiated hetero-grooming bouts	3.5 ± 1	$0 \pm 0^{***}$
Vertical rears	24 ± 1.5	$14 \pm 1.5^{***}$

* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.001$ (*U*-test) between the genotypes.

3.2. Experiment 2

Although VDR KO females mice have long been known to be infertile (Yoshizawa et al., 1997), a high calcium diet has been recently shown to restore fertility in these mice (Johnson and DeLuca, 2001). In our study, 100% VDR KO ($n = 6$) and WT ($n = 8$) females were able to conceive 2–3 weeks after mating with the heterozygous males, confirming their fertility and enabling further ethological analyses of the VDR KO maternal phenotypes. We also found significant genotype difference in the litter size, with the VDR KO mice showing the smallest litter size (3.7 ± 0.33 versus 5.3 ± 0.3 WT, $P < 0.01$, *U*-test). In addition, we observed a dramatic impairment of the VDR KO maternal behaviour, manifest in poor nest building (predominantly cup-shaped, nest completeness scores: 3.2 ± 0.2 versus more complex hooded nests, 4 ± 0 WT, $P < 0.006$), abnormal mothering, and cannibalism. While pup-killing was absent in the WT females (0%, $P < 0.01$, *U*-test), 100% of pups born by the VDR KO mice were killed by their mothers 1–5 days after birth. They also neglected their pups, often leaving them unattended and resting outside the nest, as assessed during our routine homecage observations (data not shown). These impairments were not observed in the WT group, displaying extensive ''hooded'' nests (completeness score: 4 ± 0, $P < 0.05$, *U*-test) and normal maternal behaviours, including regular grooming and arched-back nursing of their pups.

Using two-way ANOVA to assess the nest building activity in single-housed mice, we found significant genotype and sex effects ($F(3,27) = 24.84$, $P = 0.00005$; $F(3,27) = 6.34$, $P = 0.019$, respectively; sex × genotype interaction $F(3,27) = -3.02$, $P = 0$) for nest quality. Overall, the WT males (average scores: 2.9 ± 0.1 WT, $n = 9$, $P < 0.007$, *U*-test), but not female mice (2 ± 0.3 WT, $n = 5$, NS), tend to build more complex, complete and accurate nests, than do their respective VDR KO littermates (1.6 ± 0.4 KO males, $n = 7$, 1.6 ± 0.4 KO females, $n = 7$). Fig. 1 shows representative nests of the WT and KO mice, including the WT cup-shaped nests with well-assembled walls, compared to the pad-shaped and only slightly wrapped KO nests, consisting of untouched nesting material.

Using two-way ANOVA (factors: genotype, sex) to analyse working out nesting material (''paperwork''), we found significant genotype, but not sex or sex × genotype effects ($F(3,39) = 7.02$, $P = 0.012$; $F(3,39) = 1.50$, $P = 0.23$, $F(3,39) = 1.00$, $P = 0.27$) in these mice. Overall, the WT males (average score: 2.6 ± 0.15, $n = 11$), but not females (2 ± 0.2, $n = 10$), generally performed significantly more ''paperwork'', compared to the VDR KO male (1.5 ± 0.4, $n = 8$) and female (1.5 ± 0.3, $n = 11$) mice, $P < 0.04$. Collectively, this indicates that VDR KO mice, especially males, display impaired nest building behaviour (Fig. 1).

3.3. Experiment 3

Examining barbering and aggressive behaviour in socially housed mice, we note that the VDR KO mice (Table 2) displayed reduced barbering severity and occurrence in group-

