Akt2 Deficiency is Associated with Anxiety and Depressive Behavior in Mice

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Abstract

Background: The economic burden associated with major depressive disorder and anxiety disorders render both disorders the most common and debilitating psychiatric illnesses. To date, the exact cellular and molecular mechanisms underlying the pathophysiology, successful treatment and prevention of these highly associated disorders have not been identified. Akt2 is a key protein in the phosphatidylinositide-3 (PI3K) / glycogen synthase 3 kinase (GSK3) signaling pathway, which in turn is involved in brain-derived neurotrophic factor (BDNF) effects on fear memory, mood stabilisation and action of several antidepressant drugs. The present study thus explored the impact of Akt2 on behaviour of mice. Methods: Behavioural studies (Open-Field, Light-Dark box, O-Maze, Forced Swimming Test, Emergence Test, Object Exploration Test, Morris Water Maze, Radial Maze) have been performed with Akt2 knockout mice (akt−/−) and corresponding wild type mice (akt+/+). Results: Anxiety and depressive behavior was significantly higher in akt−/− than in akt+/+ mice. The akt−/− mice were cognitively unimpaired but displayed increased anxiety in several behavioral tests (O-Maze test, Light-Dark box, Open Field test). Moreover, akt−/− mice spent more time floating in the Forced Swimming test, which is a classical feature of experimental depression. Conclusion: Akt2 might be a key factor in the pathophysiology of depression and anxiety.

Key Words
Anxiety • Behavior • Depression • Akt2 • PI3K • GSK3 • BDNF

Introduction

Phosphatidylinositide-3 kinase (PI3K) dependent signaling participates in the regulation of several fundamental neuronal processes, including neuronal growth, survival and differentiation, formation of dendrites as well as synaptic plasticity [1].

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PI3K signaling is triggered by a variety of growth factors including brain-derived neurotrophic factor (BDNF) [2, 3], which has been implicated in bipolar disorder, depression, depression-related personality traits and anxiety [2, 4-7]. Decreased expression of BDNF contributes to stress-related mood disorders and the upregulation of BDNF plays a role in the actions of different antidepressant treatments [8, 9].

Downstream targets of the PI3K include phosphoinositide dependent kinases and Akt [3, 10]. Akt phosphorylates and thus inhibits glycogen synthase 3 (GSK3). PI3K-Akt signaling has been discussed to mediate antidepressant effects and constitutes an important signaling pathway in the subcellular integration of synaptic neurotransmission [11, 12]. This pathway also modulates neuronal cell proliferation, migration, and plasticity [12]. Lithium, valproate, olanzapine and clozapine are at least in part effective via PI3K-Akt signalling [13-16]. Moreover, PI3K is involved in behavioral sensitization to cocaine [17], the extinction of fearful memories [18] and hippocampal plasticity [18]. Disruption of GSK3 phosphorylation by Akt decreases anxiety and reduces proneness to depression in mice [19]. Conversely, decreased expression of phosphoinositide dependent kinase 1 (PDK1), leads to increased anxiety [20].

The three Akt isoforms do not serve identical functions [21]. Akt1 participates in the regulation of body and cell size [22, 23] and has been implicated in bipolar disorders [24-27]. Akt3 participates in the signaling underlying developmental brain malformations [28-32]. Akt2 is particularly important for glucose metabolism [33, 34] and participates in the regulation of neuronal differentiation and survival [21, 35, 36] and dopamine transporter cell surface expression [37]. To the best of our knowledge, however, nothing is known about a role of Akt2 in the regulation of behavior and mood.

The present study thus aimed to define the role of the isoform Akt2 in mouse behavior. To this end, behavioral studies have been performed in Akt2 knockout (Akt2–/–) and wild type mice (akt+/+).

Animals and Methods

Animals

The generation, properties and genotyping of Akt2 deficient mice were described earlier [33]. Sex and age matched mice with age more than 2 months were used for the experiments. All animal experiments were conducted according to the German law for the care and use of laboratory animals and were approved by local authorities. For the present study 59 Akt2 knockout mice (akt2–/–) and 64 wild type mice (akt+/+) were used. The akt2–/– and akt2+/+ mice were divided into four sets (first set: 14 akt2–/–, 16 akt2+/+ mice, second set: 8 akt2–/–, 10 akt2+/+ mice, third set: 12 akt2–/–, 12 akt2+/+ mice and fourth set: 4 akt2–/–, 5 akt2+/+ mice) and tested consecutively at an interval of four months. In the radial maze 12 akt2–/– and 11 akt2+/+ mice were tested. For the Morris Water Maze 9 akt2–/– and 10 akt2+/+ mice were used. Age at the beginning of the tests was 8-12 weeks.

