Neophobia, sensory and cognitive functions, and hedonic responses in vitamin D receptor mutant mice

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Abstract

Vitamin D is a seco-steroid hormone with multiple actions in the brain, mediated through the nuclear vitamin D receptor (VDR). We have recently shown that mutant mice lacking functional VDR demonstrate altered emotional behavior and specific motor deficits. Here we further examine phenotype of these mice, testing their novelty responses, as well as cognitive and sensory (olfactory and gustatory) functions in the novel food, two-trial Y-maze and tastant consumption tests. In addition, we study depression-like behavior in these mice, using anhedonia-based sucrose preference test. Overall, VDR mutant mice showed neophobic response in several different tests, but displayed unimpaired olfactory and gustatory functions, spatial memory and baseline hedonic responses. Collectively, these data confirm that mutation of VDR in mice leads to altering emotional/anxiety states, but does not play a major role in depression, as well as in the regulation of some sensory and cognitive processes. These results support the role of the vitamin D/VDR neuroendocrine system in the regulation of behavior, and may have clinical relevance, enabling a better focus on psychiatric and behavioral disorders associated with dysfunctions in this neuroendocrine system.

Keywords: Vitamin D receptor; Anhedonia and depression; Y-maze; Olfaction; Gustation; Spatial memory; Neophobia

1. Introduction

The most active hormonal form of vitamin D is 1,25-dihydroxyvitamin D (calcitriol), which is implicated in both brain development and adult brain functioning[1–6]. Biological effects of this seco-steroid hormone are mediated through the nuclear vitamin D receptor (VDR), a ligand-activated transcription factor [7,8] widely expressed in the central nervous system [1,9–13]. Numerous brain disorders are linked to vitamin D deficits and/or VDR dysfunctions [13–15]. In both animals and humans, this hormone regulates behavioral and neuronal activity [13,16].

We have recently shown that mice lacking functional VDR (VDR mutant mice) display several behavioral abnormalities, including high anxiety and specific motor deficits [15,17], supporting the role of VDR in the regulation of behavior. In line with this, relatively high concentrations of VDR were found in the limbic system [1,9,10], the brain area that regulates emotional behaviors. Several other studies using VDR mutant mice [18,19] further strengthen the notion that vitamin D and VDR are involved in behavioral regulation.

Notably, high levels of VDR have been detected in multiple hippocampal areas [1,9–11]. As hippocampus is directly implicated in the regulation of cognitive processes, such as memory and learning, this raises the possibility that vitamin D and VDR may be involved in the regulation of these functions [2]. Indeed, while Altemus et al. [20] reported no significant impairment of cognitive functions in young adult rats deprived of vitamin D, transient prenatal vitamin D depletion has been found to affect learning and memory in rats [2]. To elucidate the role of VDR in cognitive functions further, the present study aimed to assess memory in VDR mutant mice in a two-trial Y-maze.

Since the assessment of general physiological status and sensory systems is a key part of behavioral phenotyping of
The genotype of the animals was confirmed by polymerase chain reaction (PCR) on DNA prepared from tail tissue. Primers were used to amplify a 400-bp Neo band and 166-bp VDR band from the targeted allele. All animal care and experimental procedures in this study were conducted in accordance with European legislation.

2.2. Apparatus and procedures

All behavioral tests were performed between 12.00 and 19.00 h in a dimly lit room. To assess the mouse working memory (Experiment 1), we used a two-trial version [40] of the Y-maze test, a traditional model of spatial working memory and exploration [13]. The Y-maze, made of Plexiglas, was elevated to a height of 70 cm and consisted of three walled arms (30 cm long, 10 cm wide, walls: 15 cm), radiating at an angle of 120° from each other. The study of spatial memory consisted of two Y-maze trials, with a 30-min interval between trials. During trial 1 (acquisition phase), one arm of the Y-maze (chosen randomly among the three arms) was closed, and remained unavailable for mice to explore. Each mouse was placed in one of the other two arms, and allowed to explore them for 5 min. The duration (s) and the number of visits (four-paw criterion) to the two arms were recorded by an experienced observer sitting 2 m away from the apparatus. The apparatus was thoroughly cleaned with ethanol solution after each animal. During trial 2 (retrieval phase), the animals had free access to all three arms, and the number and the duration (s) of visits to each arm were recorded for 5 min, as described earlier.