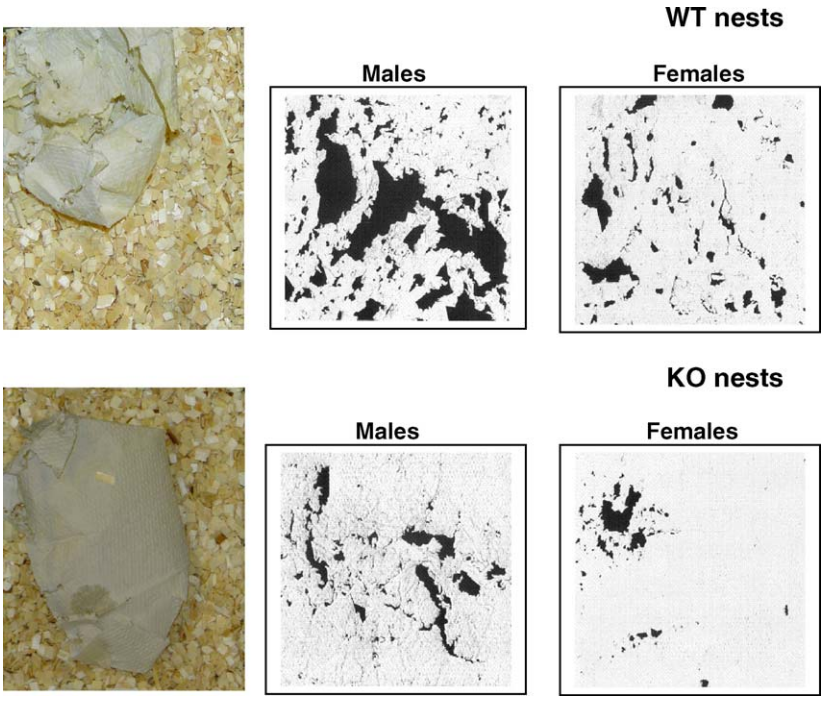

Fig. 1. Impaired nest building behaviour in the VDR knockout (KO) mice. Photographs (left) show examples of nests built by a wild type (WT) and a KO male mice 3 days after introduction of nesting material (paper towel) into the cage. Nest building activity (right) was measured as working out (biting, thorning, chewing) of the nesting material by representative male and female mice of each genotype (white paper towel photographed against black background; black colour reflects the amount of chewed nesting material).

housed cages, and a similar tendency, if housed in pairs with their WT littermates (also see Fig. 2). As can be seen in Table 2, these mutants also demonstrated significantly reduced aggression in both situations.

Table 2
Inter-male aggression and barbering phenotypes in the VDR knockout mice (KO) and their wild type (WT) controls

Behaviours	Socially housed since birth, five same-genotype cages		Housed in pairs (WT + KO) for 4 weeks, seven cages	
	WT ($n = 15$)	KO ($n = 15$)	WT ($n = 7$)	KO ($n = 7$)
Aggression				
% animals with scarring	47 ± 13	0 ± 0*	0 ± 0	29 ± 18*
% cages with scarring	80 ± 20	0 ± 0*	–	86 ± 14
Average scarring score	1.8 ± 0.4	0 ± 0*	0 ± 0	1.5 ± 0.3*
Barbering				
% cages with barbering	60 ± 24	0 ± 0	–	43 ± 20
Average severity score	1.7 ± 0.3	0 ± 0*	0 ± 0	0.6 ± 0.3
% barbered animals	53 ± 13	0 ± 0*	0 ± 0	43 ± 20
% barbers	20 ± 11	0 ± 0	0 ± 0	43 ± 20

* $P < 0.05$ (U-test) compared to the respective WT group.

4. Discussion

Analyzing depressive-like behaviours in our mice, we note that in the tail suspension test, a widely used mouse model of depression (Crawley, 2000; Cryan et al., 2005), the VDR KO male mice showed less immobility compared to all other groups. Although this observation suggests that depressiveness was not increased in the mutant group (and therefore unaffected by the mutation), it seems to contradict human data linking various Vitamin D dysfunctions to mood disorders (Stumpf and Privette, 1989; Lansdowne and Provost, 1998), thus, meriting further in-depth investigation. Since poor swimming of VDR KO mice (Kalueff et al., 2004d) makes it impossible to use the forced swim depression test, more sophisticated paradigms may be needed to assess depressive phenotypes of these mutants more fully. Nevertheless, our results do not seem to support the idea that VDR KO mice might have higher depressive-like behaviour.