Housing and handling

Mice were bred and housed in Tuebingen. They had access to a standard mouse chow and tap water ad libitum and were maintained at a 12:12 h inverted cycle with lights on between 7 p.m. and 7 a.m. Behavioral testing occurred between 8 a.m. and 8 p.m. Only one type of experiment was done on the same day and the home cage rack was brought to the test room at least 30 min before each experiment. Dry surfaces of apparatus were thoroughly cleaned with 70% ethanol before releasing the animal. Experiments extended over a total of 3 months and were done in the following order: open-field, light dark (LD) box, O-maze, forced swimming test, emergence test, novel object. In addition, a further cohort of mice was tested on the 8-arm radial maze and another group on the Morris Water Maze.

Video tracking and illumination

For data acquisition animals were video tracked by the camera 302050-SW-KIT-2-CAM at a resolution of 0.62 to 0.72 pixel (TSE-Systems, Bad Homburg, Germany). Raw data were transferred to Excel for further analysis. Experiments were performed with diffuse indirect room light produced by dimmable bulbs,
adjusted to yield less than 30 lux in the center of the experimental arena. The only exception was the LD-box test where full room light was switched on to obtain approximately 500 lux in the lit chamber.

**Open-Field**

The quadratic arena had side lengths of 50 cm, a white plastic floor and 40 cm high sidewalls. Each subject was released near the wall and observed for 30 min. For evaluation, a border with 10 cm distance to the wall was considered. The layout is illustrated in Fig. 1A. Data were collected in the entire arena which was further divided into border- and center area. Corners as a subsegment of the border area were studied separately. Global distance and speed as well as distance and time spent in the different regions were assessed. In addition the number of crosses over the lines of the virtual grid drawn on the arena surface were counted (horizontal activity). A frame generating a photoelectric barrier in the Open Field arena allowed the registration of rearings (vertical activity). Since some of the mice showed low numbers of rearings but long overall rearing times we also provide the rearing time normalized to the number of rearings (ratio time/rearing) indicating the average time for a single rearing.

**Light-Dark (LD-) box**

An infrared permeable box made of black acryl (40 cm high) was inserted into the Open Field arena and covered 33% of the surface area (Fig. 2A). An aperture of 10 cm length and 11 cm height with rounded down corners led from the lighted arena to the dark box. The experiment was performed under direct room light. Each subject was released in the corner of the lit compartment and observed for 10 min. The illuminated part of the arena was divided in subregions as well. As shown in the ground plan in Fig. 2A, data were also collected for corners and the entrance area of the box. Gathered were time spent in the different regions and covered distances as well as the number of transitions between lit chamber and dark box. Rearings were counted by the same photoelectric barrier used in the Open Field experiment.

**O-Maze**

A 5.5 cm wide annular runway was constructed using grey polyvinyl-chloride. It had an outer diameter of 46 cm and was placed 40 cm above the floor. The two opposing 90° closed sectors were protected by 11 cm high inner and outer walls of grey polyvinyl-chloride, while the remaining two open sectors had a border of 5 mm. Animals were released in one of the closed sectors and observed for 10 min. A photograph illustrates the experimental set-up in Fig. 3A. The mice were not only confronted with open space, but also with height. After an acclimatization period mice advance onto an unprotected runway and dip their heads to explore the cliff. This behavior was classified as protected headdip when the body was still in the protected zone. When the protected zone was left completely the exploration of the height was classified as unprotected headdip. Assessed were the numbers of protected and unprotected headdips, time and covered distance in protected and unprotected zones and the number of transitions between the areas.

**Forced Swimming Test**

In the Forced Swimming Test mice were placed in a container filled with water of temperatures between 24 and 26 °C (Fig. 4A). The diameter of the container was 20 cm. The mouse was placed in the water without being able to touch the ground. Mice were observed during 6 min and the time they spent without movement, called floating, was recorded.

**Emergence Test**

For the Emergence Test, the procedure was modified after Dulawa et al. [21]. An infrared permeable box, which was previously in the home cage of the mouse during more than 24 hours, was inserted into the arena. The home box had a base area of 9x10 cm with two apertures (3 cm length, 3.5 cm height with rounded down corners) on the long sides. The mouse was observed for 30 min. A diagram shows the experimental setup in Fig. 5 A. The time, which the mice spent in their homebox and the number of entries into the open arena were recorded.