In a separate study (performed 2 weeks later, Experiment 2), we food-deprived the mice for 24 h, and then analyzed their spatial memory in the two-trial Y-maze with food reinforcement and a 2-h inter-trial interval. To minimize novelty factor in this experiment, the animals were allowed to explore the Y-maze freely for 10 min prior to testing. During the first trial, a 0.2-g pellet of familiar food (chow) was placed in a randomly selected arm, and the latency (s) to eat food, as well as the number of correct entries (arm with food), incorrect entries (empty arm) and total number of entries were recorded for 5 min. In addition, we calculated the percentage of incorrect entries (correct/total entries × 100%) for each animal in both trials. During trial 2, the location of food pellets was the same for each animal as during the initial trial. Food pellets were changed between animals and trials.

To study behavioral response to novel food (Experiment 3), we used a combination of familiar food versus novel food. A piece of white bread (2.3 g), vanilla (1.6 g) or onion (1.7 g) was used as novel food in this study. The protocol was as used previously in Experiment 2. Two weeks later, the animals were re-tested, using a combination of novel food (vanilla) versus familiar food. In the last test, performed 2 weeks later, a novel aversive food (onion) was given in combination with the familiar food (chow). The procedures were as described above, with a 4-h interval between trials. All food pellets were changed between animals and trials.

To determine whether the VDR mutant mice have altered olfactory function, we assessed their performance in the
buried food pellet test [41] (Experiment 4). Briefly, the mice were placed on a food-restricted diet (0.2 g standard chow per mouse) for 24 h. On the following day, a food pellet was buried approximately 0.5 cm deep under the bedding in a clean cage. The mouse was then placed in the diagonally opposite corner, and the latency (s) to find the buried food (as an index of olfactory function) was measured with a 5-min cut off time. The food was considered found if the animal was holding it in its forepaws (the mice were then allowed to consume food, and returned to their home cages). The bedding material was changed between animals.

To assess in parallel the mouse gustatory function and their baseline hedonic responses (Experiment 5), we used the two-bottle “preference” test [38], in which individually housed animals were given a free choice between two bottles (one with taste solution and another one with tap water). The solutions used in the present study included: sweet (1% sucrose, BDH Laboratory Supplies, England; 7 days), sour (50 mM citric acid 1-hydrate, Riedel-de Haen A.G., Germany), bitter (100 μM cycloheximide, Sigma–Aldrich, Germany), and salty (0.3 M NaCl, J.T. Baker, Holland), 4 days each. The position of bottles was switched every 24 h to avoid side preferences. The consumption of water and taste solutions was estimated simultaneously in both genotypes by weighing the bottles daily, and calculating daily preference (%) as total tastant solution/total water intake × 100%. No food or water deprivation was used in this experiment.

2.3. Statistical analyses

All results are expressed as mean ± S.E.M. Differences between genotypes were analyzed by the Mann–Whitney U-test. Difference between trials was analyzed using the Wilcoxon signed ranks test. A probability less than 0.05 was considered statistically significant in all tests.

3. Results

Table 1 summarizes the results of Experiment 1. In the spatial memory test, there were no genotype differences in the latency to enter the novel arm, the number of entries to the novel arm, and time spent exploring the novel arm.

In Experiment 2, using the Y-maze task with food reinforcement, there were also no genotype differences in the latency to eat in either trial (Table 2). However, during the first trial, the VDR mutant mice demonstrated significantly more “incorrect” entries to empty arms, and displayed more total entries, than did their WT littermates. In contrast, during the second trial, the number of entries was similar in both genotypes (Table 2).

Assessing behavioral responses to novel food (Experiment 3), we found no differences between the groups in the latency to eat bread on trial 1, whereas this measure on trial 2 was significantly shorter in the WT mice than in the mutant group (Table 3). Total number of entries was unaltered in both genotypes in all trials. As can be seen in Table 3, testing the mouse response to vanilla food revealed no genotype differences in the latency to eat on trial 1. In contrast, during the second trial, VDR mutant mice showed a shorter latency to eat compared to their WT controls. WT mice also displayed more entries to the food arm (correct entries) and to the empty arms (incorrect entries) during the initial trial, compared to the mutant group. In both trials, the WT mice demonstrated more total entries than did the VDR mutant mice (Table 3). Finally, in test with onion, the WT mice showed a longer latency to eat food, and made more entries, compared to the VDR mutant mice. Again, these behavioral parameters did not differ significantly between the genotypes during the second trial (Table 3).