Social interaction with non-aggressive NMRI mice did not reveal genotype differences between the WT and KO groups, suggesting that either this aspect may be unaffected by the mutation, or was masked by the ‘‘floor effect’’ due to hypoactive anxious phenotypes of both WT and KO groups, compared to non-anxious active NMRI strain. In a striking contrast, in social confrontation test, representing a modification of social interaction test (File and Seth, 2003), the WT males were more aggressive members of WT–KO pairs, initiating significantly more sniffing and follows, than did their KO counterparts. In line with this interpretation, the WT

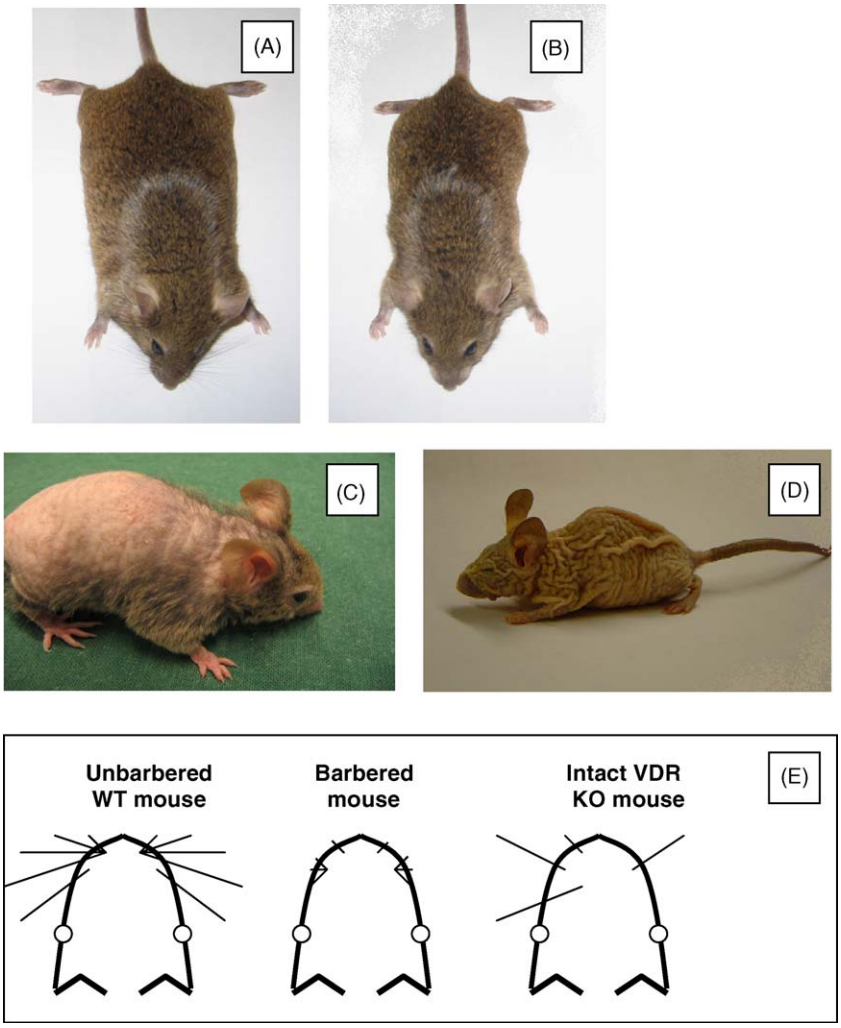

Fig. 2. Phenotypes of the wild type (WT) and the VDR knockout (KO) mice. (A) WT 129S1 mouse with normal fur and whiskers. (B) Barbered WT mouse without whiskers. (C) Intact 4-month old VDR KO mouse, with pronounced progressing alopecia (balding patches with rare hairs and sparse uncut whiskers). (D) Nude 1-year old VDR KO mouse, with no fur and whiskers. (E) Schematic diagram showing the difference between alopecia and barbering-evoked whisker loss.

males also displayed frequent hetero-grooming bouts, mounting and holding their submissive KO partners.