**Object Exploration Test**

For the object exploration test, the procedure was modified after Dulawa et al. [21]. The Arena was the same as for the Open Field and the Emergence Test. The mouse was first observed for 30min in the Open
Field arena. During that time mice became familiar with their environment. Afterwards, a falcon tube was additionally put into the arena and fixed with adhesive tape. Thereby the animals were confronted with a novel stimulus while offering the option to retreat into a familiar, safe area. The mice were then observed for another 30min and the contact with the nose on the falcon tube was taken as criterion to analyze the reaction on a novel object. Figure 6A shows the layout of the arena. The number of approaches and the average distance to the novel object were determined.

**Morris Water Maze**

We adapted the original procedure for the use with mice [38, 39]. A round grey poly-propylene swimming tank with a diameter of 150 cm was filled with water (temperature 24-26° C, depth 15cm) that was made opaque by addition of a white dye. The white round goal platform (d = 12 cm) was hidden 0.5cm below the water surface. Salient distant cues such as wall-mounted laboratory shelves and various posters were available in room. Animals performed 30 training trials, 6 per day with intertrial intervals of 30-60min and pseudo-randomly varying starting positions. To minimize handling, they were transferred to the pool using a white plastic cup. The trials lasted until the mice climbed onto the platform or until 120s had elapsed. After they had reached the platform and stayed on it for five seconds they were allowed to climb onto a wire mesh grid and were transferred to their cage without further handling. During the first 18 trials (acquisition phase) the hidden platform was held in the same position and then moved to the opposite quadrant for the remaining 12 trials (reversal phase). Time and distance needed to complete the task, the time spent in the border area and the average swim velocity were recorded.

**Radial Maze**

The apparatus was constructed of grey poly-vinyl chloride with smooth surface and clear Perspex side walls. Eight arms (7x38 cm) extended from the octagonal centre platform (diameter 18.5 cm) with a 47 cm distance from the platform centre to the end of each arm. The maze was elevated 38 cm from the floor and placed in a room which was rich in salient extramaze cues. Baits were small single millet pellets and placed in submerged small metal cups at the end of each arm. Firstly dietary restriction over 2 days was carried out, which led to an 85% decrease of free-feeding body weight in all subjects. For two days habituation sessions followed, where all mice had the opportunity to collect several pellets in each baiting cup over a time period of 10 minutes. In the following ten days training sessions were carried out, where every mouse had to explore the maze and to collect food until it had consumed all pellets or 15 minutes had elapsed. Assessed were the number of correct choices until the first error was made (arm entries with pellet consumption), the total number of errors (entries to arms without pellet consumption), working memory errors (re-entries to arms where the pellet has already been consumed) and time as well as distance required to complete the task.

**Statistical analysis**

For the comparison of the test results of the akt2−/− and akt2+/+ mice the statistic software Graph Pad Prism was used. Only results with P<0.05 were considered significant.

**Results**

**Open-field**

In the open-field akt2−/− mice showed the same speed as the akt2+/+ mice (Fig. 1B). However, the total distance traveled (Fig. 1C) was significantly decreased in akt2−/− mice when compared to akt2+/+ mice. The akt2−/− mice also spent more time in the border area (Fig. 1D) and travelled a shorter distance there (Fig. 1E). Also the time the akt2−/− mice spent in the corners was significantly higher in comparison to akt2+/+ mice (Fig. 1F). The akt2−/− mice visited the center area more often, spent more time there and consequently travelled a longer distance in the center (Fig. 1G-I). Rearing behavior is shown in Table 1.
The \( akt2^{-/-} \) mice spent significantly more time in the hidden area than the \( akt2^{+/+} \) mice (Fig. 2B) and visited the hidden area more often (Fig. 2C). They also showed less rearing behavior indicated by a lower number of rearings (Fig. 2D) in the light area, a shorter rearing time (Fig. 2E) and a smaller ratio of number/time rearing (Fig. 2F). Despite the fact that the \( akt2^{-/-} \) mice spent more time in the dark area the number of rearings in the hidden zone (Fig. 2G) was still decreased in comparison to \( akt2^{+/+} \) mice. The rearing time was almost the same in both groups (Fig. 2H) which consequently leads to an increased ratio of time/number of rearings (Fig. 2I).