In Experiment 4, we assessed olfactory functions in our mice, using the buried food pellets test. Overall, both geno-
Table 2
Behavioral performance of male wild type (WT) and vitamin D receptor (VDR) mutant mice in the familiar food test (two-trial Y-maze with a 2 h inter-trial interval; Experiment 2)

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 10)</th>
<th>VDR mutants (n = 10)</th>
<th>WT (n = 10)</th>
<th>VDR mutants (n = 10)</th>
<th>Between genotypes</th>
<th>Between trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-maze + food pellets interval time 2 h</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Latency (s) to eat the food</td>
<td>19.3 ± 3.07</td>
<td>57.5 ± 21</td>
<td>46.1 ± 20.4</td>
<td>22.1 ± 4.43</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Entries to the food arm</td>
<td>1.00 ± 0.00</td>
<td>1.60 ± 0.16</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Entries to the empty arms</td>
<td>0.60 ± 0.22</td>
<td>3.00 ± 0.79</td>
<td>0.60 ± 0.22</td>
<td>0.30 ± 0.15</td>
<td>P &lt; 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Total number of entries</td>
<td>1.60 ± 0.20</td>
<td>4.60 ± 0.80</td>
<td>1.60 ± 0.22</td>
<td>1.30 ± 0.15</td>
<td>P &lt; 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Incorrect entries (%)</td>
<td>26.7 ± 9.00</td>
<td>51.2 ± 9.60</td>
<td>26.7 ± 9.03</td>
<td>15.0 ± 7.64</td>
<td>P &lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M (NS: P > 0.05).

a Trial 1.
b Trial 2.
c U-test.
d Wilcoxon signed ranks test.

Table 3
Behavioral performance of male wild type (WT) and vitamin D receptor (VDR) mutant mice in the novel food test (two-trial Y-maze; Experiment 3)

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 10)</th>
<th>VDR mutants (n = 10)</th>
<th>WT (n = 10)</th>
<th>VDR mutants (n = 10)</th>
<th>Between genotypes</th>
<th>Between trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food pellets + bread bread, interval time 2 h</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Latency (s) to eat the food</td>
<td>152.0 ± 35.0</td>
<td>156.0 ± 40.0</td>
<td>67.0 ± 17.0</td>
<td>194.0 ± 38.0</td>
<td>NS</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Total number of entries</td>
<td>8.00 ± 1.00</td>
<td>9.00 ± 1.00</td>
<td>5.50 ± 1.20</td>
<td>9.00 ± 2.40</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Incorrect entries (%)</td>
<td>47.1 ± 6.5</td>
<td>55.6 ± 1.7</td>
<td>52 ± 7.6</td>
<td>51.5 ± 9.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Food pellets + vanilla interval time 4 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Latency (s) to eat the food</td>
<td>110.7 ± 27.3</td>
<td>96.7 ± 34.2</td>
<td>102.3 ± 29.2</td>
<td>53.7 ± 25.5</td>
<td>NS</td>
<td>P &lt; 0.03</td>
</tr>
<tr>
<td>Total number of entries</td>
<td>4.30 ± 0.70</td>
<td>2.40 ± 0.37</td>
<td>2.10 ± 0.59</td>
<td>1.10 ± 0.10</td>
<td>P &lt; 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Incorrect entries (%)</td>
<td>56.2 ± 1.07</td>
<td>52.6 ± 0.37</td>
<td>4.20 ± 1.81</td>
<td>1.20 ± 0.36</td>
<td>P &lt; 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Food pellets + onion interval time 4 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency (s) to eat the food</td>
<td>233.1 ± 29.0</td>
<td>138.0 ± 32.7</td>
<td>277.0 ± 21.7</td>
<td>116.6 ± 36.4</td>
<td>P &lt; 0.04</td>
<td>P &lt; 0.008</td>
</tr>
<tr>
<td>Entries to the food arm</td>
<td>6.30 ± 0.90</td>
<td>3.30 ± 0.68</td>
<td>6.80 ± 1.08</td>
<td>2.60 ± 0.65</td>
<td>P &lt; 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Entries to the empty arms</td>
<td>12.3 ± 1.58</td>
<td>5.60 ± 1.54</td>
<td>13.2 ± 2.13</td>
<td>3.80 ± 1.25</td>
<td>P &lt; 0.007</td>
<td>P &lt; 0.002</td>
</tr>
<tr>
<td>Total number of entries</td>
<td>18.6 ± 2.38</td>
<td>8.90 ± 2.16</td>
<td>20.0 ± 3.15</td>
<td>6.40 ± 1.78</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.004</td>
</tr>
<tr>
<td>Incorrect entries (%)</td>
<td>66.02 ± 1.96</td>
<td>56.94 ± 4.19</td>
<td>65.83 ± 2.23</td>
<td>55.6 ± 2.9</td>
<td>P &lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are presented as mean ± S.E.M (NS: P > 0.05).