Consistent with this, during social housing, the WT mice showed higher aggressiveness and barbering activity, commonly seen in moderately aggressive/barbering 129S1 background strain (Kalueff and Tuohimaa, 2005; Kalueff et al., 2006). In contrast, the lack of barbering and aggression in the VDR KO mice suggests that functional VDR are involved in this behaviour, and their genetic ablation may affect these domains in our mice. Taken together, these findings demonstrate affected social behaviour in VDR KO mice. Importantly, these behavioural alterations cannot be attributed to poorer motor activity or coordination in the VDR KO mice, as confirmed previously in several tests (Kalueff et al., 2004b,c,d), but are rather consistent with their general high anxiety phenotype.

Analysing several other behavioural domains, we note that Tokyo male mice fed with special rescue Ca/P-rich diet, showed no overt anomalies in their sexual behaviours, as assessed during mating with the estrous WT females (Kalueff et al., 2005). In addition, these mice were fertile in the present study, suggesting that sexual behaviours and fertility in the VDR KO mice is unaffected by the VDR genetic ablation, if mineral metabolism is corrected. In contrast, our results demonstrate robust strain differences in nest building and maternal behaviours, suggesting impairment of these behavioural domains by disruption of the VDR gene (Fig. 1). Interestingly, numerous data show the important role of VDR in the regulation of prolactin gene expression in different tissues (Delvin et al., 1990; Castillo et al., 1999), suggesting that the Vitamin D/VDR and prolactin endocrine systems may interact (Robinson et al., 1982). Thus, it was possible to suggest that such interaction is impaired in VDR KO mice, leading to their abnormal prolactin-dependent mechanisms. Given the key role of prolactin in the regulation of nest building and maternal behaviours in mice (Voci and Carlson, 1973), this hypothesis, if true, may explain both abnormal maternal and nest building behaviours observed in the VDR KO mice in the present study.

Several lines of evidence suggest that the difference between genotypes in these behaviours appears to be unrelated to different activity levels or possible physically ‘‘unhealthy’’

status of the VDR KO mice. In both groups, most of nest building occurred without delays, i.e. between Days 1 and 2 of Experiment 2 (data not shown). In our previous studies, the baseline activity levels in the actimeter and the novel object tests were unaltered in both groups (Kalueff et al., 2004b,d). Furthermore, the VDR KO group showed increased (rather than reduced) grooming activity in a battery of tests (Kalueff et al., 2004c). Collectively, this downplays possible role of the mouse activity and/or coordination differences in the present study.

Finally, we shall note that our study was limited to one of four known VDR KO mouse strains, and to only one genetic background (129S1). Therefore, it may also be interesting to assess behavioural phenotypes of other (Boston, Munich, Leuven) KO mice, as well VDR KO mice on other genetic backgrounds, markedly differing from the 129S1 strain used here. We are currently transferring the ''Tokyo'' VDR mutation on several new genetic backgrounds (C57, BALB and NMRI) in order to perform such comparative studies. Moreover, it may also be important to examine the behavioural profiles of mutant mice with other abnormalities in the Vitamin D system. For example, mice lacking 1-alpha-hydroxylase, a key enzyme of Vitamin D bioactivation, are currently available for biomedical research (Kato et al., 2002). Displaying physiological phenotypes resembling that of the VDR KO, they may be a useful tool to further dissect the role of the Vitamin D/VDR system in the regulation of behaviour.

In conclusion, our study shows that behavioural despair (depressive-like behaviour) seems to be unaffected in these mutants. In contrast, there was a dramatic reduction in barbering, aggression, nest building and maternal behaviours, implying the key role of VDR in the regulation of these important domains. Given accumulating clinical data linking various VDR mutations to a variety of psychiatric phenotypes (Ozer et al., 2004; Tajouri et al., 2005; Yan et al., 2005), our study further supports the contention that VDR KO mice may emerge as a useful animal model to study different brain disorders.

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