**Table 1.** Synopsis of rearing parameters in the open field test (arithmetic means ± SEM) of \( akt2^{-/-} \) and \( akt2^{+/+} \) mice

<table>
<thead>
<tr>
<th>Open Field</th>
<th>( akt2^{+/+} ) mice</th>
<th>( akt2^{-/-} ) mice</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rearings in border area</td>
<td>303.09 ± 6.00</td>
<td>277.11 ± 8.37</td>
<td>( P = 0.0092; ) Mann-Whitney Test</td>
</tr>
<tr>
<td>Rearing time in border area (min)</td>
<td>16.51 ± 0.40</td>
<td>12.60 ± 0.48</td>
<td>( P &lt; 0.0001; ) t-test</td>
</tr>
<tr>
<td>Ratio time/rearing in border area (s/rearing)</td>
<td>3.33 ± 0.11</td>
<td>2.74 ± 0.09</td>
<td>( P = 0.0003; ) Mann-Whitney Test</td>
</tr>
<tr>
<td>Number of rearings in center area</td>
<td>82.30 ± 4.42</td>
<td>52.92 ± 4.72</td>
<td>( P &lt; 0.0001; ) t-test</td>
</tr>
<tr>
<td>Rearing time in center area (min)</td>
<td>3.08 ± 0.18</td>
<td>1.79 ± 0.19</td>
<td>( P &lt; 0.0001; ) t-test</td>
</tr>
<tr>
<td>Ratio time/rearing in center area (s/rearing)</td>
<td>2.34 ± 0.10</td>
<td>1.93 ± 0.09</td>
<td>( P = 0.0060; ) t-test</td>
</tr>
</tbody>
</table>

**Light Dark Box**

The \( akt2^{-/-} \) mice spent significantly more time in the hidden area than the \( akt2^{+/+} \) mice (Fig. 2B) and visited the hidden area more often (Fig. 2C). They also showed less rearing behavior indicated by a lower number of rearings (Fig. 2D) in the light area, a shorter rearing time (Fig. 2E) and a smaller ratio of number/time rearing (Fig. 2F). Despite the fact that the \( akt2^{-/-} \) mice spent more time in the dark area the number of rearings in the hidden zone (Fig. 2G) was still decreased in comparison to \( akt2^{+/+} \) mice. The rearing time was almost the same in both groups (Fig. 2H) which consequently leads to an increased ratio of time/number of rearings (Fig. 2I).
As compared to akt2+/+ mice, akt2−/− mice showed significantly less protected and unprotected head dips in the O-Maze test (Fig. 3B, C). The akt2−/− mice visited the open arms less frequently and travelled a shorter distance there as well. Also the time spent in the open arms was significantly decreased in akt2−/− mice (Fig. 3D-F). Consequently, the number of
entries into the protected arms was less in akt2−/− mice (Fig. 3G). Despite the fact, that they spent more time in the closed area (Fig. 3H), the distance covered there by the akt2−/− mice was almost the same as the distance travelled by akt2+/+ mice (Fig. 3I).
Forced Swimming Test
In the Forced Swimming Test the akt2⁻/⁻ mice spent significantly more time floating on the surface of the water than the akt2⁺/+ mice (Fig. 4B).

Emergence Test
During the Emergence Test akt2⁻/⁻ mice spent significantly more time in the homebox than the akt2⁺/+ mice (Fig. 5B). The number of visits to the open area was also decreased in akt2⁻/⁻ mice (Fig. 5C).

Novel Object Test
In the Novel Object Test the curiosity of the mice was tested. akt2⁻/⁻ mice examined the novel object less often and kept a larger distance to the novel object over the whole time period than the akt2⁺/+ mice (Fig. 6B, C).
The performance of akt2\(^{-/-}\) mice in the Morris Water Maze was not different from akt2\(^{+/+}\) mice. Both groups showed that they were equally fast in learning and orientation as shown by decreasing time to complete the trials (Fig. 7A), shorter distances to reach the platform (Fig. 7B) and less time exploring the border area (Fig. 7C). The mice also acquired fast to the new challenge after the platform was moved to the opposite quadrant of the tank (trial 19-30). The swimming velocity remained approximately the same during all trials (Fig. 7D).