a Trial 1.
b Trial 2.
c U-test.
d Wilcoxon signed ranks test between trials.

types showed similar latencies to find (WT: 55 ± 9 s; VDR mutant mice: 76 ± 16 s, NS) and eat (WT: 138 ± 32 s; VDR mutant mice: 83 ± 16 s, NS) familiar food, confirming unimpaired olfaction in VDR mutant mice.

This study of mouse gustatory function and baseline hedonic responses (Table 4; Experiment 5) showed no impairments in taste discrimination in VDR mutant mice, as both genotypes demonstrated similar consumption of sweet, salt,

Table 4
Intake of different tastants in the wild type (WT) and vitamin D receptor (VDR) mutant mice (Experiment 5)

<table>
<thead>
<tr>
<th>Taste solutions</th>
<th>WT (n = 10)</th>
<th>VDR mutants (n = 10)</th>
<th>U-test</th>
<th>Wilcoxon signed ranks test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% sucrose</td>
<td>52.15 ± 1.99 (n = 9)</td>
<td>60.22 ± 5.25 (n = 10)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>0.3 M NaCl</td>
<td>28.99 ± 2.18 (n = 9)</td>
<td>29.81 ± 6.74 (n = 7)</td>
<td>NS</td>
<td>P &lt; 0.008</td>
</tr>
<tr>
<td>50 mM citric acid</td>
<td>46.27 ± 1.04 (n = 9)</td>
<td>43.01 ± 2.59 (n = 10)</td>
<td>NS</td>
<td>P &lt; 0.018</td>
</tr>
<tr>
<td>10 μM cyclohexamide</td>
<td>22.71 ± 1.05 (n = 9)</td>
<td>20.22 ± 1.86 (n = 10)</td>
<td>NS</td>
<td>P &lt; 0.008</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M. (NS: P > 0.05), calculated as the preference percentage (daily intake (%) = taste solutions daily intake/total liquid intake (taste solution intake + water intake) × 100).

sour or bitter solutions. All mice consumed markedly less NaCl solution (~30%), whereas the intake of citric acid was close to 50% in both genotypes, with a similar (~20%) aversion of cyclohexamide. Both genotypes showed unaltered intake of sucrose solution and water (Table 4).

4. Discussion

In general, the main findings of the present study can be summarized as follows: VDR mutant mice display unaltered spatial memory, olfaction, gustation and hedonic responses, but demonstrate aberrant responses to novel food in different contexts.

Despite the fact that numerous VDR were found in memory-controlling brain areas [10,11], and cognitive deficits were reported to be due to low vitamin D in both animals [2,20] and humans [35], our results showed that in Y-maze spatial memory task, both WT and VDR mutant mice explored novel arms equally well on both trials (Table 1). These findings are generally consistent with observations in VDR mutant mice tested in several other memory tasks, including spontaneous alternation and open field within-trial habituation [42,43]. Moreover, both genotypes demonstrated similar inter-trial habituation (reflecting their unaltered long-term memory) in the open field test [43]. Collectively, these and our present data support the notion that memory domain (unlike locomotion and anxiety [15,42]) is most likely unaffected by the VDR mutation in mice.

Importantly, the number of Y-maze arm entries in VDR mutant mice was similar or even higher (than in the WT mice) in some tests here (Table 2), indicating unimpeded general motor functions in these mice. Earlier studies have shown (rev. in: [44]) that increased motivation, such as hunger, may alter the animal exploratory performance in different tests, influencing both their cognitions and emotionality. In the Y-maze task with food reinforcement (Experiment 2), both groups of hungry mice showed similar ability to find familiar food in both trials, again suggesting that motor functions of VDR mutant mice in this test were relatively normal. However, the trial 1 data of this experiment, reporting higher locomotion in the VDR mutant mice, seem to be consistent with the above-mentioned effects of hunger on exploration, and may be explained by their somewhat higher initial stress-reactivity (compared to the WT mice), also in line with their increased anxiety phenotype (also see a tendency to a longer latency to eat food in trial 1, Table 2).

Since memory testing using familiar food as reinforcement (Experiment 2), revealed elevated trial 1 activity in the VDR mutant mice, we wanted to assess their responses to novel versus familiar food. As unfamiliar food is known to induce strong neophobic responses, the latency to consume novel food can be used as a measure of anxiety [45,46]. In Experiment 3, using white bread as a novel food, we noted that latency to eat was reduced in the WT mice on trial 2, but not in VDR mutant mice (showing a tendency to increase; Table 3). These observations suggest that upon second novel food exposure the VDR mutant mice were less willing to consume novel food—the response which may again be best explained by their high anxiety phenotype, as already reported [15].

Likewise, in tests with vanilla and onion, the VDR mutant mice showed reduced exploration—a profile generally seen as a sign of stress and anxiety in rodents [22]. Interestingly, the latency data from these tests yielded conflicting results, with no genotype difference on trial 1 for vanilla (resembling data from bread exposure), but not for onion, and shorter trial 2 latencies in the mutant group (Table 3). Although it is difficult to interpret such latency data, in our present study we aimed at a more accurate interpretation of mouse phenotypes, following recommendations to avoid conclusions based on a single measure [21], and trying to assess several different indices. It is also possible that varying latency responses in this experiment represent a complex interplay between different factors (not fully explored here), such as baseline anxiety, aversive/attractive properties of different types of food, neophobic responses, and the effects of repeated testing (including a combination of two trials, and a test battery involving several different food exposures). Importantly, as rich Ca “rescue” diet (used here in the VDR mutant mice) has been shown to normalize their plasma Ca levels [8,47], it was possible conclude that the behavioral differences reported here were not due to non-specific factors, such as hypocalcaemia. Olfactory functions of VDR mutant mice, tested here in the buried food test (Experiment 4), also appear to be unaltered, confirming that behavioral responses of these mutant mice in the novel food tests are not due to olfactory deficits.

Can genotype differences in gustatory functions affect the mouse behaviors in our study? Assessing the mouse gustatory function, we found no overt impairments in gustation in the VDR mutant mice, with a predictably strong aversion of salty and bitter solutions in all genotypes (Table 4). The fact that gustatory functions, including TRP-mediated perception of bitter taste [28,29] were normal in VDR mutant mice, suggests that VDR do not affect the TRPV system or other signaling pathways relevant to sensory mechanisms.

Finally, as human vitamin D deficiency has long been known to be accompanied by irritability, depression and psychoses [13,16], it was possible to expect altered baseline depression-like behaviors in mice lacking functional VDR. To the best of our knowledge, the present study was the first study assessing depression domain in VDR mutant mice. However, the VDR mutant mice showed unaltered hedonic responses (Table 4), negating the possibility that genetic defect in VDR per se may lead to anhedonic depression. While these data seem to contradict reports on antidepressant effects of vitamin D [14,31,32,34,35] it is also possible that such effects of vitamin D are not mediated through VDR, and may be due to its other (steroid-like) effects, resembling known antidepressant effects of other neurosteroids [48].

In conclusion, the results of the present study show that the VDR system is neither crucially important for cognitive
functions (such as memory), nor involved in the regulation of some major sensory functions. VDR genetic ablation is also unable to alter depression-like behaviors in these mice, as assessed by their unimpaired hedonic responses. However, our data demonstrate that VDR mutant mice consistently display neophobic anxiety-like responses to novelty in several different tests. Accompanied by normal motor activity levels in all these tests, our data confirm increased vulnerability to stress in VDR mutant mice, as has already been suggested [15].

Collectively, these data contribute to our understanding of the complexity of VDR mutant mouse behavioral phenotype, warranting their further in-depth analyses and the use in behavioral neuroscience as a potentially interesting genetic animal model of vitamin D-related brain disorders. These data may also be clinically relevant (enabling a better focus on human brain/behavioral disorders associated by vitamin D/VDR deficits), and contribute to the growing recognition of the importance of a neurosteroid hormone vitamin D in the regulation of brain functions and behavior [27,49].

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