**Radial Maze**

Again in the radial maze akt2\(^{+/+}\) mice and akt2\(^{/-}\) mice did not show any significant differences. The number of correct choices until the first error occurred increased (Fig. 8B) showing the learning effect in both groups. Also the number of total errors (Fig. 8C) and working memory errors (Fig. 8D) dropped in both groups during the sessions. The mice needed less time to collect the hidden baits (Fig. 8E) and also had to travel shorter distances (Fig. 8F) to complete the task.

**Discussion**

In order to explore the *in vivo* functional significance of Akt2 in the regulation of behavior, we assessed the performance of Akt2 knockout mice (*Akt2\(^{-/-}\)*) and C57Bl/6J wild type mice in a place navigation task in the water maze, radial maze and in a battery of forced and free exploration tests.

In the open-field the total distance traveled was decreased in akt2\(^{-/-}\) mice. akt2\(^{-/-}\) mice spent more time in the border area and spent significantly increased time in the corners.
In the dark light box, \( \textit{akt2}^{-/-} \) mice spent significantly more time in the hidden area, showed less rearing behavior, a shorter rearing time and a smaller ratio of number/time rearing. In the O-Maze test, \( \textit{akt2}^{-/-} \) mice showed significantly less protected and unprotected head dips, visited the open areas less frequently and travelled a larger distance there as well. In the Forced Swimming Test the \( \textit{akt2}^{-/-} \) mice spent significantly more time floating on the surface of the water. During the Emergence Test \( \textit{akt2}^{-/-} \) mice spent significantly more time in the homebox than the \( \textit{akt2}^{+/+} \) mice. The number of visits to the open area was also decreased.

In the Novel Object Test the curiosity of \( \textit{akt2}^{-/-} \) mice was decreased, i.e. \( \textit{akt2}^{-/-} \) mice examined the novel object less often and kept a longer distance to the novel object over the whole time period. In must be considered, however, that the results of the novel object test are affected by the location of the novel object in the center of the open field.

In conclusion we found decreased activity, increased anxiety, increased depressive behavior and less exploratory behavior in \( \textit{akt2}^{-/-} \) mice. Increased anxiety was observed in the O-Maze test, the dark light box and in the open field paradigm.

The present data reveal a powerful inhibitory effect of Akt2 on anxiety and depression. The present study did not address the Akt2 dependent mechanisms accounting for the impact on behavior. In theory, Akt2 could be effective through phosphorylation and thus inhibition of GSK3 [40]. However, inhibition of GSK3 reduces both depression-like and manic-like behavior [41] and transgenic mice overexpressing glycogen synthase kinase 3beta display hyperactivity and mania [42]. Moreover, mice expressing Akt-resistant GSK3 are hyperactive and curious [19]. Conversely, inhibition of GSK3 beta results in increased anxiety behavior [43]. Thus, disruption of GSK3 phosphorylation and inhibition does not explain the phenotype of the \( \textit{akt2}^{-/-} \) mice.

In view of the known effect of Akt2 on dopamine transporter cell surface expression [37], deranged dopamine transporter activity could in theory contribute to the phenotype of the \( \textit{akt2}^{-/-} \) mice. Both, Akt and GSK3 have been suggested to participate in the regulation of behavior by influencing the monoamine neurotransmitters dopamine and serotonin [44].

The behavioral phenotype of the Akt2 deficient mouse is similar to that of the PDK1 hypomorphic mouse, which displayed enhanced anxiety paralleled by significantly decreased GABA, taurine and serotonin concentrations in the amygdala [20]. As anxiety-related behavior is often connected with a clear bias towards passive coping styles, the neophobia observed in \( \textit{akt2}^{-/-} \) mice might predispose these animals to anxious behavior.

The reduced locomotion and increased floating in the forced swimming test of Akt2 knockout mice could be linked to a depression-like state in rodents, which could be attributed to disturbed BDNF and PI3K signaling via Akt2. It is tempting to speculate that Akt2 exerts some antidepressant effects and that reduced stimulation of Akt2 may lead to depressive disorders. If so, the present observations may help to resolve the apparent discrepancy on the role of BDNF in the pathophysiology of major depression [45].

Taken together, our results suggest an involvement of Akt2 in anxiety and depressive behavior in mice and identify Akt2 as a potential therapeutic target for the treatment and prevention of anxiety disorders and depression. Future studies are required to delineate the Akt2 sensitive mechanisms accounting for this powerful effect of Akt2 on behavior.

**Conflict of Interest**

All contributing authors declare no conflict of interest.

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References


